

# Chromatin-Remodeling Complex SWI/SNF Controls Multidrug Resistance by Transcriptionally Regulating the Drug Efflux Pump ABCB1

Ramin Dubey<sup>1,2</sup>, Andres M. Lebensohn<sup>1,2</sup>, Zahra Bahrami-Nejad<sup>3</sup>, Caleb Marceau<sup>4</sup>, Magali Champion<sup>5</sup>, Olivier Gevaert<sup>5</sup>, Branimir I. Sikic<sup>2</sup>, Jan E. Carette<sup>4</sup>, and Rajat Rohatgi<sup>1,2</sup>

## Abstract

Anthracyclines are among the most effective yet most toxic drugs used in the oncology clinic. The nucleosome-remodeling SWI/SNF complex, a potent tumor suppressor, is thought to promote sensitivity to anthracyclines by recruiting topoisomerase IIa (TOP2A) to DNA and increasing double-strand breaks. In this study, we discovered a novel mechanism through which SWI/SNF influences resistance to the widely used anthracycline doxorubicin based on the use of a forward genetic screen in haploid human cells, followed by a rigorous single and double-mutant epistasis analysis using CRISPR/Cas9-mediated engineering. Doxorubicin

resistance conferred by loss of the SMARCB1 subunit of the SWI/SNF complex was caused by transcriptional upregulation of a single gene, encoding the multidrug resistance pump ABCB1. Remarkably, both ABCB1 upregulation and doxorubicin resistance caused by SMARCB1 loss were dependent on the function of SMARCA4, a catalytic subunit of the SWI/SNF complex. We propose that residual SWI/SNF complexes lacking SMARCB1 are vital determinants of drug sensitivity, not just to TOP2A-targeted agents, but to the much broader range of cancer drugs effluxed by ABCB1. *Cancer Res*; 76(19); 5810–21. ©2016 AACR.

## Introduction

Anthracyclines are used to induce regressions in multiple disseminated neoplasms in both adults and children, including acute leukemias, breast and ovarian cancers, bone and soft tissue sarcomas, Hodgkin's disease, and malignant lymphomas. The most commonly used anthracyclines (doxorubicin, daunorubicin, idarubicin, and epirubicin) trap or "poison" a covalent reaction intermediate between DNA and tyrosine residues in type II topoisomerases, eventually causing double-strand breaks (DSB) and cell death (1, 2). The DNA cleavage activity of Topoisomerase II $\alpha$  (TOP2A) is thought to be required for anthracycline toxicity; indeed, lower

TOP2A levels are associated with greater resistance (3, 4). Recent studies have proposed that the SWI/SNF chromatin-remodeling complex recruits TOP2A to DNA, thereby promoting anthracycline-induced DSBs and cell death (5, 6). A second mechanism of resistance to anthracyclines (and many other chemotherapeutic drugs) is overexpression of *ABCB1*, which encodes an ATP-dependent promiscuous drug efflux pump also known as the Multidrug Resistant (MDR1) pump or P-glycoprotein (PgP) pump (7).

A forward genetic screen revealed 35 loci associated with sensitivity to doxorubicin, a commonly used anthracycline. Top hits from this screen included many chromatin regulators, including five subunits of the nucleosome-remodeling SWI/SNF complex and two subunits of the histone acetylating STAGA complex. In particular, we found that the chromatin remodeling SWI/SNF complex regulates expression of *ABCB1*, entirely explaining the source of resistance seen in cells lacking particular subunits of this complex. It follows from these findings that targeting chromatin regulators, a recent focus of therapeutic development due to their oncogenic role in many tumors, may also modulate the sensitivity of these tumors to chemotherapeutic agents.

<sup>1</sup>Department of Biochemistry, Stanford University School of Medicine, Stanford, California. <sup>2</sup>Division of Oncology, Department of Medicine, Stanford University School of Medicine, Stanford, California. <sup>3</sup>Department of Chemical and Systems Biology, Stanford University School of Medicine, Stanford, California. <sup>4</sup>Department of Microbiology and Immunology, Stanford University School of Medicine, Stanford, California. <sup>5</sup>Stanford Center for Biomedical Informatics Research, Dept. of Medicine, Stanford, California.

**Note:** Supplementary data for this article are available at Cancer Research Online (<http://cancerres.aacrjournals.org/>).

R. Dubey and A.M. Lebensohn contributed equally to this article.

**Corresponding Authors:** R. Rohatgi, Stanford University School of Medicine, 279 W. Campus Drive, Room B435, Stanford, CA 94305. Phone: 650-387-5666; Fax: 650-723-6783; E-mail: rrohathgi@stanford.edu; or J.E. Carette, Stanford University, Department of Microbiology and Immunology, 299 Campus Drive, Room D345, Stanford, CA 94305. Phone: 650-725-4468; Fax: 650-725-7282; E-mail: carette@stanford.edu

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## Materials and Methods

### Cell lines and constructs

The Hap1 cell line (8) was kindly provided by Dr. Tijn Brummelkamp, Netherlands Cancer Institute. The 293FT cell line used to generate high-titer lentiviruses was obtained from Thermo Fisher Scientific; the A549, PC3, MDA-MB-361, HCC-1954, and NCI-H1650 cell lines were purchased from the ATCC, where they were validated by short tandem repeat profiling, and used at passage numbers <5. The KOPN8 cell line was a generous gift from

Professor Michael Cleary, Stanford University. The SNU-349 cell line was obtained from Korean Cell Line Bank. The Phoenix-Ampho cell line used for retrovirus production was purchased from Allele Biotechnology.

Null alleles for genes were constructed using the CRISPR/Cas9 system. For Hap1 cells, the oligos encoding the guide RNAs were cloned into pSpCas9(BB)-2A-GFP (PX458, Addgene Plasmid #48138 from Dr. Feng Zhang; ref. 9). Single cells were sorted using flow cytometry, expanded, and clones bearing null alleles were identified by Sanger sequencing and immunoblotting. For gene disruption in cancer cell lines, the gRNAs validated in the Hap1 cells were introduced into LentiCRISPR v2 (Addgene Plasmid #52961 from Dr. Feng Zhang; ref. 10) for lentiviral-mediated delivery. The oligo sequences for guide RNAs are provided in Supplementary Methods.

### Haploid genetic screen

Methods for the execution of haploid genetic screening using insertional retroviral mutagenesis and the bioinformatic pipeline to map the distribution of these insertions in the genome have been described in detail previously (8, 11). One hundred million Hap1 cells mutagenized using a gene-trap (GT) bearing retrovirus were treated with doxorubicin (17.5 nmol/L) for 4 days, followed by 1 week of expansion in the absence of doxorubicin prior to harvest. Genome-wide mapping of insertions was performed in a pool of 30 million doxorubicin-resistant cells and compared with insertions mapped in an equal number of unselected, but mutagenized cells. Clonal cell lines carrying a gene trap insertion in a specific location were isolated from the resistant cell pool using a nested PCR strategy.

### Western blotting

Antibodies used for immunoblotting are as follows: Rabbit anti-ABCB1 (D3H1Q, 1:1,000) and rabbit anti-TOP2A (D10G9, 1:1,000) from Cell Signaling Technologies; rabbit anti-SMARCB1 (A301-087A-T, 1:1,000), goat anti-SMARCA4 (A303-877A-T, 1:1,000), and rabbit anti-ARID1A (A301-041A-T; 1:1,000) from Bethyl Laboratories; Mouse anti- $\alpha$ -tubulin (T6199, 1:10,000) from Sigma-Aldrich. A detailed description of our cell lysis and Western blotting protocol is given in Supplementary Materials and Methods.

### Short-term cell viability (MTT) assay

A total of 5,000 cells were plated in each well of 96-well plate, allowed to grow for 24 hours, and then treated with various concentrations of doxorubicin or other anticancer drugs in quadruplicate for 96 hours prior to being subjected to a standard MTT assay.

### Long-term growth assay

A total of  $10^5$  cells were plated in each well of a 6-well plate. After 24 hours, the cells were treated with 17.5 nmol/L or 30 nmol/L doxorubicin for 10 days. After 10 days, the cells were fixed with 4% paraformaldehyde and stained with 0.05% Crystal violet solution for 1 hour.

### Statistical analysis

All graphing, curve-fitting, and statistical analysis was performed in SigmaPlot. Error bars denote SD derived from 3 to 4 replicates. Details of statistical analyses, including identity of test

used, replicate numbers, and *P* values for relevant comparisons from all figure panels, are given in Supplementary Table S4.

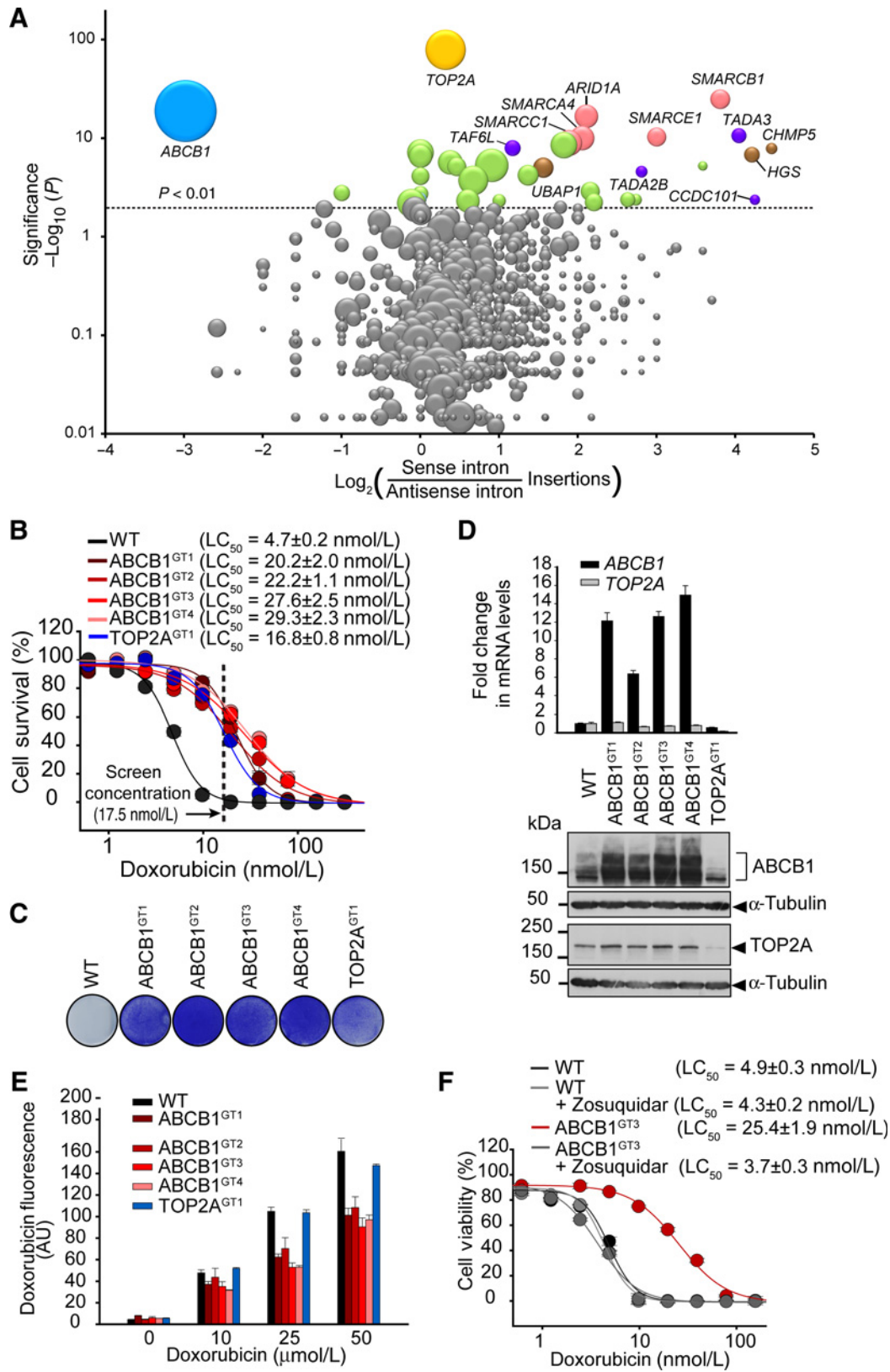
## Results

### A haploid genetic screen for mediators of doxorubicin sensitivity

We conducted a genetic screen (12) in Hap1 cells (8), a near-haploid human cell line that enables the generation of null alleles in most genes by insertional mutagenesis (11). One hundred million Hap1 cells were mutagenized by infecting them with a retrovirus carrying a GT construct (Supplementary Fig. S1A), and then the entire population was treated with a concentration of doxorubicin (17.5 nmol/L) that approximates levels achieved in patients (1). This regimen killed 99% of the cells ( $LC_{99}$ ) after 4 days of continuous treatment (Supplementary Fig. S1B). Cells that survived, presumably because they had acquired an inactivating GT insertion in a gene promoting sensitivity to doxorubicin, were pooled, and the genomic location and orientation of GT insertions were determined by deep sequencing as described previously (Supplementary Fig. S1A; ref. 8).

Genes whose inactivation conferred doxorubicin resistance were identified using two criteria: (i) an enrichment of inactivating insertions (defined as all insertions in exons plus sense insertions in introns) in the selected population compared with the unselected population and (ii) a bias toward sense insertions over antisense insertions in introns in the selected population (Supplementary Table S1 and Supplementary Fig. S1C; ref. 13). A volcano plot based on these two parameters (Fig. 1A) identified two well-established regulators of doxorubicin resistance, *ABCB1* and *TOP2A*, multiple subunits of the SWI/SNF (also known as the SMAR or BAF) nucleosome remodeling complex (14, 15), and multiple subunits of the histone acetylating STAGA complex (16, 17). A total of 35 genes exceeded our FDR-corrected *P* value threshold of 0.01 (Supplementary Table S1; full results are provided in Supplementary Table S3).

The top two hits in our screen were *TOP2A* and *ABCB1*, genes implicated in the leading causes of doxorubicin resistance. GT insertions in *TOP2A* were mostly found in a sense orientation and thus predicted to interrupt transcription. Paradoxically, GT insertions in *ABCB1* were predominantly in an antisense orientation, such that the splice acceptor element would be incapable of interrupting transcription (Supplementary Fig. S1C and S1D). To understand the molecular basis of resistance caused by the unexpected orientation of GT insertions in *ABCB1*, we isolated a clonal cell line carrying a sense insertion near the start of *TOP2A* (*TOP2A*<sup>GT1</sup>) and four independent clonal cell lines carrying antisense insertions in *ABCB1* (*ABCB1*<sup>GT1-4</sup>; Supplementary Fig. S1E). In both short-term (96-hour) viability assays (Fig. 1B) and long-term (10-day) growth assays (Fig. 1C), all five gene-trapped clones showed substantially increased resistance to doxorubicin. In MTT assays, the 50% lethal concentration ( $LC_{50}$ ), defined as the doxorubicin concentration at which cell viability was reduced by 50% compared with an untreated population, was increased by 4- to 6-fold compared with the parental wild-type (WT) Hap1 cells (Fig. 1B). Analysis of *TOP2A* and *ABCB1* RNA and protein levels in these clonal cell lines revealed the causes of resistance (Fig. 1D). The sense GT insertions significantly reduced *TOP2A* protein levels, whereas the antisense GT insertions caused a dramatic increase in *ABCB1* protein, both through transcriptional



mechanisms (Fig. 1D). All four cell lines carrying antisense insertions in the *ABCB1* locus accumulated less doxorubicin in a fluorescence-based uptake assay (Fig. 1E). Most importantly, the doxorubicin-resistant phenotype of *ABCB1*<sup>GT3</sup> cells could be reversed by Zosuquidar, a potent and specific *ABCB1* inhibitor (Fig. 1F; ref. 18). Reversal of the phenotype by Zosuquidar makes it unlikely that doxorubicin resistance in *ABCB1*<sup>GT3</sup> cells was caused by passenger insertions at other loci.

In summary, our screen in Hap1 cells identified two leading causes of doxorubicin resistance, decreased *TOP2A* and increased *ABCB1* expression levels, establishing the physiologic relevance of this system. These results also demonstrate the ability of retroviral GT mutagens to generate both hypomorphic and gain-of-function alleles in haploid cells, highlighting their potential to uncover a wider range of genes (including essential genes like *TOP2A*) compared with strict loss-of-function screens.

### Proteins that regulate chromatin structure can influence sensitivity to doxorubicin

To validate the SWI/SNF and STAGA complex subunits identified in our screen (Supplementary Table S1), we introduced CRISPR/Cas9-mediated frameshift mutations into *SMARCB1*, *SMARCA4*, *ARID1A*, and *TADA3* using two different guide RNAs (gRNAs) targeting each gene (refs. 9, 19; Supplementary Table S2). Clonal cell lines carrying mutations were isolated and are hereafter denoted as "CR1" or "CR2" following the gene name. We confirmed depletion of *SMARCB1*, *SMARCA4*, and *ARID1A* by immunoblotting (Fig. 2A). As expected, protein levels of some of the SWI/SNF complex subunits were dependent on each other—loss of *SMARCB1* led to a decline in *ARID1A* protein levels and vice versa.

Depletion of *SMARCB1*, *ARID1A*, and *TADA3* increased resistance to doxorubicin in both short-term MTT assays (Fig. 2B) and long-term growth assays (Fig. 2C). Depletion of *SMARCB1* gave the strongest phenotype, leading to a 4-fold increase in the LC<sub>50</sub> of doxorubicin (Fig. 2B). The loss of *SMARCA4* did not significantly change the doxorubicin LC<sub>50</sub> in MTT assays. However, in longer-term growth assays, *SMARCA4*<sup>CR</sup> cells had a growth advantage in the presence of doxorubicin (Fig. 2C), which explains the enrichment of retroviral insertions in *SMARCA4* seen in the screen.

### Loss of *SMARCB1*, but not *SMARCA4*, increases the expression of *ABCB1*

Loss of *SMARCB1* caused the largest increase in the LC<sub>50</sub> of doxorubicin (Fig. 2B). *SMARCB1* is a core subunit of the SWI/SNF complex and a potent tumor suppressor lost in the

majority of malignant rhabdoid tumors (MRT; refs. 20–25). *SMARCA4* is one of the two alternative ATPase subunits required for the energy-dependent nucleosome remodeling activity of the complex, the other being *SMARCA2* (or *BRM*; ref. 15). Given that both *SMARCB1* and *SMARCA4* were identified in the screen and that both proteins are core subunits of the SWI/SNF complex, we expected that loss of either would regulate doxorubicin sensitivity by the same mechanism.

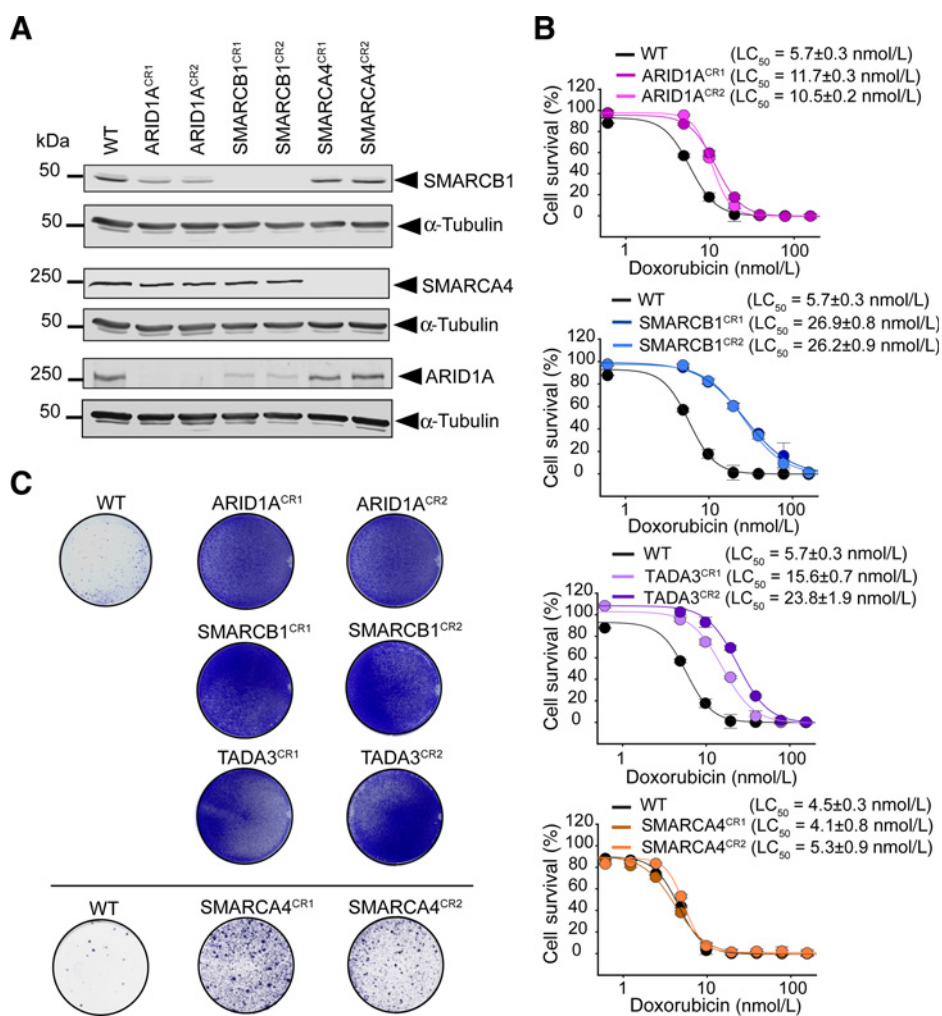
We compared the transcriptional profile of WT Hap1 cells with that of their isogenic counterparts lacking either *SMARCB1* or *SMARCA4* (Fig. 3 and Supplementary Table S5). Though both proteins are part of the same complex, there were striking differences in the genes upregulated by the loss of each protein (Fig. 3). Expression of the most highly upregulated genes in the *SMARCB1*<sup>CR</sup> cells compared with WT cells was only minimally altered in *SMARCA4*<sup>CR</sup> cells ("CR" following the gene name denotes both "CR1 and CR2"). There was more overlap between the *SMARCA4*<sup>CR</sup> and *SMARCB1*<sup>CR</sup> expression profiles looking at downregulated genes (Fig. 3A and B). *ABCB1* was the 12th most highly upregulated gene (5.8-fold) in *SMARCB1*<sup>CR</sup> cells compared with WT cells (Fig. 3B and Supplementary Table S5). Conversely, *ABCB1* mRNA was 1.7-fold reduced in *SMARCA4*<sup>CR</sup> cells compared with WT cells. This suggested that increased *ABCB1* levels could cause doxorubicin resistance in *SMARCB1*<sup>CR</sup> cells, but that the mechanism whereby *SMARCA4* loss confers doxorubicin resistance was most likely a different one. Among a panel of ABC transporters that have been implicated in doxorubicin resistance, the loss of *SMARCB1* also led to the transcriptional upregulation of *ABCG2* (Supplementary Fig. S5C); however, absolute mRNA levels of *ABCG2* (even after induction) were much lower than those of *ABCB1* in HAP1 cells.

We measured levels of *ABCB1* mRNA by qRT-PCR and protein by immunoblotting (Fig. 4A) in cell lines lacking individual SWI/SNF components. In agreement with the RNAseq data, *ABCB1* mRNA was upregulated in two independent cell lines (*SMARCB1*<sup>CR1</sup> and *SMARCB1*<sup>CR2</sup>), and this change was translated into increased *ABCB1* protein levels. In contrast, *SMARCA4* loss resulted in reduced *ABCB1* protein levels, supporting opposing roles of these two core SWI/SNF subunits in *ABCB1* gene regulation. Thus, *SMARCA4* must confer doxorubicin resistance independently of *ABCB1* regulation; however, we did not further pursue this alternative mechanism because of the subtle effects of *SMARCA4* loss on the growth of Hap1 cells in our assays (Fig. 2B and C).

When epitope-tagged *SMARCB1*-3xHA was stably reintroduced into either *SMARCB1*<sup>CR1</sup> or *SMARCB1*<sup>CR2</sup> cells, *ABCB1* expression

### Figure 1.

A forward genetic screen in human haploid cells for mediators of doxorubicin sensitivity. **A**, volcano plot depicting the results of the screen. Each circle represents a gene, with the diameter scaled according to the number of independent retroviral insertions, plotted based on the *P* value for enrichment of insertions in the doxorubicin-selected population over the control population (*y*-axis) and the bias toward inactivating intronic insertions (*x*-axis). Genes with a FDR-corrected *P* value smaller than 0.01 are colored. Pink, SWI/SNF complex genes; violet, STAGA complex genes; brown, MVB (multivesicular body) trafficking genes; green, other significant genes. Intron-less genes were assigned a sense/antisense insertion ratio (*x*-axis) of 1. The full set of significant genes (*P* < 0.01) with enumeration of insertions is provided in Supplementary Table S1, and the entire dataset for the screen is provided in Supplementary Table S3. **B**, an MTT assay (96 hours) was used to determine the LC<sub>50</sub> for doxorubicin in cell lines carrying independent gene-trap (GT) insertions in *ABCB1* or *TOP2A*. **C**, crystal violet staining was used to assess cell growth after 10 days (d) of exposure to 17.5 nmol/L doxorubicin. **D**, *ABCB1* and *TOP2A* mRNA (top) and protein levels (bottom) in the indicated GT clones, measured by qRT-PCR and immunoblotting, respectively. Tubulin is shown as a loading control for each of the two separate blots. **E**, doxorubicin accumulation in the indicated cell lines measured by the intrinsic fluorescence of doxorubicin after exposure of cells to various concentrations of the drug for 2 hours. **F**, sensitivity of WT cells or cells with an antisense insertion at the *ABCB1* locus to doxorubicin in the absence or presence of Zosuquidar (100 nmol/L). Error bars, which in many cases are smaller than the diameter of the circles used to denote the mean value, represent the SD (*n* = 4 for **B** and **F** and *n* = 3 for **D** and **E**); *P* values for relevant comparisons are shown in Supplementary Table S4.



**Figure 2.** Disrupting genes that encode components of the SWI/SNF and STAGA chromatin regulatory complexes confers resistance to doxorubicin. **A**, immunoblot showing protein levels of SMARCB1, SMARCA4, and ARID1A in clonal cell lines carrying CRISPR/Cas9-mediated frameshift mutations in the indicated genes. Each gene was targeted with two different guide RNAs (CR1 and CR2; see Supplementary Table S2). Doxorubicin sensitivity in each cell line was determined by MTT assays (96 hours; **B**) or by crystal violet staining after 10 days of exposure to 17.5 nmol/L doxorubicin (**C**).

significantly declined and approached the level of WT cells (Fig. 4B). Importantly, these rescued cells were resensitized to doxorubicin in both short-term (Fig. 4C) and long-term (Fig. 4D) growth assays. This experiment established that the changes in *ABCB1* levels and doxorubicin sensitivity measured in *SMARCB1*<sup>CR1</sup> and *SMARCB1*<sup>CR2</sup> cell lines were indeed caused by the loss of *SMARCB1*, rather than by cryptic mutations.

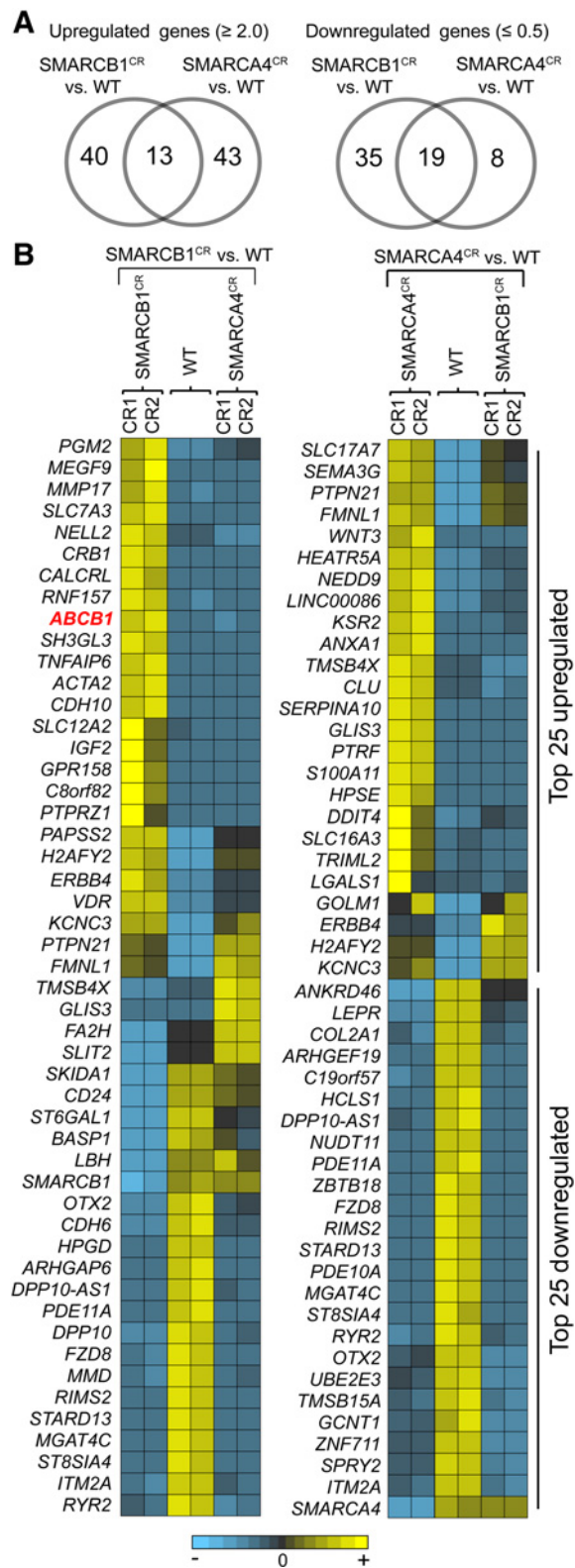
We correlated expression levels of *SMARCB1* and *ABCB1* across 12 human cancers from The Cancer Genome Atlas (TCGA) portal (Supplementary Fig. S2). In ten of twelve cancers, a significant negative correlation was observed between the expression of these two genes, with a Pearson coefficient of linear correlation around  $-0.2$ . *ABCB1* mRNA levels were consistently higher among tumors in the bottom quartile of *SMARCB1* expression compared with those in the top quartile. This correlative data are consistent with the negative effect of *SMARCB1* on *ABCB1* expression seen in Hap1 cells and suggest this regulatory relationship may be relevant in some human cancers. Across several of these cancers, the expression of *SMARCB1* was also negatively correlated with a gene-expression signature associated (26) with resistance to doxorubicin (Supplementary Fig. S3).

To provide more direct evidence, *SMARCB1* was depleted in five human cancer cell lines infected with lentivirus-based CRISPR/Cas9 (LentiCR) constructs carrying gRNAs validated in

Hap1 cells (Supplementary Fig. S4A). In each case, *ABCB1* mRNA was significantly upregulated, ranging from approximately 2-fold in PC3 cells to approximately 17.5-fold in NCI-H1650 cells (Fig. 4E). Conversely, overexpression of *SMARCB1*-3xHA in the SNU-349 renal cancer cell line, chosen because it expresses high levels of *ABCB1*, led to downregulation of *ABCB1* protein (Fig. 4F). Thus, both gain and loss of *SMARCB1* function have corresponding opposing effects on *ABCB1* expression in cancer cell lines, consistent with the analysis in Hap1 cells (Fig. 4B) and the correlative observations from TCGA data (Supplementary Figs. S2 and S3).

#### Doxorubicin resistance in cells lacking *SMARCB1* can be reversed by the pharmacologic or genetic ablation of *ABCB1*

*SMARCB1* has been implicated in diverse genomic processes, including control of developmental (25) and lineage-specific gene expression (27), nucleosome positioning at promoters (28), and TOP2A recruitment (5), all mechanisms that could contribute to altered sensitivity to doxorubicin. To test whether *ABCB1* overexpression was the cause of doxorubicin resistance in cell lines lacking SWI/SNF subunits, we measured doxorubicin sensitivity in the presence of Zosuquidar. Zosuquidar neutralized the effect of *SMARCB1* loss almost completely—the doxorubicin  $LC_{50}$  in *SMARCB1*<sup>CR1</sup> cells treated with Zosuquidar was reduced



**Figure 3.** Gene expression changes in Hap1 cells lacking SMARCB1 or SMARCA4. **A**, Venn diagram showing the number of genes whose expression is increased (left) or decreased (right) in cells lacking SMARCB1 or SMARCA4 compared with WT

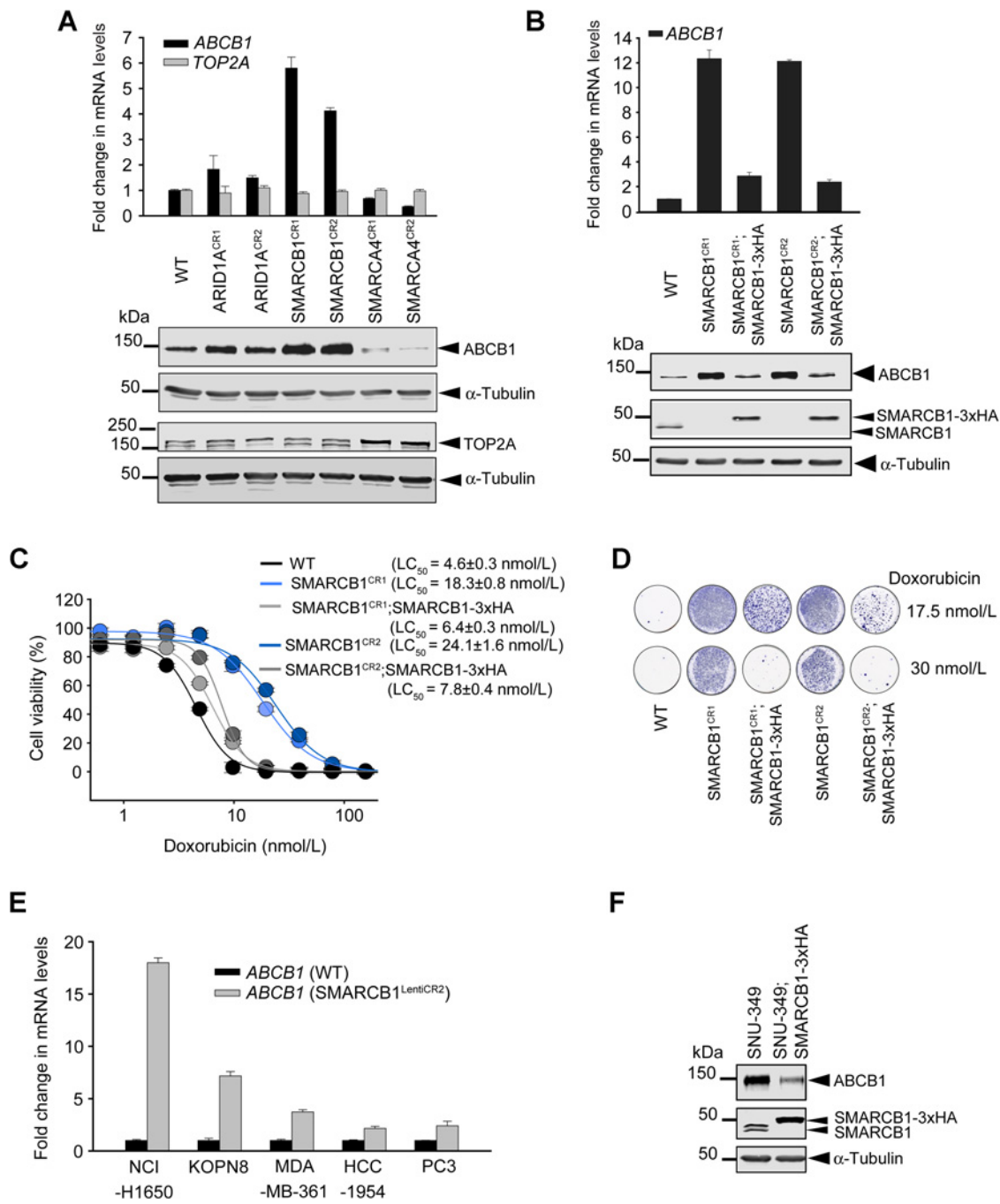
by 4-fold, nearly down to that measured in WT cells (Fig. 5A). A similar effect was seen in ARID1A<sup>CR1</sup> cells (Fig. 5A).

Because small molecules can have off-target effects, we probed the epistatic relationship between SMARCB1 and ABCB1 with respect to doxorubicin resistance through an independent genetic approach. We knocked out both SMARCB1 and ABCB1 in two independent cell lines (ABCB1<sup>CR1.1</sup>;SMARCB1<sup>CR2.1</sup> and ABCB1<sup>CR1.1</sup>;SMARCB1<sup>CR2.2</sup>) and compared their doxorubicin sensitivity with that of the corresponding single knock-out cell lines (Fig. 5B). In both short-term (Fig. 5C) and long-term (Fig. 5D) growth assays, ABCB1 was epistatic to SMARCB1—the doxorubicin sensitivity of cells lacking both ABCB1 and SMARCB1 was very similar to that of WT cells or cells lacking only ABCB1, but much greater than that of cells lacking SMARCB1 alone. This result places ABCB1 downstream of SMARCB1 and indicates that doxorubicin resistance in cells devoid of SMARCB1 is mediated by ABCB1.

Our results are at odds with a prevailing model (6) in the field that doxorubicin resistance in cells lacking SMARCB1 is due to impaired recruitment of TOP2A to chromatin, a previously described activity of the SWI/SNF complex (5), and a consequent reduction in doxorubicin-mediated DNA damage. Because our analysis was performed in exactly the same Hap1 cell line, it was essential to experimentally address the fundamental differences between our studies.

While both studies used Hap1 cells, the doxorubicin treatment regimens were quite different. We exposed cells continuously to low nanomolar doses of doxorubicin (0–100 nmol/L), comparable with the steady-state concentrations attained by all dosage regimens used in patients (1), whereas the other study treated cells with a two-hour pulse of micromolar concentrations of doxorubicin, presumably to mimic the high peak doxorubicin concentrations achieved by bolus dosing regimens. To determine whether different resistance mechanisms may operate under these two regimes, we exposed WT, SMARCB1<sup>CR2</sup>, ABCB1<sup>CR1.1</sup>, and ABCB1<sup>CR1.1</sup>;SMARCB1<sup>CR2</sup> cells to precisely the same doxorubicin treatment protocol described in the other study (6)—a two-hour pulse of 0, 0.25, 0.5, or 1  $\mu$ mol/L doxorubicin followed by a 10-day growth assay. In agreement with their results, loss of SMARCB1 alone increased resistance to doxorubicin (Fig. 5E and Supplementary Fig. S4B). However, this effect could be reversed by the depletion of ABCB1: the doxorubicin sensitivity of ABCB1<sup>CR1.1</sup>;SMARCB1<sup>CR2</sup> cells was comparable with that of ABCB1<sup>CR1.1</sup> cells and significantly higher than that of SMARCB1<sup>CR2</sup> cells (Fig. 5E and Supplementary Fig. S4B). Again, ABCB1 was epistatic to SMARCB1, supporting a mechanism for doxorubicin resistance in cells lacking SMARCB1 that depends on ABCB1. The identical doxorubicin sensitivity of ABCB1<sup>CR1.1</sup> and ABCB1<sup>CR1.1</sup>;SMARCB1<sup>CR2</sup> cells discounts an additional or alternative role for SMARCB1, such as the proposed effects on DNA damage.

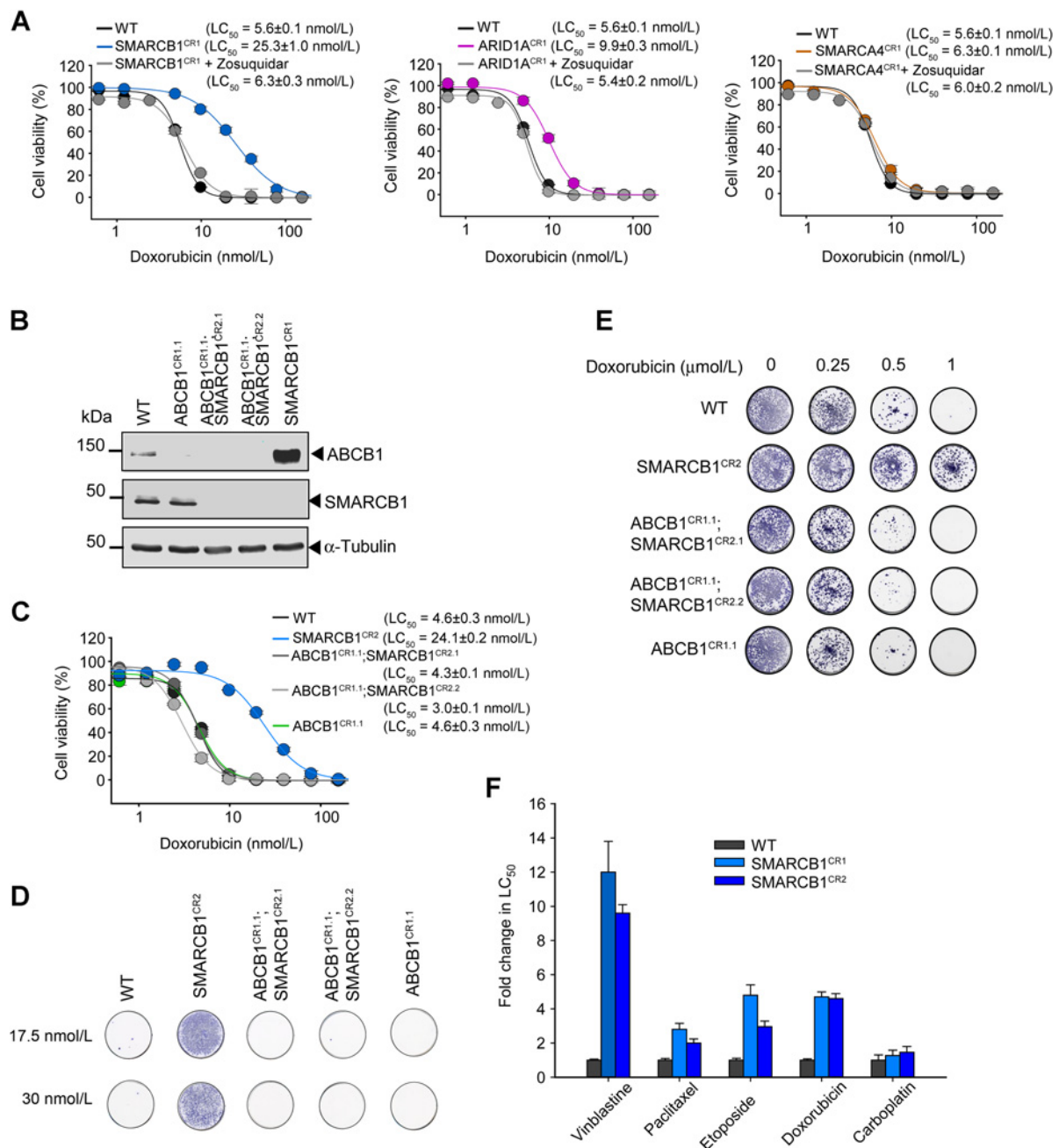
Hap1. **B**, heatmaps showing relative expression levels across all three cell types for the 25 most up- and downregulated genes in SMARCB1<sup>CR</sup> compared with WT cells (left) and in SMARCA4<sup>CR</sup> compared with WT cells (right). Genes are arranged based on hierarchical clustering. Biological replicates for the SMARCB1- and SMARCA4-null cell lines represent two independent clonal cell lines generated by CRISPR methods using two different guide RNAs (CR1 and CR2, see Supplementary Table S2). Replicates for the WT RNAseq data were generated from two different passages of Hap1 cells. Only genes that passed a FDR-corrected  $P$  value of  $<0.001$  for the indicated comparison and were represented by  $>1,000$  total reads were included in the analyses. The full RNAseq dataset is given in Supplementary Table S5.



**Figure 4.** SMARCB1 disruption increases expression of ABCB1 in Hap1 cells. **A**, ABCB1 and TOP2A mRNA (top) and protein levels (bottom) in the indicated cell lines, measured by qRT-PCR and immunoblotting, respectively. **B**, re-expression of SMARCB1 (epitope-tagged with 3xHA) in two cell lines (CR1 and CR2) lacking endogenous SMARCB1 leads to a decrease in both ABCB1 mRNA (top) and protein (bottom). Both cell lines expressing 3xHA-SMARCB1 show increased sensitivity to doxorubicin in 96-hour MTT assays (**C**) and 10-day growth assays (**D**). **E**, ABCB1 mRNA was measured by qRT-PCR in several cancer cell lines infected with a control lentivirus or a lentivirus carrying a validated gRNA (Lenti<sup>CR2</sup>) against SMARCB1. Levels of SMARCB1 were assessed by immunoblotting, as shown in Supplementary Fig. S4A. **F**, ABCB1 protein levels were measured by immunoblotting in SNU-349 cells stably overexpressing epitope-tagged SMARCB1. Error bars, SD ( $n = 3$  in **A**, **B**, and **E**;  $n = 4$  in **C**);  $P$  values for relevant comparisons are shown in Supplementary Table S4.

These two mechanisms of resistance to doxorubicin, ABCB1 overproduction, or reduced TOP2A recruitment predict very different therapeutic consequences. Our mechanism, ABCB1 over-

expression, predicts that cells lacking SMARCB1 will be resistant, not only to TOP2A-targeted drugs like doxorubicin, but also to other classes of chemotherapeutics that are substrates of ABCB1,

**Figure 5.**

ABCB1 is required for doxorubicin resistance in Hap1 cells lacking SMARCB1. **A**, doxorubicin  $LC_{50}$  was measured using an MTT assay (96 hours) in cell lines lacking SMARCB1 (left), ARID1A (middle), and SMARCA4 (right) in the absence or presence of Zosuquidar (100 nmol/L). **B**, immunoblotting was used to compare the protein levels of ABCB1 in SMARCB1<sup>CR2</sup> cells, ABCB1<sup>CR1</sup> cells, and in two independent clonal cell lines carrying frameshift mutations in both *SMARCB1* and *ABCB1*. Doxorubicin sensitivity in each of these single and double mutant cell lines was measured using a 96-hour MTT assay (**C**) or a 10-day growth assay (**D**). **E**, single and double mutant cell lines were treated with a transient 2-hour pulse of doxorubicin, followed by drug removal, and growth for 10 days. Crystal violet staining used to assess cell growth after this treatment regimen is shown in **E** and quantified in Supplementary Fig. S4B. Error bars, SD ( $n = 4$  in **A** and **C**). **F**, fold increases in the  $LC_{50}$  for various drugs in two cell lines carrying independent frameshift mutations in SMARCB1. Vinblastine and paclitaxel are microtubule-targeted ABCB1 substrates, etoposide and doxorubicin are TOP2A-targeted ABCB1 substrates, and carboplatin is not considered an ABCB1 substrate. Drug resistance induced by SMARCB1 loss can be reversed by adding the ABCB1-inhibitor Zosuquidar (Supplementary Fig. S4C).

such as the microtubule-targeted taxanes or vinca alkaloids. The alternative mechanism predicts that resistance should be restricted to TOP2A-targeted drugs; sensitivity to microtubule-targeted

agents should be unaffected. Indeed, two independent cell lines lacking SMARCB1 show increased resistance to both paclitaxel and vinblastine, consistent with a multidrug resistant (MDR)



phenotype conferred by ABCB1 overexpression (Fig. 5F and Supplementary Fig. S4C).

The myriad lines of experimental data presented above prove that doxorubicin resistance in cells lacking SMARCB1 is due to increased expression of the drug efflux pump ABCB1. The diminished DNA damage measured in the previous study (6) when SMARCB1 is lost is likely to be a secondary consequence of the reduced intracellular accumulation of doxorubicin and the other TOP2A poisons used in that study (all of which are ABCB1 substrates). Indeed, Hap1 cells lacking SMARCB1 accumulated lower levels of two structurally distinct ABCB1 substrates, doxorubicin itself, and the nontoxic Rhodamine-123 (ref. 29; Supplementary Fig. S4D and S4E).

#### Doxorubicin resistance in cells lacking SMARCB1 depends on SMARCA4

Given that SMARCB1 and SMARCA4 are two core components of the same protein complex, how can they have opposing effects on *ABCB1* gene expression (Fig. 4A)? To understand their epistatic relationship, we engineered a double mutant Hap1 cell line (SMARCB1<sup>CR1</sup>;SMARCA4<sup>CR2</sup>) lacking expression of both SMARCB1 and SMARCA4. The elevated ABCB1 protein and mRNA levels seen in SMARCB1<sup>CR1</sup> cells were markedly reduced by the additional depletion of SMARCA4, returning to levels approximating those found in WT cells (Fig. 6A). The reduction in ABCB1 observed in SMARCB1<sup>CR1</sup>;SMARCA4<sup>CR2</sup> cells also led to increased doxorubicin sensitivity in both short-term (Fig. 6B) and long-term (Fig. 6C) growth assays. The low *ABCB1* expression and doxorubicin sensitivity measured in SMARCB1<sup>CR1</sup>;SMARCA4<sup>CR2</sup> cells resembled the phenotype of SMARCA4<sup>CR2</sup> cells, consistent with a model in which SMARCA4 function is epistatic to SMARCB1 function.

We tested whether SMARCA4 also functions downstream of SMARCB1 in the lung cancer cell line A549, which does not express endogenous SMARCA4 (Fig. 6D). Loss of SMARCB1 in A549 cells did not alter ABCB1 levels, consistent with the observation from Hap1 cells that *ABCB1* upregulation in this setting depends on the function of SMARCA4. Indeed, the expression of SMARCA4-FLAG in A549 cells lacking SMARCB1 led to the induction of *ABCB1* protein and mRNA (Fig. 6E). These results in Hap1 and A549 cells demonstrate that SMARCA4 is a critical barrier to *ABCB1* expression, and consequently drug sensitivity, when the tumor suppressor SMARCB1 is lost.

Transcriptional profiling of the double mutant SMARCB1<sup>CR1</sup>;SMARCA4<sup>CR2</sup> cells, compared with the corresponding single mutant cell lines, revealed a larger set of "ABCB1-like" genes whose increased expression when SMARCB1 is lost depends on the continued function of SMARCA4 (Supplementary Fig. S5). More generally, genes could be classified into distinct groups based on whether their expression was modulated by SMARCA4, SMARCB1, or both proteins (Supplementary Fig. S5 and Supplementary Table S6), suggesting that SWI/SNF complexes composed of different combinations of these core subunits can regulate distinct sets of genes.

## Discussion

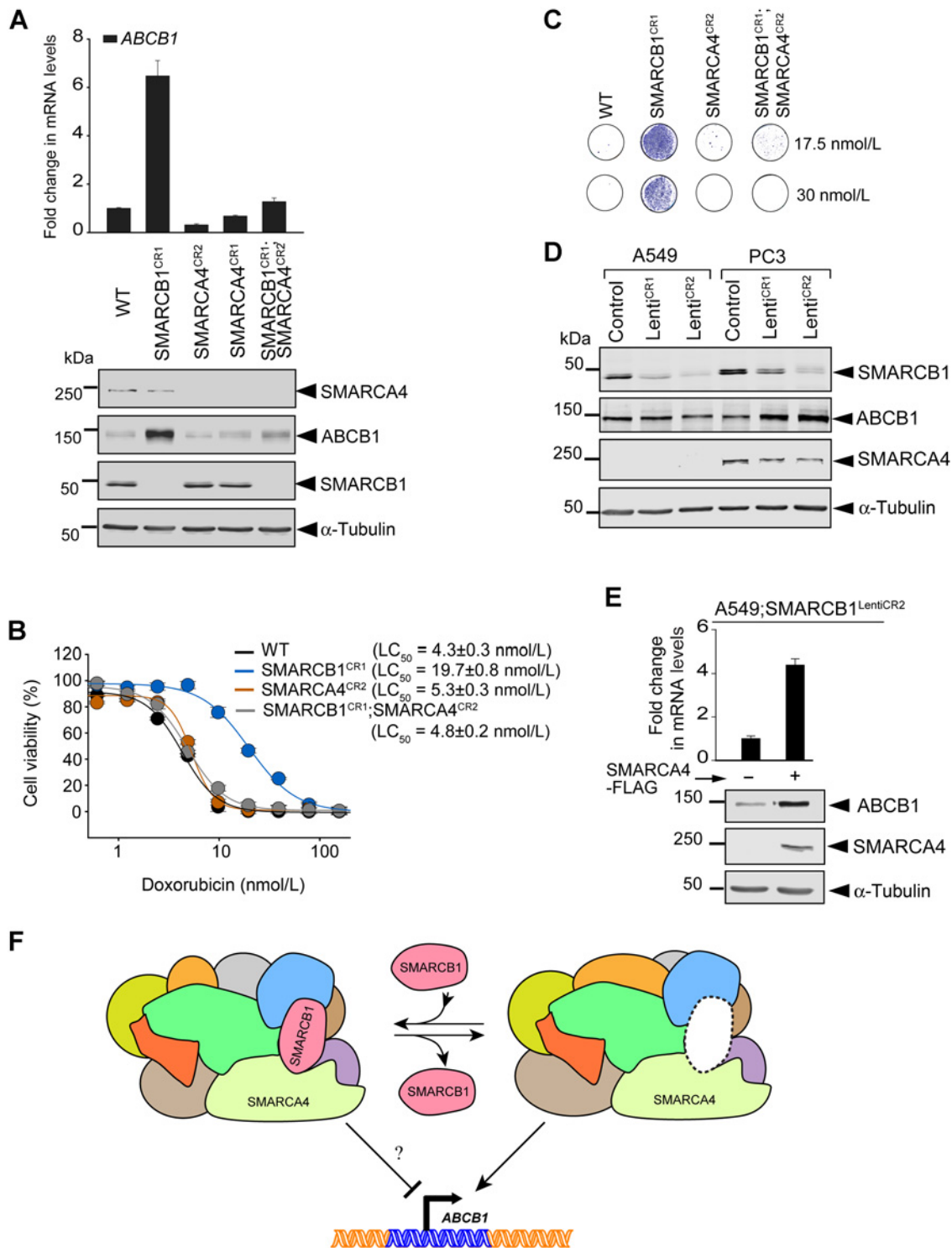
The SWI/SNF complex is a tumor suppressor mutated in up to 20% of human cancers (30–32), comparable with mutation rates for iconic tumor suppressors such as *P53* or *PTEN*. We uncovered a novel mechanism by which SWI/SNF complex subunits can

influence the efficacy of cancer drugs—through transcriptional regulation of the major multidrug resistance pump implicated in human cancer, ABCB1. ABCB1 substrates include anthracyclines, used as the selective agent in our haploid screen, but also other classes of commonly used drugs in oncology such as the taxanes, vinca alkaloids, and epipodophyllotoxins (33). The loss of SMARCB1, the SWI/SNF subunit with the greatest impact on doxorubicin sensitivity, leads to transcriptional upregulation of *ABCB1* and resistance to both TOP2A-targeted and microtubule-targeted drugs. Both effects can be completely reversed by the genetic or pharmacologic ablation of ABCB1 (Fig. 5C). Among a panel of ABC transporters that have been implicated in doxorubicin resistance, the loss of SMARCB1 also led to the transcriptional upregulation of *ABCG2* (Supplementary Fig. S5C); however, absolute mRNA levels of *ABCG2* (even after induction) were much lower than those of *ABCB1* in HAP1 cells. While *ABCG2* upregulation does not play a major role in HAP1 cells, confirmed by our genetic analysis, it may be important in other tumor contexts. Our results also do not support the model (6) that SMARCB1 loss contributes to doxorubicin resistance by reducing the recruitment of TOP2A to DNA. We speculate that the reduced DNA DSBs observed when SMARCB1 is lost are not due to impaired recruitment of TOP2A to DNA, but rather simply due to reduced intracellular levels of doxorubicin.

Our specific analysis of the *ABCB1* locus (Fig. 6) and a more global analysis of gene expression profiles (Supplementary Fig. S5) suggest a complex interaction between SMARCA4 and SMARCB1 in regulating the activity of the SWI/SNF complex. The most parsimonious model, one in which *ABCB1* is repressed by the SWI/SNF complex, is inconsistent with the observation that depletion of SMARCA4 in SMARCB1-null cells leads to reduction of ABCB1 back to WT levels. Instead, this result points to an activating, gain-of-function role for SWI/SNF complexes in the absence of SMARCB1. The picture that emerges is one in which residual SWI/SNF complexes lacking SMARCB1 have different, and even opposite, functions in transcriptional regulation compared with intact complexes. Indeed, in the context of SMARCB1-loss, SMARCA4 becomes a vulnerability because its loss sensitizes cells to doxorubicin by downregulating ABCB1 transcription (Fig. 6).

Our results are consistent with the emerging view that aberrant SWI/SNF complexes play important pathogenic roles when one subunit is lost (33–37), an unexpected concept that first emerged from the discovery that oncogenesis driven by the loss of SMARCB1 could be prevented by the concomitant loss of SMARCA4 (34). This is analogous to our observation that *ABCB1* overexpression and doxorubicin resistance driven by the loss of SMARCB1 could be reversed by eliminating SMARCA4. As a consequence, strategies being developed to target these pathogenic residual SWI/SNF complexes may also improve sensitivity to chemotherapeutics that are substrates of ABCB1.

While we cannot distinguish whether *ABCB1* is directly or indirectly regulated by SWI/SNF complexes, a simple model (Fig. 6F) consistent with the data postulates two populations of SMARCA4-containing SWI/SNF complexes, those with or without SMARCB1, that have opposing effects on *ABCB1* expression. The former would either have no influence on *ABCB1* expression or could have a repressive role, whereas the latter must have an activating role because additional removal of SMARCA4 reverts the activation of target genes like *ABCB1*. The levels of SMARCB1 could regulate the relative proportion of these complexes.



**Figure 6.** SMARCA4 is required for elevated *ABCB1* expression in cells lacking SMARCB1. **A**, *ABCB1* mRNA (top) and protein levels (bottom) in clonal cell lines carrying frameshift mutations in *SMARCB1*, *SMARCA4*, or both genes (*SMARCB1*<sup>CR1</sup>;*SMARCA4*<sup>CR2</sup> cells). Doxorubicin sensitivity in each of these single and double mutant cell lines was measured using a 96-hour MTT assay (**B**) or a 10-day growth assay (**C**). **D**, *ABCB1* protein levels were measured to assess the effect of SMARCB1 depletion (using two independent gRNAs, Lenti<sup>CR1</sup>, and Lenti<sup>CR2</sup>) in A549 cells, which do not express endogenous SMARCA4, or PC3 cells, which do express endogenous SMARCA4. **E**, *ABCB1* mRNA (top) and protein (bottom) levels were measured in A549 cells depleted of endogenous SMARCB1 (A549; *SMARCB1*<sup>LentiCR2</sup> cells) before or after the stable expression of epitope-tagged SMARCA4-FLAG. **F**, a model for the regulation of *ABCB1* by SWI/SNF complexes assembled with or without SMARCB1. See the main text for a description. Error bars, SD ( $n = 3$  in **A**;  $n = 4$  in **B**).

When SMARCB1 is lost, the activating complexes would predominate and drive expression of *ABC B1* and other target genes subject to similar regulation (Supplementary Fig. S5B). Thus, loss of SMARCB1 would increase the abundance (or activity) of SWI/SNF complexes that drive a gene expression program promoting both oncogenic transformation and drug resistance. More generally, our gene expression data suggest that SWI/SNF complexes that vary in their occupancy by SMARCB1 or SMARCA4 regulate target genes differently, even though both are considered core subunits (Supplementary Fig. S5A). We speculate that levels of these subunits could be used to regulate large sets of genes during development or lineage specification, by controlling the composition or activity of SWI/SNF complexes.

In addition to the SWI/SNF complex, our genome-wide haploid genetic screen in cultured human cells revealed 35 candidate genetic loci (Supplementary Table S1) that may regulate sensitivity to doxorubicin, including several other genes that encode chromatin regulatory proteins. A majority of these genes have not been implicated in resistance to anthracyclines and thus represent an important resource for further investigations into how cells respond to this important class of drugs that remains one of the backbones of current chemotherapy regimens.

#### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

#### Authors' Contributions

**Conception and design:** R. Dubey, A.M. Lebensohn, Z. Bahrami-Nejad, J.E. Carette, R. Rohatgi

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- Development of methodology: R. Dubey, A.M. Lebensohn, Z. Bahrami-Nejad, C. Marceau, O. Gevaert, R. Rohatgi
- Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): R. Dubey, A.M. Lebensohn, C. Marceau, R. Rohatgi
- Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): R. Dubey, A.M. Lebensohn, M. Champion, O. Gevaert, B.I. Sikic, J.E. Carette, R. Rohatgi
- Writing, review, and/or revision of the manuscript: R. Dubey, A.M. Lebensohn, O. Gevaert, B.I. Sikic, R. Rohatgi
- Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): R. Dubey, C. Marceau, R. Rohatgi
- Study supervision: R. Dubey, J.E. Carette, R. Rohatgi

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