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Residual Complexes Containing SMARCA2 (BRM) Underlie the Oncogenic Drive of *SMARCA4* (*BRG1*) Mutation

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Collectively, genes encoding subunits of the SWI/SNF (BAF) chromatin remodeling complex are mutated in 20% of all human cancers, with the *SMARCA4* (*BRG1*) subunit being one of the most frequently mutated. The SWI/SNF complex modulates chromatin remodeling through the activity of two mutually exclusive catalytic subunits, *SMARCA4* and *SMARCA2* (BRM). Here, we show that a *SMARCA2*-containing residual SWI/SNF complex underlies the oncogenic activity of *SMARCA4* mutant cancers. We demonstrate that a residual SWI/SNF complex exists in *SMARCA4* mutant cell lines and plays essential roles in cellular proliferation. Further, using data from loss-of-function screening of 165 cancer cell lines, we identify *SMARCA2* as an essential gene in *SMARCA4* mutant cancer cell lines. Mechanistically, we reveal that *Smarca4* inactivation leads to greater incorporation of the nonessential *SMARCA2* subunit into the SWI/SNF complex. Collectively, these results reveal a role for *SMARCA2* in oncogenesis caused by *SMARCA4* loss and identify the ATPase and bromodomain-containing *SMARCA2* as a potential therapeutic target in these cancers.

Growing evidence indicates that subunits of the SWI/SNF (BAF) complex serve essential roles in the initiation and progression of cancer. At least eight genes that encode SWI/SNF subunits are frequently mutated in a variety of different cancers (1–5). The *SMARCA4* (*BRG1*) subunit is mutated in 10 to 35% of non-small-cell lung carcinoma, 15% of Burkitt's lymphoma, 5 to 10% of childhood medulloblastoma, and occasionally in pancreatic adenocarcinoma, ovarian clear cell carcinoma, and melanoma (2, 6–10). *SMARCA4* has been validated as a bona fide tumor suppressor, as haploinsufficient mice are tumor prone (11–13). Other SWI/SNF subunits also have potent tumor suppressor functions. Recently, a comprehensive analysis of whole-exome and whole-genome sequencing studies revealed that collectively subunits of the SWI/SNF complex are specifically mutated in one-fifth of all human cancers (2). Given that *SMARCA4* is one of the most broadly mutated subunits (3), developing an understanding of the mechanisms by which mutation of *SMARCA4* drives cancer and of the vulnerabilities created carries major disease relevance.

A catalytic ATPase subunit in the SWI/SNF complex, *SMARCA4*, has been shown to mediate nucleosome repositioning and to regulate transcription of its targets. *SMARCA2* (BRM), a homolog 75% identical to *SMARCA4*, is similarly capable of regulating chromatin structure and is mutually exclusive of *SMARCA4* in the SWI/SNF complex (14–17). These subunits function together with other core subunits, which include *SMARCC1*, *SMARCC2*, and *SMARCB1*, as well as with a number of variant lineage-restricted subunits, and collectively contribute to the control of cell fate and lineage specification (18–20). However, *SMARCA4* and *SMARCA2* have important differences in expression and function. In human and mouse tissues, *SMARCA4* and *SMARCA2* are often expressed at different stages of development and in distinct cell and tissue types (21, 22). Homozygous inactivation of *Smarca4* in mice leads to early embryonic lethality, whereas

SMARCA2-deficient mice are viable and survive into adulthood (11, 23).

While *SMARCA4* has emerged as a critical tumor suppressor, the mechanisms by which its mutation contributes to tumorigenesis, and whether its mutation creates cellular vulnerabilities, have been unknown. Given that at least eight SWI/SNF subunits are recurrently mutated in cancer, one possibility is that each of these mutations inactivates the SWI/SNF complex, resulting in mutational equivalency. However, based upon distinct associated cancer spectra and distinct mutational phenotypes in mice, we hypothesize that cancer driven by SWI/SNF mutations results from aberrant activity of the remaining subunits, which assemble into a residual complex. Indeed, we have previously shown that a residual SWI/SNF complex is essential in *SMARCB1* mutant rhabdoid tumors (24). Here, we evaluate the role of residual SWI/SNF complexes in *SMARCA4* mutant cancers.

MATERIALS AND METHODS

Cell culture. NCI-H1299 (CRL-5803) and A549 (CCL-185) cell lines were purchased from the ATCC. NCI-H2122, H460, and HCC-827 were obtained from Jeffrey Shapiro's laboratory at Dana-Farber Cancer Institute. Derivation and manipulation of mouse embryonic fibroblasts (MEFs) were described previously (25). Transduced cells were selected in puromycin for 48 to 72 h before counting and seeding for colony formation assays. To evaluate colony-forming ability, cells were seeded at low density

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TABLE 1 Cell lines with inactivating SMARCA4 mutations used in vulnerability screen

Cell line	Tissue	SMARCA4 protein change	Variant classification	Heterozygosity
A549 ^a	Lung	p.L728fs	Frame shift deletion	Homozygous
HEC1A	Endometrium	p.V268fs	Frame shift insertion	Heterozygous
NCIH23 ^a	Lung	p.1598_1599KE>N ^a	Nonsense mutation	Homozygous
NCIH661 ^a	Lung	p.G1159fs	Frame shift deletion	Homozygous
NCIH1299 ^a	Lung	p.Tyr560fs	Frame shift	Homozygous
JHOC5 ^b	Ovary	Large focal deletion	Deletion	Homozygous
SK-MEL-5 ^b	Skin	p.F1053fs	Frame shift	Heterozygous
TYKNU	Ovary	Large focal deletion	Deletion	Homozygous

^a A previously reported SMARCA4 mutant (9).

^b Previously reported to be SMARCA4 deficient (29, 30).

and incubated under standard conditions for 10 to 14 days before staining with crystal violet staining solution (0.05% crystal violet, 1% formaldehyde, 1% phosphate-buffered saline [PBS], 1% methanol) for 20 min.

shRNA-mediated knockdown of SWI/SNF subunits. Doxycycline-inducible short hairpin RNAs (shRNAs) targeting SMARCB1 (V3LHS_367694, ACCAGTGTGACCCTGTTAA; V3LHS_367696, AGACAGCA GATCGAGTCCCT; Open Biosystems), cloned into the pTRIPZ vector (RHS4750; Open Biosystems) or nonsilencing control shRNA (pTRIPZ-NS; RHS4743; Open Biosystems), were transduced into SMARCA4 mutant cell lines (NCI-H1299 and A549) using lentivirus. Expression was initiated by the addition of doxycycline (2.5 µg/ml; 631311; Clontech). Cell medium was replaced with fresh medium containing doxycycline every other day. shRNAs targeting SMARCA2 (TRCN0000358828, GGCCATCGAAGACGGCAA TTT; TRCN0000330445, CTATATCATCATCGTCTATAA) and a nonsilencing control (TRCN0000072240, TCGTATTACAACGTGCTGACT) were obtained from the RNA interference (RNAi) screening facility at the Dana-Farber Cancer Institute and transduced into SMARCA4 mutant cell lines (NCI-H1299 and A549) and SMARCA4 wild-type cell lines (NCI-2122, NCI-H460, and HCC827). SMARCA2 and nonsilencing control shRNAs are in the pLKO lentiviral expression vector backbone.

Project Achilles. Data from Project Achilles were used to identify differentially essential genes in SMARCA4 mutant cell lines. Project Achilles is a systematic approach aimed at determining cancer-specific vulnerabilities in cancer cell lines using genome-scale shRNA screens (<http://www.broadinstitute.org/achilles>) (26). We used the Achilles version 2.4 data set, consisting of 216 cell lines that were screened according to an unpublished method. Briefly, for the Achilles screens, cells were transduced with pooled shRNA libraries containing 54,020 shRNAs targeting 11,194 genes and propagated for at least 16 doublings. The abundance of remaining individual shRNAs was determined at the endpoint relative to an initial reference pool using next-generation sequencing. The subsequent analysis of Achilles data included the following steps. (i) Gene-level shRNA scores were derived from individual shRNA scores using ATARiS. ATARiS is a computational method that enriches for related phenotypic effects caused by individual shRNAs (27). (ii) The gene mutation status for SMARCA4 in cancer cell lines was next determined using information from the Cancer Cell Line Encyclopedia (www.broadinstitute.org/ccle) and prior publications (Table 1). Cell lines without hybrid capture sequencing data were removed from the analysis; thus, 165 of the 216 cell lines were used in SMARCA4 mutant and SMARCA4 wild-type comparisons. These data were used to generate a classifier file that was used in subsequent comparisons. (iii) Differentially essential genes were identified using the GenePattern module PARIS, which ranked ATARiS values based on the mutation status of the cell lines using the mutual information-based metric RNMI (rescaled normalized mutual information) (unpublished data; the PARIS GenePattern module will be available at <http://www.broadinstitute.org/cancer/software/genepattern>).

Immunoblots and coimmunoprecipitation experiments. Whole-cell extracts for immunoblotting were prepared by incubating cells on ice in 1% NP-40 lysis buffer (50 mM Tris-HCl, pH 7.4, 5 mM EDTA, 12% glycerol, 50 mM NaCl, 1% NP-40) plus protease inhibitors (11836170001;

Complete, Mini, EDTA-free; Roche) for 30 min. Supernatants were collected following centrifugation (10 min at 17,900 × g) at 4°C in a Legend 17R microcentrifuge (ClickSeal rotor 75003424). Protein concentrations were determined using the Bradford reagent (Bio-Rad). SDS-polyacrylamide gel electrophoresis was used to separate proteins, which were subsequently transferred to polyvinylidene difluoride (PVDF) membranes (Millipore). Antibodies used for immunoblotting included SMARCC1 (9746; Santa Cruz), ARID1A (A301-041A; Bethyl Laboratories), PBRM1 (A301-591A; Bethyl Laboratories), SMARCA4 (17796; Santa Cruz), SMARCC2 (A301-039A; Bethyl Laboratories), SMARCD1 (A301-595A; Bethyl Laboratories), SMARCE1 (A300-810A; Bethyl Laboratories), SMARCA2 (6889; Cell Signaling Technology), ACTL6A (A301-391A; Bethyl Laboratories), and actin (5125; Cell Signaling Technology) antibodies. Adobe Photoshop was used to prepare digital images (cropping and brightness/contrast) for publication.

Nuclear extracts for immunoprecipitation were prepared using the NE-PER nuclear and cytoplasmic extraction kit (78833; Thermo Scientific). Nuclear extracts (125 µg) were incubated in low-salt IP buffer (50 mM Tris-HCl [pH 7.5], 1 mM EDTA, 10% glycerol, 100 mM NaCl, 0.05% Tween 20, and protease inhibitors; the total volume was 300 µl) with antibodies (SMARCC1, 9746; Santa Cruz; SMARCA4, 17796; Santa Cruz; IgG, 2028; Santa Cruz) overnight at 4°C. Protein G Dynabeads (40 µl; 10004D; Life Technologies) were added and incubated at 4°C for 3.5 h. Beads were then washed twice with low-salt IP buffer and once with high-salt IP buffer (50 mM Tris-HCl [pH 7.5], 1 mM EDTA, 10% glycerol, 250 mM NaCl, 0.05% Tween 20). The beads were then resuspended in reducing SDS gel loading buffer.

Gene expression data. Whole-genome expression data from SMARCA4-deficient and wild-type mouse embryonic fibroblasts was generated as previously described (28). Robust multiarray (RMA)-normalized expression values for *Smarca2* in these samples were evaluated using Prism data analysis software. The *P* value was calculated using an unpaired *t* test with Welch's correction.

RESULTS

Residual SWI/SNF complexes are present in SMARCA4 mutant cell lines. As SMARCA4 is widely mutated in cancer and is an essential and core ATPase of the SWI/SNF complex, we sought to investigate the nature of the SWI/SNF complex following SMARCA4 mutation. We first evaluated the integrity of the SWI/SNF complex using human cancer cell lines that contain biallelic inactivating mutations in SMARCA4. We utilized SMARCC1 to purify the complex because it is present in all known variants of the SWI/SNF complex. Despite the absence of SMARCA4, SMARCC1 coprecipitated all 11 tested SWI/SNF subunits in the SMARCA4 mutant cell lines A549 and H1299 (Fig. 1A). Immunoprecipitation with antibodies to either SMARCA4 itself or control IgG did not yield SWI/SNF subunits, indicating that these associations are not due to nonspecific binding. Collectively, these find-

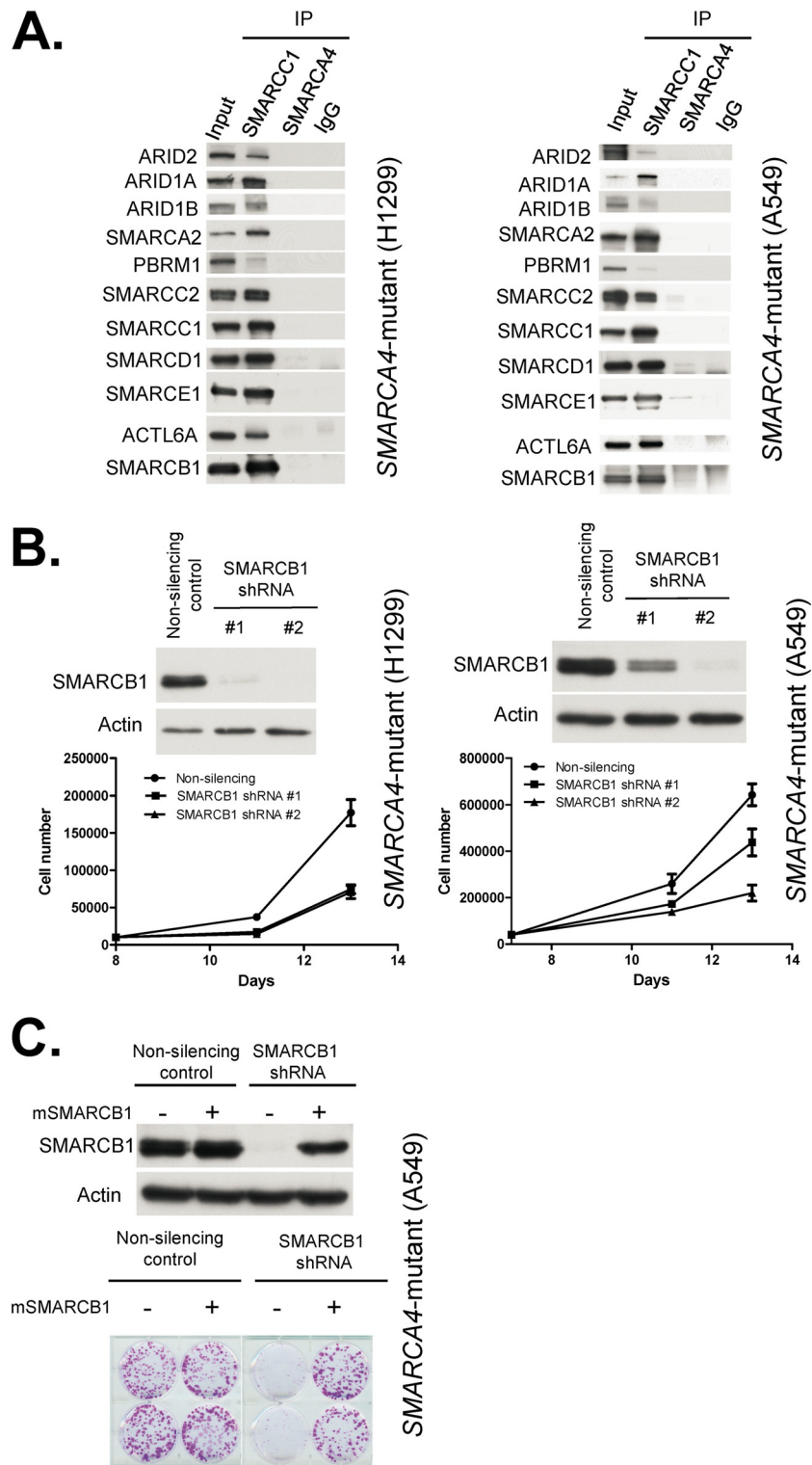


FIG 1 Residual SWI/SNF complexes exist in *SMARCA4* mutant cells and are essential for proliferation. (A) SMARCC1 associates with other SWI/SNF subunits in *SMARCA4* mutant cancer cell lines. Immunoblots of SWI/SNF subunits before (input, 10%) and after precipitation (IP) with SMARCC1, SMARCA4, and IgG antibodies. Nuclear extracts used in these experiments are derived from the *SMARCA4* mutant cell lines NCI-H1299 and A549. (B) SMARCB1 knockdown impairs proliferation of *SMARCA4* mutant cancer cells. shRNA-mediated knockdown of SMARCB1 in *SMARCA4* mutant NCI-H1299 and A549 cell lines is shown. Immunoblotting was used to determine the efficiency of knockdown. Cell proliferation was determined by cell count. Data are represented as means \pm SEM from three biological replicates. (C) Reexpression of SMARCB1 rescues the growth defect. Shown is a colony formation assay following reexpression of a mouse derivative of SMARCB1 (mSMARCB1) that is not targeted by human SMARCB1 shRNAs. Images are from duplicate biological replicates. Immunoblotting was used to determine the efficiency of SMARCB1 knockdown.

ings establish that in *SMARCA4* mutant cancer cell lines, other SWI/SNF subunits exist in a residual complex.

Residual SWI/SNF complexes are essential in *SMARCA4* mutant cell lines. We next investigated whether residual SWI/SNF complexes were contributing to the growth of *SMARCA4* mutant cancer cell lines. Using two different shRNAs, we knocked down the core SWI/SNF subunit, SMARCB1 (SNF5/IN11/BAF47), in *SMARCA4* mutant cancer cell lines. We reasoned that since SMARCB1 is found in all variants of the SWI/SNF complex and is itself a tumor suppressor, reducing its levels would allow us to determine whether residual SWI/SNF complexes have a role in the viability of *SMARCA4* mutant cancers. Using two different shRNAs targeting SMARCB1, we first tested the effects of SMARCB1 knockdown in two separate *SMARCA4* mutant non-small-cell lung carcinoma cell lines. In both cases, reduced SMARCB1 levels markedly decreased proliferation, and the magnitude of effect correlated with the degree of knockdown (Fig. 1B). Reexpression of a mouse derivative of SMARCB1 not recognized by the human SMARCB1 shRNAs rescued the growth defects caused by SMARCB1 loss, indicating that the effect was on target (Fig. 1C). This finding establishes that SMARCB1 remains essential in the absence of *SMARCA4*.

SMARCA2 is differentially essential in *SMARCA4* mutant cancer cell lines. Independent of these experiments, we performed a large-scale screen to search for cancer-specific vulnerabilities created by *SMARCA4* mutations. To do this, we used data from Project Achilles, a large-scale effort to identify essential genes in human cancer cell lines. Specifically, using data from the Cancer Cell Line Encyclopedia and prior publications (Table 1), we identified 21 cell lines in the Project Achilles screen that harbored mutations in *SMARCA4* (<http://www.broadinstitute.org/achilles>) (26). We compared dependencies identified by screening 54,020 shRNAs targeting 11,194 genes in these 21 cancer cell lines to 144 *SMARCA4* wild-type cell lines and identified SMARCA2 as the ninth most differentially essential gene in cancer cell lines containing mutations in *SMARCA4* ($P = 0.00069$; false discovery rate [FDR], 0.69). As it is difficult to know whether individual missense mutations are driver or passenger changes, we next reanalyzed the data using only those cell lines that contained clear inactivating *SMARCA4* mutations (frameshift, nonsense, and large focal deletions). When we compared dependencies in these 8 cell lines to the remaining 157 cell lines, SMARCA2 was the most differentially essential gene (P value = 7.366×10^{-06} ; FDR, <0.001) (Fig. 2A). Consistent with this, cell lines with inactivating *SMARCA4* mutations were more sensitive to reduced levels of SMARCA2 than cell lines that have missense or noninactivating mutations (Fig. 2B). Using data from the Cancer Cell Line Encyclopedia, we next evaluated whether cell lines contained monoallelic or biallelic inactivating mutations in *SMARCA4*. In six of the eight cell lines, mutations were biallelic (Table 1). In SK-MEL-5, only one allele has been found mutated, but this cell line has previously been shown to lack *SMARCA4* expression, suggesting complete loss of function (29). In HEC1A, only one allele was mutated, and this cell line has previously been shown to express the *SMARCA4* protein (30). Notably, HEC1A was the sole cell line with an inactivating *SMARCA4* mutation that did not score as sensitive to SMARCA2 depletion. The *SMARCA4* mutant cell lines used in these analyses were from various lineages (lung, ovary, endometrium, and skin), suggesting the dependency upon SMARCA2 is not a lineage-specific effect but occurs broadly in

SMARCA4 mutant cancers. To verify the finding that *SMARCA4* mutant cell lines were dependent upon SMARCA2 expression, we used independently derived shRNAs to suppress SMARCA2 levels in three *SMARCA4* wild-type cell lines and two *SMARCA4* mutant cell lines. While reduced SMARCA2 levels led to modest/no effects on the proliferation of *SMARCA4* wild-type cell lines, *SMARCA4* mutant cell lines were exquisitely sensitive to reduced SMARCA2 levels, as detected by colony formation assays (Fig. 2C and D). Despite reduced proliferation, we detected no significant change in apoptosis, senescence, or cell cycle (not shown), suggesting that combined loss of the ATPase subunits causes defects in multiple phases of the cell cycle. Indeed, the SWI/SNF complex has previously been shown to have functions during all four cell cycle phases (31–35).

SMARCA2 is largely dispensable for assembly of the SWI/SNF complex in *SMARCA4* mutant cells. To gain insight into the specific vulnerability for SMARCA2 in *SMARCA4* mutant cells, we next investigated whether knockdown of SMARCA2 affected the integrity of the residual SWI/SNF complex. Using coimmunoprecipitation of SMARCC1, we evaluated complex assembly following depletion of SMARCA2 in the A549 *SMARCA4* mutant lung cancer cell line. Reduced levels of SMARCA2 had virtually no effect on the association between SMARCC1 and other SWI/SNF subunits, with a single exception (Fig. 3A). ACTL6A (BAF53) is a subunit of the SWI/SNF complex previously shown to bind directly to *SMARCA4* (36). Following suppression of SMARCA2, ACTL6A no longer associated with SMARCC1. As a negative control to evaluate the specificity of these interactions, we evaluated BMI1, a subunit of the Polycomb chromatin complex not known to interact with the SWI/SNF complex, and found no association with SMARCC1 (Fig. 3B).

We next used genetically engineered *Smarca4* conditional murine embryonic fibroblasts to evaluate the consequences of *Smarca4* deletion upon expression of *Smarca2* and incorporation of SMARCA2 into residual SWI/SNF complexes in primary cells. While the expression of other core SWI/SNF components was not affected by *Smarca4* inactivation, SMARCA2 levels increased following *Smarca4* inactivation, and SMARCA2 was incorporated in larger amounts into SWI/SNF complexes (Fig. 4A). This was due, at least in part, to increased transcription of the *Smarca2* gene ($P = 0.029$) (Fig. 4B). Conversely, in *SMARCA4* wild-type cells, knockdown of SMARCA2 led to a reciprocal effect of increased expression of *SMARCA4*, as well as increased assembly of *SMARCA4* into the SWI/SNF complex (Fig. 4C). Collectively, these results indicate reciprocal expression and assembly of SMARCA2 and *SMARCA4* into SWI/SNF complexes.

DISCUSSION

One-fifth of all human cancers have mutations in genes encoding subunits of the SWI/SNF chromatin remodeling complex (2). As *SMARCA4* is recurrently mutated in several cancer types, the identification of cancer-specific vulnerabilities created by mutations in the *SMARCA4* subunit carries substantial relevance to human disease. Given that many *SMARCA4* mutations are clearly inactivating, a key question is whether mutations in *SMARCA4* lead to a complete loss of function of the SWI/SNF complex or whether the remaining SWI/SNF subunits assemble into residual complexes that can serve as therapeutic targets in *SMARCA4* mutant cancers. Here, we demonstrate that SMARCB1, a core SWI/SNF subunit found in all variant SWI/SNF complexes, is essential

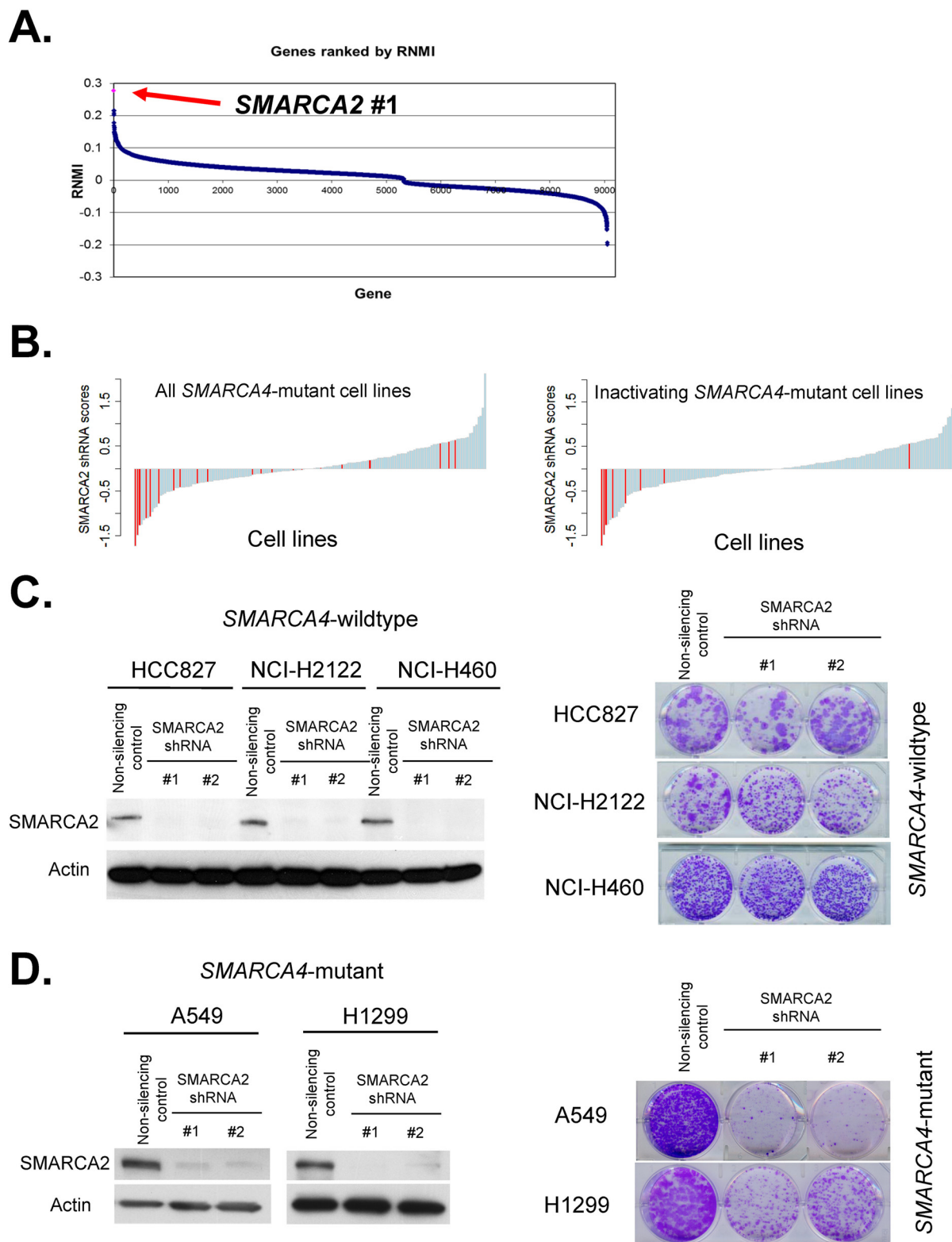


FIG 2 *SMARCA2* is differentially essential in *SMARCA4* mutant cancer cells. (A) Achilles shRNA screen identifies *SMARCA2* as the number one vulnerability in *SMARCA4*-inactivated cancer cell lines. Gene scores from comparison of inactivating *SMARCA4* mutant cancer cell lines to *SMARCA4* wild-type cell lines are shown. (B) Cell lines with inactivating mutations in *SMARCA4* are most sensitive to reduced levels of *SMARCA2*. Waterfall plot of Achilles *SMARCA2* shRNA scores. Cell lines containing all *SMARCA4* mutations are displayed in the left panel, and cell lines with inactivating *SMARCA4* mutations (nonsense, frameshift, and large focal deletions) are displayed in the right panel. Red columns indicate *SMARCA4* mutant cancer cell lines. (C) Reduced levels of *SMARCA2* do not affect the growth of *SMARCA4* wild-type cell lines. *SMARCA2* knockdown in *SMARCA4* wild-type cell lines HCC827, NCI-H2122, and H460. Immunoblotting is used to determine the efficiency of knockdown. Cell proliferation is evaluated using colony formation assays. Representative images are shown from one of two biological replicates for each cell line. (D) Reduced levels of *SMARCA2* impair the growth of *SMARCA4* mutant cell lines. *SMARCA2* knockdown in *SMARCA4* mutants NCI-H1299 and A549 leads to decreased proliferation. Immunoblotting is used to determine efficiency of knockdown. Cell proliferation is evaluated using colony formation assays. Representative images are shown from one of two biological replicates for each cell line.

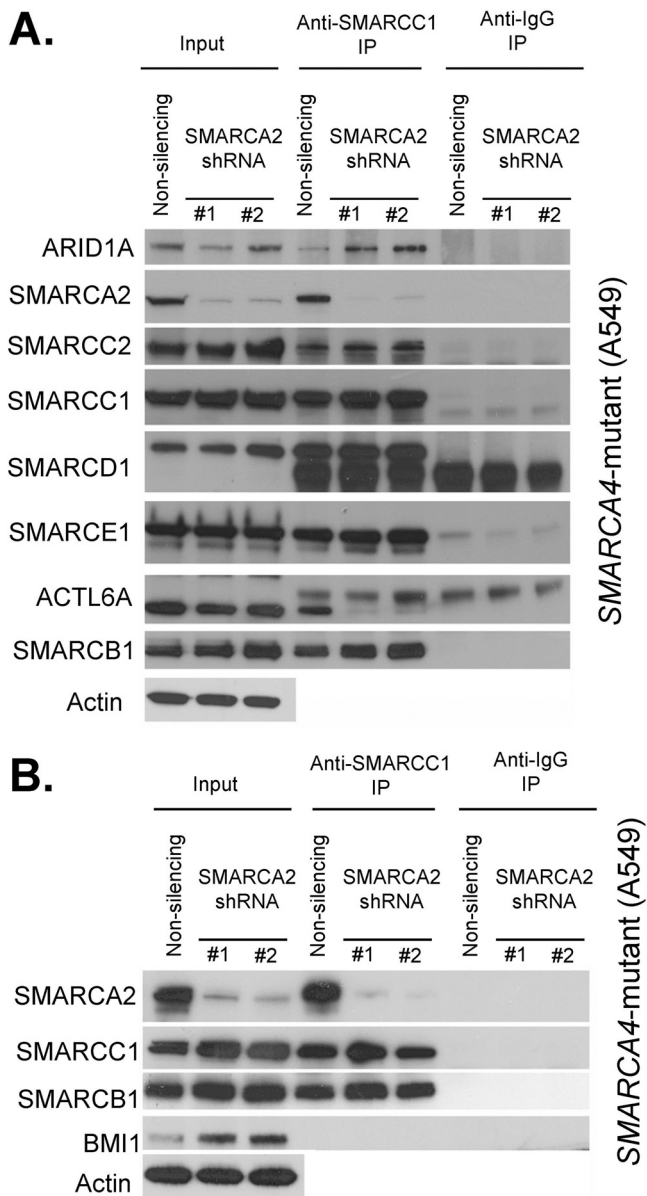


FIG 3 SWI/SNF complex assembles in the absence of SMARCA2 and SMARCA4. (A) SMARCC1 associates with other SWI/SNF subunits following SMARCA2 knockdown in *SMARCA4* mutant cells. Immunoblots of SWI/SNF subunits before (input, 10%) and after precipitation (IP) with SMARCC1 and IgG antibodies are shown. Nuclear extracts used in these experiments are derived from the *SMARCA4* mutant cell line A549 treated with SMARCA2 shRNAs or nonsilencing control shRNAs. A representative image from duplicate biological replicates is shown. (B) SMARCC1 does not associate with the Polycomb-group protein BMI1. Immunoblots of inputs (input, 5%) and SMARCC1 and IgG precipitates (IP) following SMARCA2 knockdown in the *SMARCA4* mutant cell line A549 are shown.

in *SMARCA4* mutant cancer cells, suggesting important roles for residual complexes in the tumorigenesis of *SMARCA4* mutant cancers. We further demonstrate a specific dependency upon the mutually exclusive SWI/SNF subunit SMARCA2 in these cancers, implicating SMARCA2-containing residual complexes as essential for tumorigenesis following *SMARCA4* loss. As we were preparing our manuscript, a study that tested the hypothesis that

SMARCA2 takes over for SMARCA4 in cancer cells was published, and it similarly concluded that SMARCA2 was specifically essential in *SMARCA4* mutant cancer cells (37). Notably, SMARCB1 depletion did not score as differentially essential for *SMARCA4* mutant cell lines in our vulnerability screen, consistent with its essential role in early mouse development and its essential role in most normal lineages (25, 38–40). Collectively, our work identifies SMARCA2-containing residual complexes as a specific vulnerability in *SMARCA4* mutant cancers and implicates SMARCA2 as a therapeutic target in these cancers. In future experiments, it will be of interest to investigate the mechanism underlying the oncogenic drive. Is it loss of some aspect of SMARCA4 function and SMARCA2 simply rescues the loss of viability that would otherwise be caused by SMARCA4 loss, or does the expression of SMARCA2 bring neomorphic protransformation activity to the complex?

Our studies also indicate a reciprocal assembly of SMARCA2 into SWI/SNF complexes following genetic inactivation of SMARCA4, providing insight into the mechanisms driving tumorigenesis. Previous studies have suggested compensatory roles for SMARCA2 and SMARCA4 (23, 24, 41, 42). Our studies similarly implicate a reciprocal regulation of SMARCA2 and SMARCA4 that contributes to tumorigenesis. However, we also demonstrate distinct roles for SMARCA2 and SMARCA4 during oncogenic transformation by showing essential roles for SMARCA2 in *SMARCA4* mutant cancers. Further highlighting functional differences between SMARCA4 and SMARCA2, homozygous inactivation of SMARCA4 is embryonic lethal, whereas SMARCA2-deficient mice are viable (11, 23). SMARCA2 and SMARCA4 are also expressed differentially during development and have distinct chromatin binding profiles and differential effects on gene regulation (21, 43). SMARCA4 also associates with transcription factors that are distinct from those that associate with SMARCA2 (43). Unlike SMARCA4, missense mutations of SMARCA2 that have unknown functional impact, whether gain of function, loss of function, or dominant negative, occur in Nicolaides-Baraitser syndrome (44–46). Lastly, during osteoblast differentiation, SMARCA2 and SMARCA4 have been shown to serve opposing functions with respect to differentiation (47). Collectively, our work demonstrates reciprocal regulation and assembly of SMARCA4 and SMARCA2 and demonstrates partial redundancy such that SMARCA2 becomes a specific vulnerability in *SMARCA4* mutant cancers.

SMARCA4 is frequently mutated in cancer. Inactivating *SMARCA2* mutations are rare in cancer, but cell lines and primary cancers have been identified that contain little or no SMARCA2 (48, 49). It is unclear whether there are low but essential levels of SMARCA2 in these cases, contributing to vulnerability, or whether in some cases cancers can adapt to the complete absence of both SMARCA4 and SMARCA2. Future studies are needed to determine whether functional residual complexes exist in these cancers and whether they contribute to tumorigenesis. Further work is also needed to uncover the mechanisms driving tumorigenesis in cells that may have become resistant to SMARCA2 loss despite the absence of SMARCA4.

Therapeutic targets for *SMARCA4* mutant cancers have remained elusive. Our studies suggest that therapeutic inhibition of SMARCA2-containing residual complexes is a promising approach for the treatment of *SMARCA4* mutant cancers. Notably, SMARCA2-deficient mice are viable, suggesting that SMARCA2

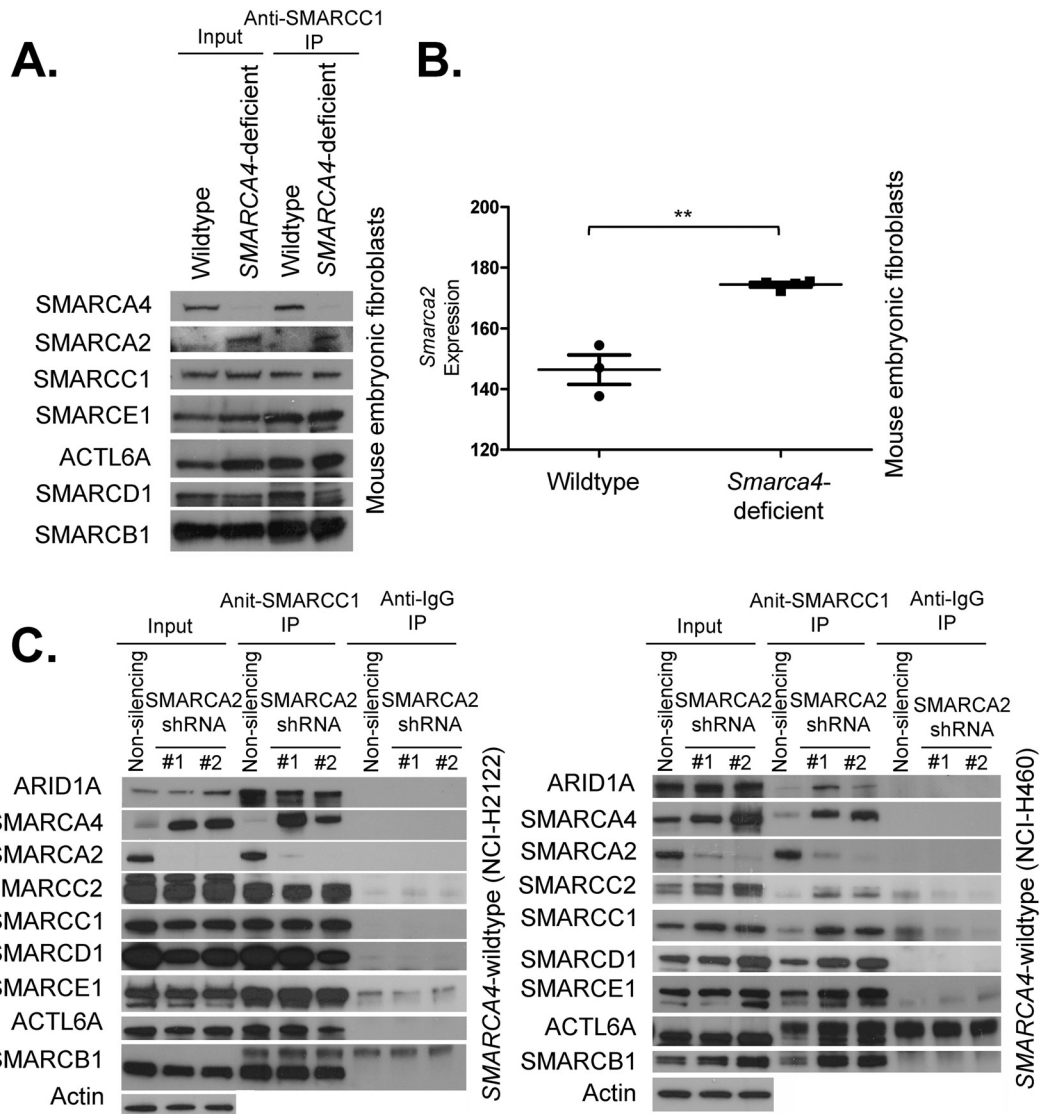


FIG 4 Reciprocal compensatory functions for SMARCA2 and SMARCA4. (A) *Smarca4* inactivation in primary cells leads to specific upregulation of *Smarca2* and increased association of SMARCA2 with other SWI/SNF subunits. An immunoblot of SWI/SNF subunits before (input, 10%) and after precipitation (IP) with SMARCC1 is shown. Nuclear extracts used in these experiments are derived from wild-type and *Smarca4*-deficient mouse embryonic fibroblasts. A representative image from duplicate biological replicates is shown. (B) *Smarca2* transcript levels are elevated following *Smarca4* inactivation. A plot of *Smarca2* expression levels in wild-type and *Smarca4*-deficient mouse embryonic fibroblasts is shown. **, $P = 0.029$. (C) SMARCA2 knockdown leads to SMARCA4 upregulation and increased association of SMARCA4 with other SWI/SNF subunits. Immunoblots of SWI/SNF subunits before (input, 10%) and after precipitation (IP) with SMARCC1 and IgG antibodies. Nuclear extracts used in these experiments are derived from the SMARCA4 wild-type cell lines NCI-H2122 and H460 treated with SMARCA2 shRNAs or nonsilencing control shRNAs. Representative images from duplicate biological replicates are shown.

generally is not required for cellular proliferation. SMARCA2 harbors at least two potentially targetable domains, an enzymatic ATPase domain and a bromodomain. Bromodomains have recently emerged as particularly good targets for anticancer therapy (50). Collectively, these studies identify SMARCA2 as a viable therapeutic target in SMARCA4 mutant cancers.

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We declare no conflicts of interest.

REFERENCES

- Wilson BG, Roberts CW. 2011. SWI/SNF nucleosome remodellers and cancer. *Nat. Rev. Cancer* 11:481–492. <http://dx.doi.org/10.1038/nrc3068>.
- Kadoch C, Hargreaves DC, Hodges C, Elias L, Ho L, Ranish J, Crabtree GR. 2013. Proteomic and bioinformatic analysis of mammalian SWI/SNF

- complexes identifies extensive roles in human malignancy. *Nat. Genet.* 45:592–601. <http://dx.doi.org/10.1038/ng.2628>.
3. Shain AH, Pollack JR. 2013. The spectrum of SWI/SNF mutations, ubiquitous in human cancers. *PLoS One* 8:e55119. <http://dx.doi.org/10.1371/journal.pone.0055119>.
 4. Weissman B, Knudsen KE. 2009. Hijacking the chromatin remodeling machinery: impact of SWI/SNF perturbations in cancer. *Cancer Res.* 69:8223–8230. <http://dx.doi.org/10.1158/0008-5472.CAN-09-2166>.
 5. Reisman D, Glaros S, Thompson EA. 2009. The SWI/SNF complex and cancer. *Oncogene* 28:1653–1668. <http://dx.doi.org/10.1038/onc.2009.4>.
 6. Love C, Sun Z, Jima D, Li G, Zhang J, Miles R, Richards KL, Dunphy CH, Choi WW, Srivastava G, Lugar PL, Rizzieri DA, Lagoo AS, Bernal-Mizrachi L, Mann KP, Flowers CR, Naresh KN, Evens AM, Chadburn A, Gordon LI, Czader MB, Gill JI, Hsi ED, Greenough A, Moffitt AB, McKinney M, Banerjee A, Grubor V, Levy S, Dunson DB, Dave SS. 2012. The genetic landscape of mutations in Burkitt lymphoma. *Nat. Genet.* 44:1321–1325. <http://dx.doi.org/10.1038/ng.2468>.
 7. Hodis E, Watson IR, Kryukov GV, Arold ST, Imielinski M, Theurillat JP, Nickerson E, Auclair D, Li L, Place C, Dicara D, Ramos AH, Lawrence MS, Cibulskis K, Sivachenko A, Voet D, Saksena G, Stransky N, Onofrio RC, Winckler W, Ardlie K, Wagle N, Wargo J, Chong K, Morton DL, Stemke-Hale K, Chen G, Noble M, Meyerson M, Ladbury JE, Davies MA, Gershenwald JE, Wagner SN, Hoon DS, Schadendorf D, Lander ES, Gabriel SB, Getz G, Garraway LA, Chin L. 2012. A landscape of driver mutations in melanoma. *Cell* 150:251–263. <http://dx.doi.org/10.1016/j.cell.2012.06.024>.
 8. Parsons DW, Li M, Zhang X, Jones S, Leary RJ, Lin JC, Boca SM, Carter H, Samayoa J, Bettegowda C, Gallia GL, Jallo GI, Binder ZA, Nikolsky Y, Hartigan J, Smith DR, Gerhard DS, Fuhs DW, Vandenberg S, Berger MS, Marie SK, Shinjo SM, Clara C, Phillips PC, Minturn JE, Biegel JA, Judkins AR, Resnick AC, Storm PB, Curran T, He Y, Rasheed BA, Friedman HS, Keir ST, McLendon R, Northcott PA, Taylor MD, Burger PC, Riggins GJ, Karchin R, Parmigiani G, Bigner DD, Yan H, Papadopoulos N, Vogelstein B, Kinzler KW, Velculescu VE. 2011. The genetic landscape of the childhood cancer medulloblastoma. *Science* 331:435–439. <http://dx.doi.org/10.1126/science.1198056>.
 9. Medina PP, Romero OA, Kohno T, Montuenga LM, Pio R, Yokota J, Sanchez-Cespedes M. 2008. Frequent BRG1/SMARCA4-inactivating mutations in human lung cancer cell lines. *Hum. Mutat.* 29:617–622. <http://dx.doi.org/10.1002/humu.20730>.
 10. Shain AH, Giacomini CP, Matsukuma K, Karikari CA, Bashyam MD, Hidalgo M, Maitra A, Pollack JR. 2012. Convergent structural alterations define SWI/SNF chromatin remodeler as a central tumor suppressive complex in pancreatic cancer. *Proc. Natl. Acad. Sci. U. S. A.* 109:E252–E259. <http://dx.doi.org/10.1073/pnas.1114817109>.
 11. Bultman S, Gebuhr T, Yee D, La Mantia C, Nicholson J, Gilliam A, Randazzo F, Metzger D, Chambon P, Crabtree G, Magnuson T. 2000. A Brg1 null mutation in the mouse reveals functional differences among mammalian SWI/SNF complexes. *Mol. Cell* 6:1287–1295. [http://dx.doi.org/10.1016/S1097-2765\(00\)00127-1](http://dx.doi.org/10.1016/S1097-2765(00)00127-1).
 12. Bultman SJ, Herschkowitz JI, Godfrey V, Gebuhr TC, Yaniv M, Perou CM, Magnuson T. 2008. Characterization of mammary tumors from Brg1 heterozygous mice. *Oncogene* 27:460–468. <http://dx.doi.org/10.1038/sj.onc.1210664>.
 13. Glaros S, Cirrincione GM, Palanca A, Metzger D, Reisman D. 2008. Targeted knockout of BRG1 potentiates lung cancer development. *Cancer Res.* 68:3689–3696. <http://dx.doi.org/10.1158/0008-5472.CAN-07-6652>.
 14. Khavari PA, Peterson CL, Tamkun JW, Mendel DB, Crabtree GR. 1993. BRG1 contains a conserved domain of the SWI2/SNF2 family necessary for normal mitotic growth and transcription. *Nature* 366:170–174. <http://dx.doi.org/10.1038/366170a0>.
 15. Muchardt C, Yaniv M. 1993. A human homologue of *Saccharomyces cerevisiae* SNF2/SWI2 and *Drosophila* brm genes potentiates transcriptional activation by the glucocorticoid receptor. *EMBO J.* 12:4279–4290.
 16. Chiba H, Muramatsu M, Nomoto A, Kato H. 1994. Two human homologues of *Saccharomyces cerevisiae* SWI2/SNF2 and *Drosophila* brahma are transcriptional coactivators cooperating with the estrogen receptor and the retinoic acid receptor. *Nucleic Acids Res.* 22:1815–1820. <http://dx.doi.org/10.1093/nar/22.10.1815>.
 17. Wang W, Cote J, Xue Y, Zhou S, Khavari PA, Biggar SR, Muchardt C, Kalpana GV, Goff SP, Yaniv M, Varki M, Crabtree GR. 1996. Purification and biochemical heterogeneity of the mammalian SWI-SNF complex. *EMBO J.* 15:5370–5382.
 18. de la Serna IL, Ohkawa Y, Imbalzano AN. 2006. Chromatin remodelling in mammalian differentiation: lessons from ATP-dependent remodelers. *Nat. Rev. Genet.* 7:461–473. <http://dx.doi.org/10.1038/nrg1882>.
 19. Yoo AS, Crabtree GR. 2009. ATP-dependent chromatin remodeling in neural development. *Curr. Opin. Neurobiol.* 19:120–126. <http://dx.doi.org/10.1016/j.conb.2009.04.006>.
 20. Mohrmann L, Verrijzer CP. 2005. Composition and functional specificity of SWI2/SNF2 class chromatin remodeling complexes. *Biochim. Biophys. Acta* 1681:59–73. <http://dx.doi.org/10.1016/j.bbaexp.2004.10.005>.
 21. Kaeser MD, Aslanian A, Dong MQ, Yates JR, III, Emerson BM. 2008. BRD7, a novel PBAF-specific SWI/SNF subunit, is required for target gene activation and repression in embryonic stem cells. *J. Biol. Chem.* 283:32254–32263. <http://dx.doi.org/10.1074/jbc.M806061200>.
 22. Reisman DN, Sciarrotta J, Bouldin TW, Weissman BE, Funkhouser WK. 2005. The expression of the SWI/SNF ATPase subunits BRG1 and BRM in normal human tissues. *Appl. Immunohistochem. Mol. Morphol.* 13:66–74. <http://dx.doi.org/10.1097/00129039-200503000-00011>.
 23. Reyes JC, Barra J, Muchardt C, Camus A, Babinet C, Yaniv M. 1998. Altered control of cellular proliferation in the absence of mammalian brahma (SNF2alpha). *EMBO J.* 17:6979–6991. <http://dx.doi.org/10.1093/emboj/17.23.6979>.
 24. Wang X, Sansam CG, Thom CS, Metzger D, Evans JA, Nguyen PT, Roberts CW. 2009. Oncogenesis caused by loss of the SNF5 tumor suppressor is dependent on activity of BRG1, the ATPase of the SWI/SNF chromatin remodeling complex. *Cancer Res.* 69:8094–8101. <http://dx.doi.org/10.1158/0008-5472.CAN-09-0733>.
 25. Isakoff MS, Sansam CG, Tamayo P, Subramanian A, Evans JA, Fillmore CM, Wang X, Biegel JA, Pomeroy SL, Mesirov JP, Roberts CW. 2005. Inactivation of the Snf5 tumor suppressor stimulates cell cycle progression and cooperates with p53 loss in oncogenic transformation. *Proc. Natl. Acad. Sci. U. S. A.* 102:17745–17750. <http://dx.doi.org/10.1073/pnas.0509014102>.
 26. Cheung HW, Cowley GS, Weir BA, Boehm JS, Rusin S, Scott JA, East A, Ali LD, Lizotte PH, Wong TC, Jiang G, Hsiao J, Mermel CH, Getz G, Barretina J, Gopal S, Tamayo P, Gould J, Tsherniak A, Stransky N, Luo B, Ren Y, Drapkin R, Bhatia SN, Mesirov JP, Garraway LA, Meyerson M, Lander ES, Root DE, Hahn WC. 2011. Systematic investigation of genetic vulnerabilities across cancer cell lines reveals lineage-specific dependencies in ovarian cancer. *Proc. Natl. Acad. Sci. U. S. A.* 108:12372–12377. <http://dx.doi.org/10.1073/pnas.1109363108>.
 27. Shao DD, Tsherniak A, Gopal S, Weir BA, Tamayo P, Stransky N, Schumacher SE, Zack TI, Beroukhi R, Garraway LA, Margolin AA, Root DE, Hahn WC, Mesirov JP. 2012. ATARIS: computational quantification of gene suppression phenotypes from multi-sample RNAi screens. *Genome Res.* 23:665–678. <http://dx.doi.org/10.1101/gr.143586.112>.
 28. Tolstorukov MY, Sansam CG, Lu P, Koellhoffer EC, Helming KC, Alver BH, Tillman EJ, Evans JA, Wilson BG, Park PJ, Roberts CW. 2013. Swi/Snf chromatin remodeling/tumor suppressor complex establishes nucleosome occupancy at target promoters. *Proc. Natl. Acad. Sci. U. S. A.* 110:10165–10170. <http://dx.doi.org/10.1073/pnas.1302209110>.
 29. Keenen B, Qi H, Saladi SV, Yeung M, de la Serna IL. 2010. Heterogeneous SWI/SNF chromatin remodeling complexes promote expression of microphthalmia-associated transcription factor target genes in melanoma. *Oncogene* 29:81–92. <http://dx.doi.org/10.1038/onc.2009.304>.
 30. Guan B, Wang TL, Shih IM. 2011. ARID1A, a factor that promotes formation of SWI/SNF-mediated chromatin remodeling, is a tumor suppressor in gynecologic cancers. *Cancer Res.* 71:6718–6727. <http://dx.doi.org/10.1158/0008-5472.CAN-11-1562>.
 31. Dykhuizen EC, Hargreaves DC, Miller EL, Cui K, Korshunov A, Kool M, Pfister S, Cho YJ, Zhao K, Crabtree GR. 2013. BAF complexes facilitate decatenation of DNA by topoisomerase IIalpha. *Nature* 497:624–627. <http://dx.doi.org/10.1038/nature12146>.
 32. Versteeg J, Medjkane S, Rouillard D, Delattre O. 2002. A key role of the hSNF5/INI1 tumour suppressor in the control of the G1-S transition of the cell cycle. *Oncogene* 21:6403–6412. <http://dx.doi.org/10.1038/sj.onc.1205841>.
 33. Trouche D, Le Chalony C, Muchardt C, Yaniv M, Kouzarides T. 1997. RB and hbrm cooperate to repress the activation functions of E2F1. *Proc. Natl. Acad. Sci. U. S. A.* 94:11268–11273. <http://dx.doi.org/10.1073/pnas.94.21.11268>.

34. Xue Y, Canman JC, Lee CS, Nie Z, Yang D, Moreno GT, Young MK, Salmon ED, Wang W. 2000. The human SWI/SNF-B chromatin-remodeling complex is related to yeast rsc and localizes at kinetochores of mitotic chromosomes. *Proc. Natl. Acad. Sci. U. S. A.* 97:13015–13020. <http://dx.doi.org/10.1073/pnas.240208597>.
35. Sif S, Stukenberg PT, Kirschner MW, Kingston RE. 1998. Mitotic inactivation of a human SWI/SNF chromatin remodeling complex. *Genes Dev.* 12:2842–2851. <http://dx.doi.org/10.1101/gad.12.18.2842>.
36. Zhao K, Wang W, Rando OJ, Xue Y, Swiderek K, Kuo A, Crabtree GR. 1998. Rapid and phosphoinositol-dependent binding of the SWI/SNF-like BAF complex to chromatin after T lymphocyte receptor signaling. *Cell* 95:625–636. [http://dx.doi.org/10.1016/S0092-8674\(00\)81633-5](http://dx.doi.org/10.1016/S0092-8674(00)81633-5).
37. Oike T, Ogiwara H, Tominaga Y, Ito K, Ando O, Tsuta K, Mizukami T, Shimada Y, Isomura H, Komachi M, Furuta K, Watanabe SI, Nakano T, Yokota J, Kohno T. 2013. A synthetic lethality-based strategy to treat cancers harboring a genetic deficiency in the chromatin remodeling factor BRG1. *Cancer Res.* 73:5508–5518. <http://dx.doi.org/10.1158/0008-5472.CAN-12-4593>.
38. Gresh L, Bourachot B, Reimann A, Guigas B, Fiette L, Garbay S, Muchardt C, Hue L, Pontoglio M, Yaniv M, Klochendler-Yeivin A. 2005. The SWI/SNF chromatin-remodeling complex subunit SNF5 is essential for hepatocyte differentiation. *EMBO J.* 24:3313–3324. <http://dx.doi.org/10.1038/sj.emboj.7600802>.
39. Roberts CW, Leroux MM, Fleming MD, Orkin SH. 2002. Highly penetrant, rapid tumorigenesis through conditional inversion of the tumor suppressor gene Snf5. *Cancer Cell* 2:415–425. [http://dx.doi.org/10.1016/S1535-6108\(02\)00185-X](http://dx.doi.org/10.1016/S1535-6108(02)00185-X).
40. Wang X, Werneck MB, Wilson BG, Kim HJ, Kluk MJ, Thom CS, Wischhusen JW, Evans JA, Jesneck JL, Nguyen P, Sansam CG, Cantor H, Roberts CW. 2011. TCR-dependent transformation of mature memory phenotype T cells in mice. *J. Clin. Investig.* 121:3834–3845. <http://dx.doi.org/10.1172/JCI37210>.
41. Willis MS, Homeister JW, Rosson GB, Annayev Y, Holley D, Holly SP, Madden VJ, Godfrey V, Parise LV, Bultman SJ. 2012. Functional redundancy of SWI/SNF catalytic subunits in maintaining vascular endothelial cells in the adult heart. *Circ. Res.* 111:e111–e122. <http://dx.doi.org/10.1161/CIRCRESAHA.112.265587>.
42. Strobeck MW, Reisman DN, Gunawardena RW, Betz BL, Angus SP, Knudsen KE, Kowalik TF, Weissman BE, Knudsen ES. 2002. Compensation of BRG-1 function by Brm: insight into the role of the core SWI-SNF subunits in retinoblastoma tumor suppressor signaling. *J. Biol. Chem.* 277:4782–4789. <http://dx.doi.org/10.1074/jbc.M109532200>.
43. Kadam S, Emerson BM. 2003. Transcriptional specificity of human SWI/SNF BRG1 and BRM chromatin remodeling complexes. *Mol. Cell* 11:377–389. [http://dx.doi.org/10.1016/S1097-2765\(03\)00034-0](http://dx.doi.org/10.1016/S1097-2765(03)00034-0).
44. Wiczorek D, Bogershausen N, Beleggia F, Steiner-Haldenstatt S, Pohl E, Li Y, Milz E, Martin M, Thiele H, Altmüller J, Alanay Y, Kayserili H, Klein-Hitpass L, Bohringer S, Wollstein A, Albrecht B, Boduroglu K, Caliebe A, Chrzanowska K, Cogulu O, Cristofoli F, Czeschik JC, Devriendt K, Dotti MT, Elcioglu N, Gener B, Goecke TO, Krajewska-Walasek M, Guillen-Navarro E, Hayek J, Houge G, Kilic E, Simsek-Kiper PO, Lopez-Gonzalez V, Kuechler A, Lyonnet S, Mari F, Marozza A, Mathieu Dramard M, Mikat B, Morin G, Morice-Picard F, Ozkinay F, Rauch A, Renieri A, Tinschert S, Utine GE, Vilain C, Vivarelli R, Zweier C, Nurnberg P, Rahmann S, Vermeesch J, Ludecke HJ, Zeschne M, Wollnik B. 2013. A comprehensive molecular study on Coffin-Siris and Nicolaides-Baraitser syndromes identifies a broad molecular and clinical spectrum converging on altered chromatin remodeling. *Hum. Mol. Genet.* 22:5121–5135. <http://dx.doi.org/10.1093/hmg/ddt366>.
45. Van Houdt JK, Nowakowska BA, Sousa SB, van Schaik BD, Seuntjens E, Avonce N, Sifrim A, Abdul-Rahman OA, van den Boogaard MJ, Bottani A, Castori M, Cormier-Daire V, Deardorff MA, Filges I, Fryer A, Fryns JP, Gana S, Garavelli L, Gillessen-Kaesbach G, Hall BD, Horn D, Huylebroeck D, Klapecki J, Krajewska-Walasek M, Kuechler A, Lines MA, Maas S, Macdermot KD, McKee S, Magee A, de Man SA, Moreau Y, Morice-Picard F, Obersztyn E, Pilch J, Rosser E, Shannon N, Stolte-Dijkstra I, Van Dijck P, Vilain C, Vogels A, Wakeling E, Wiczorek D, Wilson L, Zuffardi O, van Kampen AH, Devriendt K, Hennekam R, Vermeesch JR. 2012. Heterozygous missense mutations in SMARCA2 cause Nicolaides-Baraitser syndrome. *Nat. Genet.* 44:445–449. <http://dx.doi.org/10.1038/ng.1105>.
46. Wolff D, Ende S, Azzarello-Burri S, Hoyer J, Zweier M, Schanze I, Schmitt B, Rauch A, Reis A, Zweier C. 2012. In-frame deletion and missense mutations of the C-terminal helicase domain of SMARCA2 in three patients with Nicolaides-Baraitser syndrome. *Mol. Syndromol.* 2:237–244. <http://dx.doi.org/10.1159/000337323>.
47. Flowers S, Nagl NG, Jr, Beck GR, Jr, Moran E. 2009. Antagonistic roles for BRM and BRG1 SWI/SNF complexes in differentiation. *J. Biol. Chem.* 284:10067–10075. <http://dx.doi.org/10.1074/jbc.M808782200>.
48. Reisman DN, Strobeck MW, Betz BL, Sciarriotta J, Funkhouser W, Jr, Murchardt C, Yaniv M, Sherman LS, Knudsen ES, Weissman BE. 2002. Concomitant down-regulation of BRM and BRG1 in human tumor cell lines: differential effects on RB-mediated growth arrest vs CD44 expression. *Oncogene* 21:1196–1207. <http://dx.doi.org/10.1038/sj.onc.1205188>.
49. Yamamichi N, Inada K, Ichinose M, Yamamichi-Nishina M, Mizutani T, Watanabe H, Shiogama K, Fujishiro M, Okazaki T, Yahagi N, Haraguchi T, Fujita S, Tsutsumi Y, Omata M, Iba H. 2007. Frequent loss of Brm expression in gastric cancer correlates with histologic features and differentiation state. *Cancer Res.* 67:10727–10735. <http://dx.doi.org/10.1158/0008-5472.CAN-07-2601>.
50. Filippakopoulos P, Qi J, Picaud S, Shen Y, Smith WB, Fedorov O, Morse EM, Keates T, Hickman TT, Felleter I, Philpott M, Munro S, McKeown MR, Wang Y, Christie AL, West N, Cameron MJ, Schwartz B, Heightman TD, La Thangue N, French CA, Wiest O, Kung AL, Knapp S, Bradner JE. 2010. Selective inhibition of BET bromodomains. *Nature* 468:1067–1073. <http://dx.doi.org/10.1038/nature09504>.