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ARID1B is a specific vulnerability in *ARID1A*-mutant cancers

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Summary

Recent studies have revealed that *ARID1A* is frequently mutated across a wide variety of human cancers and also has bona fide tumor suppressor properties. Consequently, identification of vulnerabilities conferred by *ARID1A* mutation would have major relevance for human cancer. Here, using a broad screening approach, we identify ARID1B, a related but mutually exclusive homolog of ARID1A in the SWI/SNF chromatin remodeling complex, as the number one gene preferentially required for the survival of *ARID1A*-mutant cancer cell lines. We show that loss of ARID1B in *ARID1A*-deficient backgrounds destabilizes SWI/SNF and impairs proliferation. Intriguingly, we also find that *ARID1A* and *ARID1B* are frequently co-mutated in cancer, but that *ARID1A*-deficient cancers retain at least one *ARID1B* allele. These results suggest that loss of *ARID1A* and *ARID1B* alleles cooperatively promotes cancer formation but also results in a unique functional dependence. The results further identify ARID1B as a potential therapeutic target for *ARID1A*-mutant cancers.

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Contributions

C. Roberts directed the study. K. Helming and X. Wang designed and performed experiments. J. Haswell and H. Manchester performed experiments. C. Roberts, K. Helming, X. Wang, B. Wilson, J. Haswell, and H. Manchester analyzed and interpreted the data. K. Helming, B. Wilson, F. Vazquez, and A. Aguirre performed analysis of Project Achilles data. G. Kryukov, M. Ghandi, and L. Garraway provided and analyzed sequencing data. Z. Wang provided *Arid1a*-conditional mice, intellectual contribution, and useful discussion. Y.Kim established *Arid1a*-conditional mouse strain. W. Hahn directs the Achilles Project, provided reagents, helped interpret Achilles data, and edited the manuscript. C. Roberts, K. Helming and X.Wang wrote the manuscript.

To search for specific dependencies created by *ARID1A* mutation, we utilized data from Project Achilles, a large-scale project focused on identifying essential genes in a wide panel of cancer cell lines using genome-scale loss-of-function genetics[1]. We compared 18 *ARID1A*-mutant and 147 cell lines wildtype for *ARID1A* (hereafter referred to as wildtype). Of 9,050 genes interrogated, *ARID1B* scored as the top candidate preferentially required for the growth of *ARID1A*-mutant cancer cell lines ($p=7.366\times10^{-6}$, FDR<0.001) (Fig. 1a, Fig. S1a). Vulnerability to ARID1B depletion was even more pronounced in the large subset of cell lines that contained inactivating *ARID1A* mutations (rather than missense mutations) (Fig. 1b), suggesting that ARID1B is specifically essential for cell lines lacking *ARID1A* (see supplementary discussion). To further evaluate this finding, we examined effects of the individual ARID1B shRNAs. Three of the four ARID1B shRNAs passed the Achilles quality control metrics[2]. These scored #1 ($p=1.211\times10^{-6}$, FDR<0.001), #4 ($p=1.211\times10^{-6}$, FDR<0.001), and #11 ($p=1.816\times10^{-5}$, FDR=0.090) of the 54,020 shRNAs in the screen. We confirmed ARID1B as a vulnerability by knocking it down in two cell lines that contained *ARID1A*-inactivating mutations, OVISE and TOV21G, and two *ARID1A* wildtype lines, ES-2 and 293T (Fig. 1c, Fig. S1b). Proliferation (Fig. 1d) and colony formation (Fig. 1e) were impaired in *ARID1A*-mutant cells but not in wildtype cells.

ARID1B and ARID1A are 60% identical, have been reported to have opposing functions in cell cycle arrest, and are mutually exclusive since individual SWI/SNF chromatin remodeling complexes can contain either ARID1A or ARID1B, but not both[3]. To investigate the relationship between ARID1A and ARID1B in cancer, we asked whether an ARID1B-containing SWI/SNF complex was present in *ARID1A*-mutant cells. Immunoprecipitation of the SMARCC1 (BAF155) core subunit of the SWI/SNF complex[4] resulted in co-precipitation of ARID1B and other SWI/SNF subunits in both wildtype and *ARID1A*-mutant cells, indicating that intact ARID1B-containing complexes are present (Fig. S2a–b) in both wildtype and *ARID1A*-mutant cells.

We next sought to determine whether ARID1B loss affects the composition of the SWI/SNF complex in *ARID1A*-mutant cancer cells. Knockdown of ARID1B in wildtype cell lines had no effect on the expression of other SWI/SNF complex subunits (Fig. 2a, Fig. S2c) or upon their incorporation into the complex (Fig. 2a, Fig. S2c). However, depletion of ARID1B in *ARID1A*-mutant cells resulted in dissociation of the core catalytic ATPase subunit SMARCA4 (BRG1) and reduced incorporation of several other subunits (Fig. 2a). Protein levels of core subunits such as SMARCA4, SMARCC2, and SMARCB1 were also decreased, particularly in the TOV21G line (Fig. 2a), while the mRNA levels were largely unaffected (Fig. S3), suggesting post-translational loss of these proteins.

To further investigate how ARID1B loss affects assembly of the SWI/SNF complex, we performed a sucrose sedimentation assay on cells treated with either control shRNA or ARID1B shRNA. Consistent with the co-immunoprecipitation results, an intact 2 MDa SWI/SNF complex is observed in *ARID1A*-mutant cells treated with control shRNA (Fig. 2b, full figure in Fig. S4–5) and in wildtype cells treated with either control or ARID1B shRNA (Fig. S6). In contrast, knockdown of ARID1B in *ARID1A*-mutant cells eliminated the intact SWI/SNF complex (Fig. 2b; additional subunits shown in Fig. S4–5), indicating that in human *ARID1A*-mutant cancer cell lines, the presence of ARID1B is essential for formation or stabilization of an intact SWI/SNF complex. Despite ARID1A/ARID1B and PBRM1 having been reported to exist in mutually exclusive versions of the SWI/SNF complex[5], our findings are consistent with a more recent publication, which found that these subunits can co-exist[6], as PBRM1 association with smaller complexes was substantially affected by the combined loss of ARID1A/B (Fig S4–5). As the SWI/SNF complex binds up to one-third of all genes[7] and several members of the SWI/SNF complex are essential in mouse

development[8–10] and for survival of many cell lineages[11,12], loss of an intact SWI/SNF complex would be predicted to be incompatible with cell viability.

In order to further validate the identification of ARID1B as a vulnerability in *ARID1A*-mutant human cancer, we sought to investigate whether inactivation of *Arid1a* creates a dependence upon Arid1b using primary MEFs conditional for *Arid1a* [13]. Deletion of *Arid1a* or knockdown of Arid1b individually had only moderate effects on proliferation, while combined loss led to substantial impairment (Fig. S7a). We similarly observed that loss of *Arid1a* or Arid1b alone had only modest effects on the composition of the complex (Fig. 2c), while loss of both led to dissociation and degradation of Smarca4 and substantial reductions in stability and incorporation of many other SWI/SNF subunits (Fig. 2d). Again, the reduced protein levels were not due to changes in transcription (Fig. S7b). Finally, sucrose sedimentation assay showed that loss of *Arid1a* and Arid1b in MEFs eliminated the intact SWI/SNF complex (Fig. 2d and Fig. S7c).

Collectively, these findings demonstrate a synthetic lethal relationship between this mutually exclusive pair of SWI/SNF subunits. Notably, however, *ARID1B* has also been reported mutated in human cancers[3,14], and has been found to be mutant in some of the same types of cancer as *ARID1A*, such as neuroblastoma[14]. Since we found ARID1B knockdown to impair the growth of *ARID1A*-mutant cell lines, we initially hypothesized that mutations in *ARID1A* and *ARID1B* would be mutually exclusive. Surprisingly, we found that *ARID1A* and *ARID1B* mutations co-occur in both cancer cell lines and primary tumors. Using data from cell line sequencing [15,16] we found that 38% of 34 *ARID1A*-mutant lines also contained *ARID1B*-inactivating mutations[16] (Supplementary Table 1, $p<1\times10^{-6}$). Notably, all lines retained at least one allele of either *ARID1A* or *ARID1B*, suggesting that retention of at least one *ARID1* allele may be essential for survival. This finding also held true in primary cancer samples. We found that of the 297 *ARID1A*-mutant primary cancer samples cataloged in the cBio Portal for Cancer Genomics[17,18], 30 (10.1%) also contained *ARID1B* mutations ($p=1.07\times10^{-7}$), significantly higher than the 3% rate in ARID1A-wildtype tumors.

The co-occurrence of *ARID1A* and *ARID1B* mutations raises the possibility that the synthetic lethality relationship could be caused simply by the high frequency of *ARID1B* mutations in *ARID1A*-mutant cancer cell lines. To evaluate this possibility, we removed all *ARID1B*-mutant cell lines and conducted a revised class comparison in which four *ARID1B*-wildtype, *ARID1A*-mutant cell lines were compared to 49 cell lines wildtype for both *ARID1A* and *ARID1B*. *ARID1B* still scored number four out of 9,000+ genes ($p=7.154\times10^{-4}$), indicating that the synthetic lethality between ARID1A and ARID1B is a result of *ARID1A* mutation and not co-occurring *ARID1B* mutations.

In this report, we show that inactivating mutations in *ARID1A,* frequent across a wide variety of cancers, create a dependency upon ARID1B (Fig. 2e). It is notable that the number one vulnerability in *ARID1A*-mutant cell lines is another member of the SWI/SNF complex. We previously showed that cancer formation in the absence of the SWI/SNF subunit SMARCB1 does not result from SWI/SNF inactivation but rather that oncogenesis was dependent upon the activity of the residual SWI/SNF complex[19]. At that time we speculated that, much like the concept of oncogene addiction, targeting the aberrant residual SWI/SNF complex might theoretically be an effective therapeutic approach for *SMARCB1*-mutant cancers. Our present study, which surveyed 9,050 genes, reveals the role of the residual complex in the growth of *ARID1A*-deficient cancers and also identifies a specific subunit as a relative vulnerability. This principle may have broad applicability to SWI/SNF-mutant cancers as Oike et al recently showed that SMARCA2, a paralog of SMARCA4, was essential in *SMARCA4*-mutant cancers[20].

Together, our findings may suggest that partial loss of ARID1 function via mutation of *ARID1A* alleles or, less frequently, *ARID1B* alleles can drive cancer growth but at the same time create a specific vulnerability compared to non-mutant cells. This suggests ARID1B as a potential therapeutic target for cancers that contain inactivating *ARID1A* mutations. Recent examples have demonstrated the feasibility and efficacy of targeting chromatin regulators such as BRD4[21,22] as well as other non-enzymatic proteins such as BCL-2[23] and molecules previously found difficult to target such as RAS[24]. ARID1B could potentially be targetable through its E3 ubiquitin ligase interaction[25]. Additionally, novel approaches using small stabilized peptides have recently been shown capable of disrupting association of EZH2 with its chromatin remodeling complex[26]. Analogous approaches may now be considered for targeting ARID1B.

Online Methods

Achilles Analysis

To find genes that are preferentially essential in mutant cell lines, we used the GenePattern module PARIS (http://www.broadinstitute.org/cancer/software/genepattern) using the default parameters except quality, which was changed to final. The gene-level Achilles dataset v2.4 was used as a data file (www.broadinstitute.org/achilles) (file name: Achilles_QC_v2.4_rnai.Gs.gct). The classifier files were generated using the gene mutation status from the Cancer Cell Line Encyclopedia (www.broadinstitute.org/ccle). Cell lines without hybrid capture sequencing data were removed from the analysis. The mutational status of *ARID1A* was annotated for 165 of the 216 cell lines in the Achilles dataset, and as a result, these 165 cell lines were used in the class comparisons.

Cell Culture

TOV21G (CRL 11730), ES-2 (CRL-1987), and 293T (CRL-3216) cell lines were purchased from ATCC. OVISE cells were obtained from William Hahn's laboratory. Mouse Embryonic Fibroblasts (MEFs) were generated as described previously[27]. Cells were transduced with shRNAs and selected with puromycin for 48–72 hours before seeding for MTT or colony formation assays. MTT assays were conducted with a Cell Proliferation Kit (Roche). Colony formation assays were conducted by staining cells for 20 minutes with crystal violet staining solution (0.05% Crystal Violet, 1% Formaldehyde, 1% PBS, 1% methanol).

shRNA-mediated knockdown of ARID1B

ARID1B shRNAs were obtained from the RNA interference (RNAi) screening facility at the Dana-Farber Cancer Institute and were lentivirally transduced into OVISE, TOV21G, ES-2, and 293T cells. ARID1B and non-silencing control shRNAs are in the pLKO.1 lentiviral expression vector backbone. Target sequences for shRNAs are available upon request.

Density Sedimentation Analysis

Nuclear extract (1 mg) was diluted in 300 μl of 0% sucrose RIPA buffer and carefully overlaid onto a 12 ml 20%–50% sucrose (in RIPA buffer) gradient prepared in a 14ml 14×95 mm polyallomer centrifuge tube (Cat. #331374, Beckman Coulter). Tubes were placed in an SW-40 Ti swing bucket rotor and centrifuged at 4°C for 16 hours at 40,000 rpm. Fractions (0.5 ml) were collected and used in gel electrophoresis and subsequent Western blotting analyses.

Immunoblots and co-immunoprecipitation 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, 5mM EDTA, 12% Glycerol, 50mM NaCl, 1% NP-40) plus protease inhibitors (Complete, Mini, EDTA-free. Roche: 11836170001) for 30 minutes. Supernatants were collected following a brief spin (10 min.) at 17900 r.c.f. to separate cellular debris in a 4° C centrifuge. Protein concentrations were determined using the Bradford reagent (Biorad). SDS-polyacrylamide gel electrophoresis was used to separate proteins, which were subsequently transferred to PVDF membranes (Millipore). ARID1B antibody (Abcam: ab54761) was used to detect efficient knockdown.

Nuclear extracts for immunoprecipitation were prepared using the NE-PER Nuclear and Cytoplasmic Extraction Kit (Thermo Scientific: 78833). Nuclear extracts were diluted with RIPA buffer (1 mg/ml, with protease inhibitors and DTT). Each IP was incubated with indicated antibodies overnight at 4° C. Protein G Dynabeads (Life Technologies: 10004D) were added and incubated at 4° C for 3 hrs. Beads were then washed three times with RIPA buffer and resuspended in reducing SDS gel loading buffer. Antibodies used in the immunoprecipitation and immunoblots are: SMARCC1/BAF155 (Santa Cruz: 9746); ARID1A (Bethyl Laboratories: A301-041A); PBRM1 (Bethyl Laboratories: A301-591A); SMARCA4 (Santa Cruz: sc17796); SMARCC2/BAF170 (Bethyl Laboratories: A301-039A); SMARCD1/BAF60A (Bethyl Laboratories: A301-595A); SMARCE1/BAF57 (Bethyl Laboratories: A300-810A); ACTL6A/BAF53A (Bethyl Laboratories: A301-391A); ACTIN (Cell Signaling Technology: 5125).

RNA purification and RT-qPCR

Total RNA was extracted using Trizol reagent (Invitrogen) following the manufacturer's instructions. 2 μg of total RNA was reverse-transcribed into first-strand cDNA using oligo$(dT)_{20}$ primers and the SuperScript III Reverse Transcriptase (Invitrogen). RT-qPCR was performed on the ViiA 7 Real-Time PCR System (Life Technologies) using SYBR Select Master Mix (Life Technologies). Reactions were performed in triplicate, and gene expression was normalized to GAPDH. Error bars represent SD of mean expression.

Cell Line Sequencing

Cell lines were sequenced as previously described[15]. Cell line sequencing data and the data from the Cancer Cell Line Encyclopedia[16] were used to identify cell lines with co-occurring mutations of ARID1A and ARID1B.

Statistical Significance of Mutation Overlap

To evaluate the statistical significance of the overlap of ARID1A and ARID1B mutations, the probability of observing at least n12 cell lines with both mutations was estimated under the null hypothesis that these two mutations are independent. For that, given n1 cell lines with ARID1A and n2 cell lines with ARID1B mutations and n12 cell lines with both mutations the following simulation was run: n1 cell lines were randomly picked with the probability for each cell line being selected set relative to its mutation rate and assigned mutation 'A' to these cell lines. Next, n2 cell lines were similarly selected and assigned mutation 'B' to those cell lines and then the number of cell lines with both mutations 'A' and 'B' was counted. This process was repeated many times to estimate the probability of observing n12 cell lines or more with both mutations.

For primary cancer samples, a contingency table was formed consisting of the counts for all the four possibilities of *ARID1A* or *ARID1B* mutation status. The Fisher's exact test was

used to calculate the statistical significance of the overlap of *ARID1A* and *ARID1B* mutations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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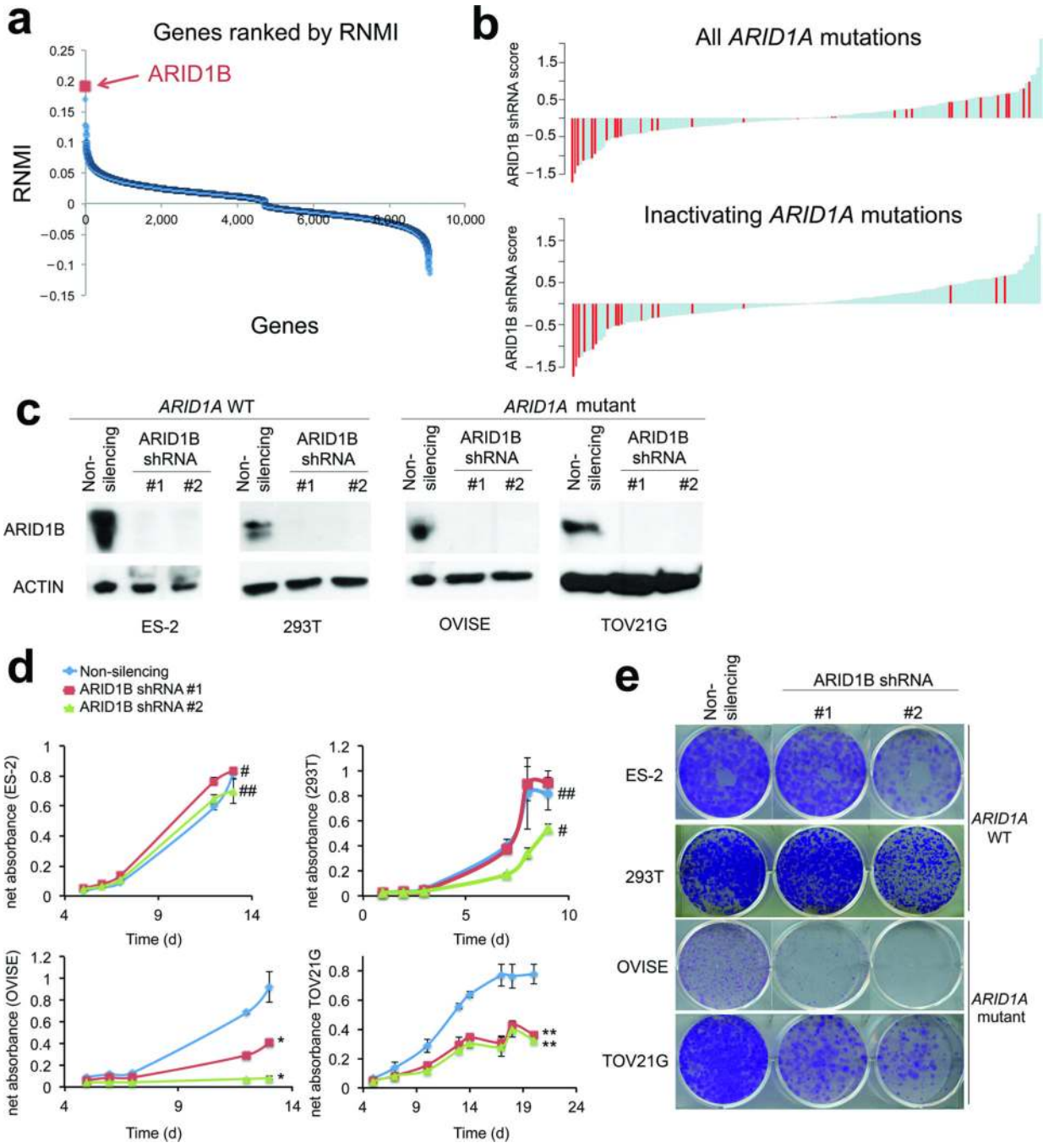

Figure 1. ARID1B is a specific vulnerability in *ARID1A*-mutant cancer cell lines

(a) Rank list of vulnerabilities identified by screen of Achilles platform cell lines. ARID1B is the #1 gene preferentially essential for the growth of *ARID1A*-mutant cancer cell lines as compared to wildtype cancer cell lines.

(b) Effects of ARID1B shRNAs across cell lines in the Achilles screen. Negative values indicate growth inhibition while positive values reflect growth enhancement. In the top panel, cell lines with any identified *ARID1A* mutation compared to the reference genome are indicated in red. In the bottom panel, only those cell lines with clear inactivating mutations in *ARID1A* are shown in red.

(c) Immunoblots showing the results of two independent shRNAs targeting ARID1B in ES-2 and 293T (*ARID1A*-wildtype lines) and in OVISE, and TOV21G (*ARID1A*-mutant lines).

(d) Proliferation of wildtype (ES-2 and 293T) and *ARID1A*-mutant (OVISE and TOV21G) cell lines in response to two independent ARID1B shRNAs. * $p<0.0002$ ** $p<4\times10^{-8}$ #$p>0.05$ ##$p<0.05$ Data are expressed as mean ± S.D.

(e) Colony formation in response to ARID1B knockdown in wildtype and *ARID1A*-mutant cell lines.

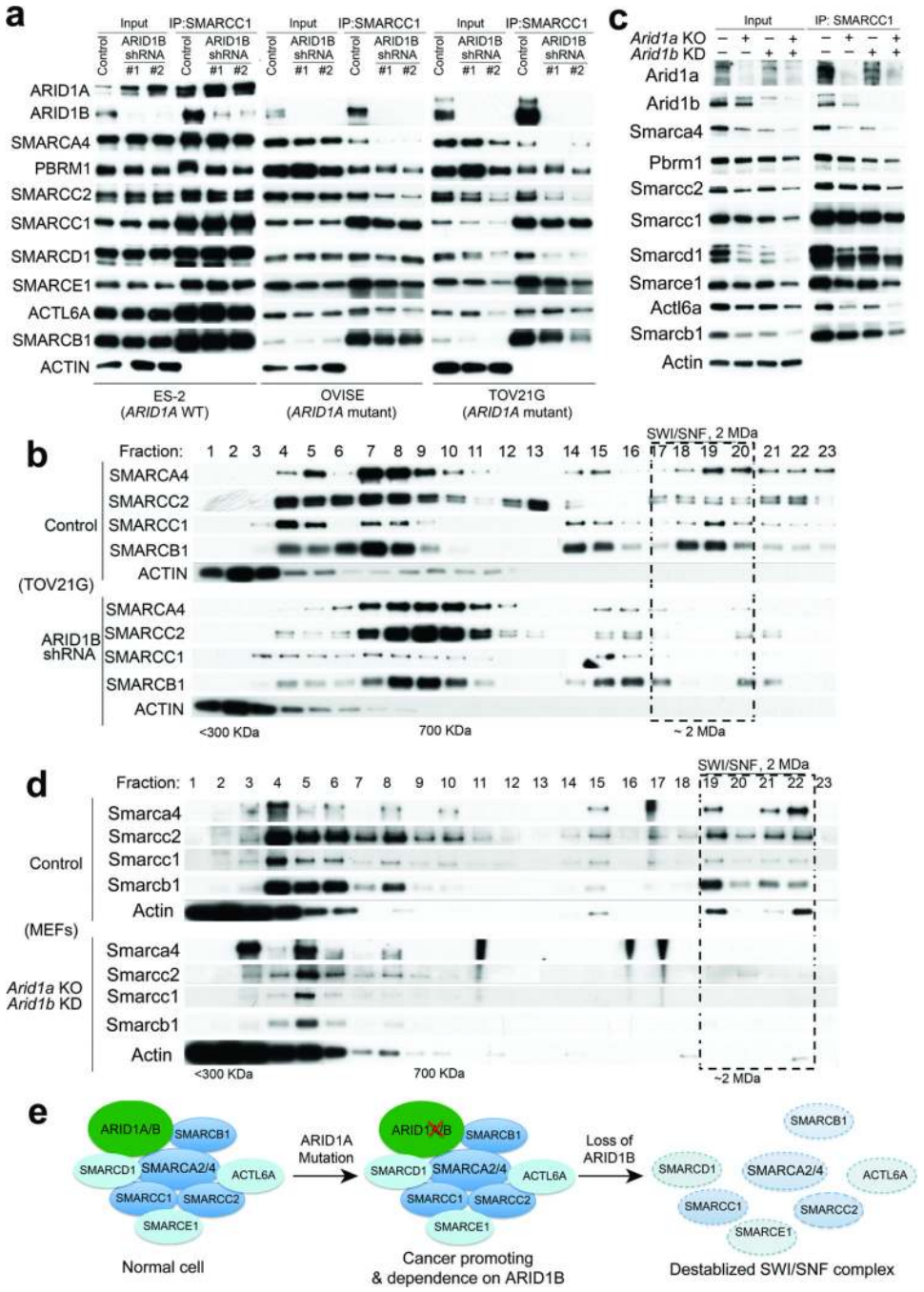

Figure 2. ARID1B is required for the maintenance of an intact SWI/SNF complex in *ARID1A*-mutant cancer cell lines and primary cells

(a) Immunoprecipitation of the SWI/SNF complex by SMARCC1 from the nuclear extract of ES-2, OVISE, and TOV21G cells upon treatment with control shRNA or two independent ARID1B shRNAs.

(b) Sucrose sedimentation (20–50%) assay of SWI/SNF complex from *ARID1A*-mutant TOV21G cells treated with either control shRNA (top half) or ARID1B shRNA (bottom half).

(c) Immunoprecipitation of the SWI/SNF complex by Smarcc1 from the nuclear extract of MEFs with indicated treatment.

(d) Sucrose sedimentation (20–50%) assay of the SWI/SNF complex from the nuclear extract of MEFs with indicated treatment: control shRNA treated MEFs (top half) or *Arid1a* knockout (KO) and Arid1b knockdown (KD) MEFs (bottom half)
(e) Model: Inactivating mutations in *ARID1A* promote oncogenic transformation but also create specific dependency on ARID1B. Inhibition of ARID1B in *ARID1A*-mutant cells destabilizes the SWI/SNF complex and results in impaired cell growth.