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DATA NOTE

A guide to selecting high-performing antibodies for ARID2 (UniProt ID: Q68CP9) for use in western blot, immunoprecipitation, and immunofluorescence

[version 1; peer review: 2 approved]

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Abstract

The AT-rich interactive domain-containing protein 2 (ARID2) is a core component of the chromatin-remodeling complex that regulates transcription by modulating nucleosome positioning. Here we have characterized six ARID2 commercial antibodies for western blot, immunoprecipitation, and immunofluorescence using a standardized experimental protocol based on comparing read-outs in knockout cell lines and isogenic parental controls. These studies are part of a larger, collaborative initiative seeking to address antibody reproducibility issues by characterizing commercially available antibodies for human proteins and publishing the results openly as a resource for the scientific community. While the use of antibodies and protocols vary between laboratories, we encourage readers to use this report as a guide to select the most appropriate antibodies for their specific needs.

Keywords

Q68CP9, ARID2, ARID2, AT-Rich Interaction Domain 2, antibody characterization, antibody validation, western blot, immunoprecipitation, immunofluorescence

Open Peer Review

Approval Status

	1	2
version 1		
24 Apr 2026	view	view
1. Karen Bowman , University of Leicester, Leicester, UK		
2. Deborah Moshinsky , Institute for Protein Innovation, Boston, USA		
Any reports and responses or comments on the article can be found at the end of the article.		



This article is included in the **YCharOS (Antibody Characterization through Open Science)** gateway.

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Competing interests: For this project, the authors developed partnerships with leading antibody manufacturers and KO cell line providers. The partners provide antibodies and KO cell lines to this project at no cost. These partners include: Abcam, ABCD antibodies, ABclonal, Addgene, Aviva Systems Biology, BioTechne, Cell Signaling Technology, Developmental Studies Hybridoma Bank, GeneTex, Horizon Discovery, Institute for Protein Innovation, MilliporeSigma, Proteintech, Synaptic Systems, Thermo Fisher Scientific.

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Introduction

ARID2 is a key component of the Polybromo, Brg1-Associated Factors (PBAF) subtype of the SWI/SNF chromatin-remodeling complex, where it contributes to the regulation of gene expression through interactions with DNA and nucleosomes.¹ As a structural and regulatory subunit, ARID2 helps recruit the PBAF complex to specific genomic loci, enabling precise control of transcriptional programs involved in cell differentiation, development, and tissue homeostasis. Mutations and dysregulation of *ARID2* have been increasingly linked to human disease, including cancer and neurodevelopmental disorders, highlighting its importance for maintaining normal chromatin architecture and genomic stability.²

This research is part of a broader collaborative initiative in which academics, funders and commercial antibody manufacturers are working together to address antibody reproducibility issues by characterizing commercial antibodies for human proteins using standardized protocols, and openly sharing the data.³ It consists of identifying human cell lines with adequate target protein expression and the development/contribution of equivalent knockout (KO) cell lines, followed by antibody characterization procedures using most commercially available renewable antibodies against the corresponding protein.³ Here we characterized six commercial ARID2 antibodies, selected and donated by participant antibody manufacturers, for use in western blot, immunoprecipitation, and immunofluorescence (also referred to as immunocytochemistry), enabling biochemical and cellular assessment of ARID2 properties and function.

The authors do not engage in result analysis or offer explicit antibody recommendations. Our primary aim is to deliver top-tier data to the scientific community, grounded in Open Science principles. This empowers experts to interpret the characterization data independently, enabling them to make informed choices regarding the most suitable antibodies for their specific experimental needs. Guidelines on how to interpret antibody characterization data found in this study are featured on the YCharOS gateway⁴ and in [Table 4](#) of this data note.³

Results and discussion

Our standard protocol involves comparing readouts from wild type (WT) and KO cells.^{5,6} The first step was to identify a cell line(s) that expresses sufficient levels of a given protein to generate a measurable signal using antibodies. To this end, we examined the DepMap (Cancer Dependency Map Portal, RRID:SCR_017655) transcriptomics database to identify all cell lines that express the target at levels greater than $2.5 \log_2$ (transcripts per million “TPM” + 1), which we have found to be a suitable cut-off.⁷ The HAP1 expresses the *ARID2* transcript at $3.5 \log_2$ TPM + 1, and an *ARID2* KO in the HAP1 cell line was obtained from Horizon Discovery ([Table 1](#)). Moreover, as seen on DepMap, the HAP1 does not carry mutations in the *ARID2* that could affect antibody–epitope binding.

To screen the six by western blot, WT and *ARID2* KO protein lysates were ran on SDS-PAGE, transferred onto nitrocellulose membranes, and then probed with all six ARID2 antibodies in parallel ([Figure 1](#)).

We then assessed the capability of all six antibodies to capture ARID2 from HAP1 protein extracts using immunoprecipitation techniques, followed by western blot analysis. For the immunoblot step, a specific ARID2 antibody identified previously (refer to [Figure 1](#)) was selected. Equal amounts of the starting material (SM) and the unbound fractions (UB), as well as the whole immunoprecipitate (IP) eluates were separated by SDS-PAGE ([Figure 2](#)).

For immunofluorescence, six antibodies were screened using a mosaic strategy. First, HAP1 WT and *ARID2* KO cells were labelled with different fluorescent dyes in order to distinguish the two cell lines, and the ARID2 antibodies were evaluated. Both WT and KO lines imaged in the same field of view to reduce staining, imaging and image analysis bias ([Figure 3](#)). Quantification of immunofluorescence intensity in hundreds of WT and KO cells was performed for each antibody tested, and the images presented in [Figure 3](#) are representative of this analysis.³

In conclusion, we have screened six ARID2 commercial antibodies by western blot, immunoprecipitation, and immunofluorescence by comparing the signal produced by the antibodies in human HAP1 WT and *ARID2* KO cells. To assist users in interpreting antibody performance, [Table 4](#) outlines various scenarios in which antibodies may perform in all three applications.⁷ High-quality and renewable antibodies that successfully detect ARID2 were identified in all applications. Researchers who wish to study ARID2 in a different species are encouraged to select high-quality

Table 1. Summary of the cell lines used.

Institution	Catalog number	RRID (Cellosaurus)	Cell line	Genotype
Horizon Discovery	C631	CVCL_Y019	HAP1	WT
Horizon Discovery	HZGHC000907c009	CVCL_SD47	HAP1	<i>ARID2</i> KO

Table 2. Summary of the ARID2 antibodies tested.

Company	Catalog number	Lot number	RRID (Antibody Registry)	Clonality	Clone ID	Host	Concentration (µg/µL)	Vendors recommended applications
Cell Signaling Technology	82342**	3	AB_2799992	recombinant mono	D8D8U	rabbit	0.24	Wb, IP
Developmental Studies Hybridoma Bank (DSHB)	PCRP-ARID2-1A1*	12/13/18	AB_2618409	monoclonal	PCRP-ARID2-1A1	mouse	0.06	IP
GeneTex	GTX129443	41551	AB_2885997	polyclonal	-	rabbit	1.00	Wb, IP, IF
GeneTex	GTX129444	41493	AB_2885998	polyclonal	-	rabbit	1.00	Wb, IP, IF
GeneTex	GTX632011*	44517	AB_2888275	monoclonal	GT7311	mouse	1.00	Wb, IF
Proteintech	23406-1-AP	00025260	AB_2918080	polyclonal	-	rabbit	0.23	Wb

Wb = western blot; IF = immunofluorescence; IP = immunoprecipitation,

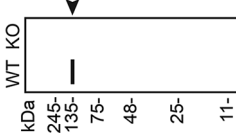
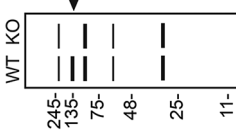
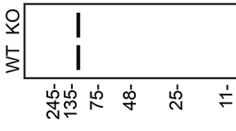
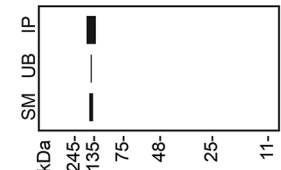
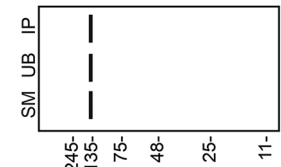
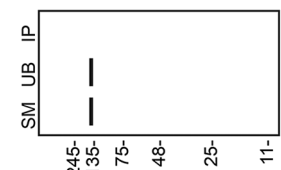
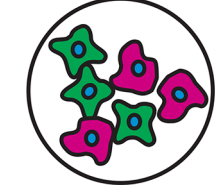
**= recombinant antibody,

*= monoclonal antibody

Table 3. Table of secondary antibodies used.

Company	Secondary antibody	Catalog number	RRID (Antibody Registry)	Clonality	Concentration (µg/µL)	Working concentration (µg/mL)
Proteintech	HRP-Goat Anti-Rabbit Antibody (H + L)	RGAR001	AB_3073505	recombinant polyclonal	1.0	0.05
Proteintech	HRP-Goat Anti-Mouse Antibody (H + L)	RGAM001	AB_3068333	recombinant polyclonal	1.0	0.5
Cell Signaling Technology	Protein A, HRP conjugate	12291	NA	polyclonal	0.125	0.5
Proteintech	Coralite Plus 555-Goat Anti-Rabbit Antibody (H + L)	RGAR003	AB_3073507	recombinant polyclonal	0.5	0.5
Proteintech	Coralite Plus 555-Goat Anti-Mouse Antibody (H + L)	RGAM003	AB_3068539	recombinant polyclonal	0.5	0.5

Table 4. Illustrations to assess antibody performance in all western blot, immunoprecipitation and immunofluorescence.

Western blot	Immunoprecipitation			Immunofluorescence				
Successful antibody WT KO  245- 135- 75- 48- 25- 11- Target protein detected (arrowhead) among others	Successful antibody WT KO  245- 135- 75- 48- 25- 11- Target protein detected (arrowhead) among others	Unsuccessful antibody WT KO  245- 135- 75- 48- 25- 11- Target protein not detected	Successful antibody SM UB IP  245- 135- 75- 48- 25- 11- Target protein captured in the IP	Successful antibody SM UB IP  245- 135- 75- 48- 25- 11- Target protein captured in the IP	Unsuccessful antibody SM UB IP  245- 135- 75- 48- 25- 11- Target protein not captured in the IP	WT/KO cell mosaic 	Successful antibody WT KO  Target protein detected (white staining)	Unsuccessful antibody WT KO  Target protein not detected

antibodies, based on the results of this study, and investigate the predicted species reactivity of the manufacturer before extending their research.

Limitations

Inherent limitations are associated with the antibody characterization platform used in this study. Firstly, the YCharOS project focuses on renewable (recombinant and monoclonal) antibodies and does not test all commercially available ARID2 antibodies. YCharOS partners provide approximately 80% of all renewable antibodies, but some top-cited polyclonal antibodies may not be available through these partners. We encourage readers to consult vendor documentation to identify the specific antigen each antibody is raised against, where such information is available.

Secondly, the YCharOS effort employs a non-biased approach that is agnostic to the protein for which antibodies have been characterized. The aim is to provide objective data on antibody performance without preconceived notions about how antibodies should perform or the molecular weight that should be observed in western blot. As the authors are not experts in ARID2, only a brief overview of the protein's function and its relevance in disease is provided. ARID2 experts are invited to analyze and interpret observed banding patterns in western blots and subcellular localization in immunofluorescence.

Thirdly, YCharOS experiments are not performed in replicates primarily due to the use of multiple antibodies targeting various epitopes. Once a specific antibody is identified, it validates the protein expression of the intended target in the selected cell line, confirms the lack of protein expression in the KO cell line and supports conclusions regarding the

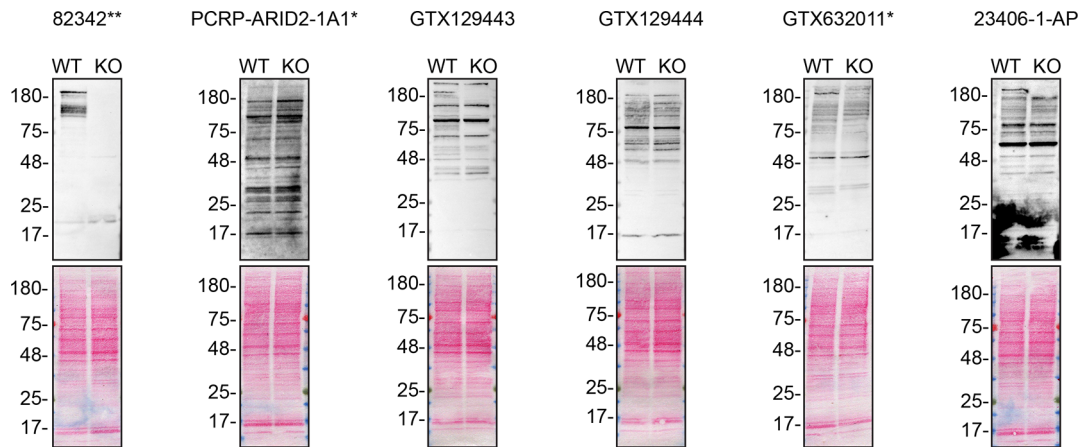


Figure 1. ARID2 antibody screening by western blot. Lysates of HAP1 WT and ARID2 KO were prepared, and 30 µg of protein were processed for western blot with the indicated ARID2 antibodies. The Ponceau stained transfers of each blot are presented to show equal loading of WT and KO lysates and protein transfer efficiency from the acrylamide gels to the nitrocellulose membrane. Antibody dilutions were chosen according to the recommendations of the antibody supplier. Antibody dilutions used: 82342** at 1/1000; PCRP-ARID2-1A1* at 1/100; GTX129443 at 1/500; GTX129444 at 1/500; GTX632011* at 1/500; 23406-1-AP at 1/500. Predicted band size: 197.4 kDa. ** = recombinant antibody, * = monoclonal antibody.

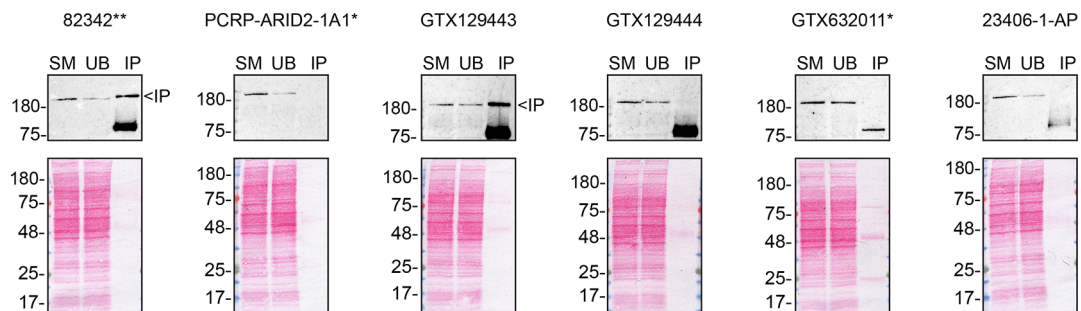


Figure 2. ARID2 antibody screening by immunoprecipitation. HAP1 lysates were prepared, and immunoprecipitation was performed for 1 h using 0.5 mg of lysate and 2.0 µg of the indicated ARID2 antibodies pre-coupled to Dynabeads protein A or protein G. Samples were washed and processed for western blot with the ARID2 antibody 82342** used at 1/200. The Ponceau stained transfers of each blot are shown. SM = 6% starting material; UB = 6% unbound fraction; IP = immunoprecipitate, ** = recombinant antibody, * = monoclonal antibody.

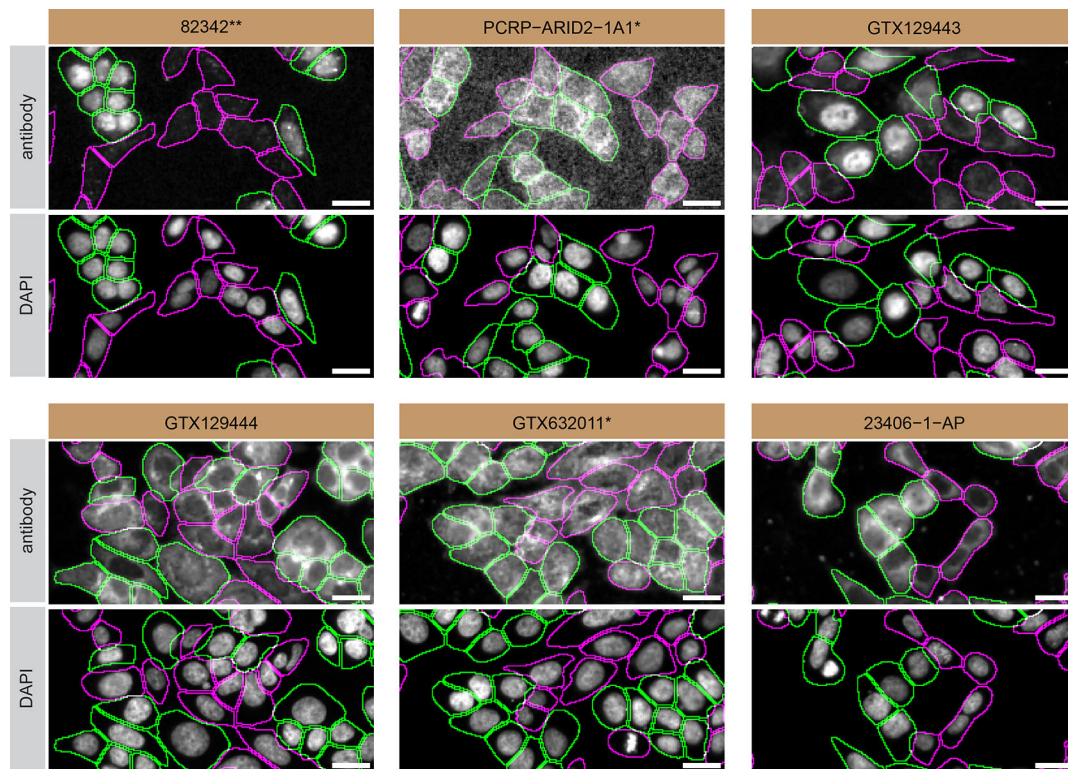


Figure 3. ARID2 antibody screening by immunofluorescence. HAP1 WT and *ARID2* KO cells were labelled with a green or a far-red fluorescent dye, respectively. WT and KO cells were mixed and plated to a 1:1 ratio on 96-well plate with optically clear flat-bottom. Cells were stained with the indicated ARID2 antibodies and with the corresponding Alexa-fluor 555 coupled secondary antibody including DAPI. Acquisition of the blue (nucleus-DAPI), green (WT), red (antibody staining) and far-red (KO) channels was performed. Representative images of the blue and red (grayscale) channels are shown. WT and KO cells are outlined with green and magenta dashed line, respectively. When an antibody was recommended for immunofluorescence by the supplier, we tested it at the recommended dilution. The rest of the antibodies were tested at 1 and 2 $\mu\text{g}/\text{ml}$, and the final concentration was selected based on the detection range of the microscope used and a quantitative analysis not shown here. Antibody dilutions used: 82342** at 1/100; PCRP-ARID2-1A1* at 1/100; GTX129443 at 1/1000; GTX129444 at 1/1000; GTX632011* at 1/500; 23406-1-AP at 1/100. Bars = 10 μm . ** = recombinant antibody, * = monoclonal antibody.

specificity of the other antibodies. All experiments are performed using master mixes, and meticulous attention is paid to sample preparation and experimental execution. In IF, the use of two different concentrations serves to evaluate antibody specificity and can aid in assessing assay reliability. In instances where antibodies yield no signal, a repeat experiment is conducted following titration. Additionally, our independent data is performed subsequently to the antibody manufacturers internal validation process, therefore making our characterization process a repeat.

Lastly, as comprehensive and standardized procedures are respected, any conclusions remain confined to the experimental conditions and cell line used for this study. The use of a single cell type for evaluating antibody performance poses as a limitation, as factors such as target protein abundance significantly impact results. Additionally, the use of cancer cell lines containing gene mutations poses a potential challenge, as these mutations may be within the epitope coding sequence or other regions of the gene responsible for the intended target. Such alterations can impact the binding affinity of antibodies. This represents an inherent limitation of any approach that employs cancer cell lines.

Method

The standardized protocols used to carry out this KO cell line-based antibody characterization platform was established and approved by a collaborative group of academics, industry researchers and antibody manufacturers. The detailed materials and step-by-step protocols used to characterize antibodies in western blot, immunoprecipitation and immunofluorescence are openly available on Protocols.io ([protocols.io/view/a-consensus-platform-for-antibody-characterization](https://www.protocols.io/view/a-consensus-platform-for-antibody-characterization)).³ Brief descriptions of the experimental setup used to carry out this study can be found below.

Cell lines and antibodies

The cell lines, primary and secondary antibodies used in this study are listed in Table 1, 2, and 3, respectively. To ensure consistency with manufacturer recommendations and account for proprietary formulations (where antibody concentrations are not disclosed), antibody usage is reported as dilution ratios rather than absolute concentrations. To facilitate proper citation and unambiguous identification, all cell lines and antibodies are referenced with their corresponding Research Resource Identifiers (RRIDs).^{8,9} All cell lines used in this study were regularly tested for mycoplasma contamination and were confirmed to be mycoplasma-free.

Antibody screening by western blot

HAP1 WT and *ARID2* KO cells were collected in RIPA buffer (25 mM Tris-HCl pH 7.6, 150 mM NaCl, 1% NP-40, 1% sodium deoxycholate, 0.1% SDS) (Thermo Fisher Scientific, cat. Number 89901) supplemented with 1x protease inhibitor cocktail mix (MilliporeSigma, cat. number P8340). Lysates were sonicated briefly and incubated 30 min on ice. Lysates were spun at $\sim 110,000 \times g$ for 15 min at 4 °C and equal protein aliquots of the supernatants were analyzed by SDS-PAGE and western blot. BLUelf prestained protein ladder (GeneDireX, cat. number PM008–0500) was used.

Western blots were performed precast midi 4–20% Tris-Glycine polyacrylamide gels (Thermo Fisher Scientific, cat. number WXP42012BOX) ran with Tris/Glycine/SDS buffer (Bio-Rad, cat. number 1610772), loaded in Laemmli loading sample buffer (Thermo Fisher Scientific, cat. number AAJ61337AD) and transferred on nitrocellulose membranes. Proteins on the blots were visualized with Ponceau S staining (Thermo Fisher Scientific, cat. number BP103–10) which is scanned to show together with individual western blot. Blots were blocked with 5% milk for 1 hr, and antibodies were incubated O/N at 4 °C with 5% milk in TBS with 0.1% Tween 20 (TBST) (Cell Signalling Technology, cat. number 9997). Following three washes with TBST, the peroxidase conjugated secondary antibody was incubated in TBST with 5% milk for 1 hr at room temperature followed by three washes with TBST. Membranes were incubated with Pierce ECL (Thermo Fisher Scientific, cat. number 32106) prior to detection with the iBright™ CL1500 Imaging System (Thermo Fisher Scientific, cat. number A44240).

Antibody screening by immunoprecipitation

Antibody-bead conjugates were prepared by adding 2 µg to 500 µl of Pierce IP Lysis Buffer from Thermo Fisher Scientific (cat. number 87788) in a microcentrifuge tube, together with 30 µl of Dynabeads protein A- (for rabbit antibodies) or protein G- (for mouse antibodies) (Thermo Fisher Scientific, cat. number 10002D and 10004D, respectively). Tubes were rocked for ~ 1 h at 4 °C followed by two washes to remove unbound antibodies.

HAP1 WT were collected in Pierce IP buffer (25 mM Tris-HCl pH 7.4, 150 mM NaCl, 1 mM EDTA, 1% NP-40 and 5% glycerol) supplemented with protease inhibitor. Lysates were rocked 30 min at 4 °C and spun at $110,000 \times g$ for 15 min at 4 °C. 0.5 ml aliquots at 1 mg/ml of lysate were incubated with an antibody-bead conjugate for ~ 1 h at 4 °C. The unbound fractions were collected, and beads were subsequently washed three times with 1.0 ml of IP buffer and processed for SDS-PAGE and western blot on precast midi 4–20% Tris-Glycine polyacrylamide gels.

Antibody screening by immunofluorescence

HAP1 WT and *ARID2* KO cells were labelled with a green and a far-red fluorescence dye, respectively (Thermo Fisher Scientific, cat. number C2925 and C34565). The nuclei were labelled with DAPI (Thermo Fisher Scientific, cat. Number D3571) fluorescent stain. WT and KO cells were plated on 96-well plate with optically clear flat-bottom (Perkin Elmer, cat. number 6055300) as a mosaic and incubated for 24 hrs in a cell culture incubator at 37 °C, 5% CO₂. Cells were fixed in 4% paraformaldehyde (PFA) (VWR, cat. number 100503-917) in phosphate buffered saline (PBS) (Wisent, cat. number 311–010-CL). Cells were permeabilized in PBS with 0.1% Triton X-100 (Thermo Fisher Scientific, cat. number BP151–500) for 10 min at room temperature and blocked with PBS with 5% BSA, 5% goat serum (Gibco, cat. number 16210–064) and 0.01% Triton X-100 for 30 min at room temperature. Cells were incubated with IF buffer (PBS, 5% BSA, 0.01% Triton X-100) containing the primary ARID2 antibodies overnight at 4 °C. Cells were then washed 3 $\times$ 10 min with IF buffer and incubated with corresponding Alexa Fluor 555-conjugated secondary antibodies in IF buffer at a dilution of 1.0 µg/ml for 1 hr at room temperature with DAPI. Cells were washed 3 $\times$ 10 min with IF buffer and once with PBS.

Images were acquired on an ImageXpress micro confocal high-content microscopy system (Molecular Devices), using a 20x NA 0.95 water immersion objective and scientific CMOS cameras, equipped with 395, 475, 555 and 635 nm solid state LED lights (lumencor Aura III light engine) and bandpass filters to excite DAPI, Cellmask Green, Alexa-555 and Cellmask Red, respectively. Images had pixel sizes of 0.68 $\times$ 0.68 microns, and a z-interval of 4 microns. For analysis and visualization, shading correction (shade only) was carried out for all images. Then, maximum intensity projections were generated using 3 z-slices. Segmentation was carried out separately on maximum intensity projections of Cellmask channels using CellPose 1.0, and masks were used to generate outlines and for intensity quantification.¹⁰ Figures were assembled with Adobe Illustrator.

Data availability

Underlying data

Zenodo: Dataset for the ARID2 antibody screening study <[10.5281/zenodo.19070027](https://doi.org/10.5281/zenodo.19070027)>.

Data are available under the terms of the [Creative Commons Attribution 4.0 International license \(CC-BY 4.0\)](https://creativecommons.org/licenses/by/4.0/).

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ABIF consortium: Claire M. Brown and Joel Ryan.

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Deborah Moshinsky 

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ARID2 contributes to the regulation of gene expression through interactions with DNA and nucleosomes. 6 ARID2 commercial antibodies were characterized by Western Blot, IP, and IF using standardized protocols against cells and lysates with wild type antigen in addition to those with the antigen knocked out. The results were clearly shown for the readers' interpretation. The authors purposely did not engage in result interpretation, but rather left the results for the reader to use in addition to guidance on result interpretation. These data are useful in determining which commercial antibodies are active under the conditions tested.

Is the rationale for creating the dataset(s) clearly described?

Yes

Are the protocols appropriate and is the work technically sound?

Yes

Are sufficient details of methods and materials provided to allow replication by others?

Yes

Are the datasets clearly presented in a useable and accessible format?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Antibody Characterization and Validation

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 13 May 2026

<https://doi.org/10.5256/f1000research.197872.r479994>

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The study involved characterisation of six commercial antibodies, from four different companies, for the AT-rich interactive domain-containing protein 2 (ARID2) protein for use in western blot, immunoprecipitation, and immunofluorescence. The study formed part of a wider YCharOS collaboration to characterise commercial antibodies, which are renewable (recombinant and monoclonal), for human proteins using standardised protocols with the intention of improving antibody reproducibility issues. As a Data Note, it allows the direct comparison of the performance of commercially available antibodies to the ARID2 protein to aid scientists in choosing the most appropriate antibody for their studies. For example, in understanding how disease-related mutations affect the protein's biochemical and cellular characteristics. This comparison would be of particular use in the study of chromatin architecture and genomic stability, as well as the study of cancer and neurodevelopmental disorders.

A committee of industry and academic representatives have endorsed the platform used, and the protocols are consistent with those in general use. The platform consisted of identification of a human cell line suitable for antibody characterisation studies i.e., with adequate levels of the ARID2 protein to generate a measurable signal. Using the DepMap (Cancer Dependency Map Portal, RRID:SCR_017655) transcriptomics database, they found that the commercially available HAP1 WT cell line expresses *ARID2* transcript at a level above the average range for cancer cells analysed, and therefore, was a logical choice for their study. Knockdown of the target gene in HAP1 cells was used to provide an appropriate negative control HAP1 cell line (*ARID2* KO). The final step was a series of antibody characterisation procedures, limited to the most common research uses of antibodies. The interpretation of the results is left to the reader, with the study providing unbiased guidance. However, it gave some indication of the optimum dilutions of commercial antibodies. Limitations of the study are clearly stated.

It would be useful to know if any of the antibodies bound to mutant versions of ARID2. Since only six antibodies were studied, perhaps a comparison of the antibodies with other cell lines with mutations in *ARID2* could have been added to determine effects on antibody-epitope binding.

Is the rationale for creating the dataset(s) clearly described?

Yes

Are the protocols appropriate and is the work technically sound?

Yes

Are sufficient details of methods and materials provided to allow replication by others?

Yes

Are the datasets clearly presented in a useable and accessible format?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Anti-cancer drug discovery and development. Diagnostics.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

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