



# ARID1A and CEBP $\alpha$ cooperatively inhibit UCA1 transcription in breast cancer

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## Abstract

As one of the primary members of SWI/SNF chromatin remodeling complexes, ARID1A contains frequent loss-of-function mutations in many types of cancers. However, the molecular mechanisms underlying ARID1A deficiency in cancer biology remain to be investigated. Using breast cancer as a model, we report that silencing ARID1A significantly increased cellular proliferation and migration. Mechanistically, primarily functioning as a transcriptional repressor, loss of ARID1A profoundly alters histone modifications and the transcriptome. Notably, ARID1A inhibited the expression of a long non-coding RNA, *UCA1*, by regulating chromatin access of the transcription factor CEBP $\alpha$ . Restoration experiments showed that *UCA1* mediates the functions of ARID1A that induces loss of cellular proliferation and migration. Together, our findings characterize *ARID1A* as a key tumor-suppressor gene in breast cancer through cooperation with *CEBP $\alpha$* , and loss-of-function mutations of ARID1A activates *UCA1*.

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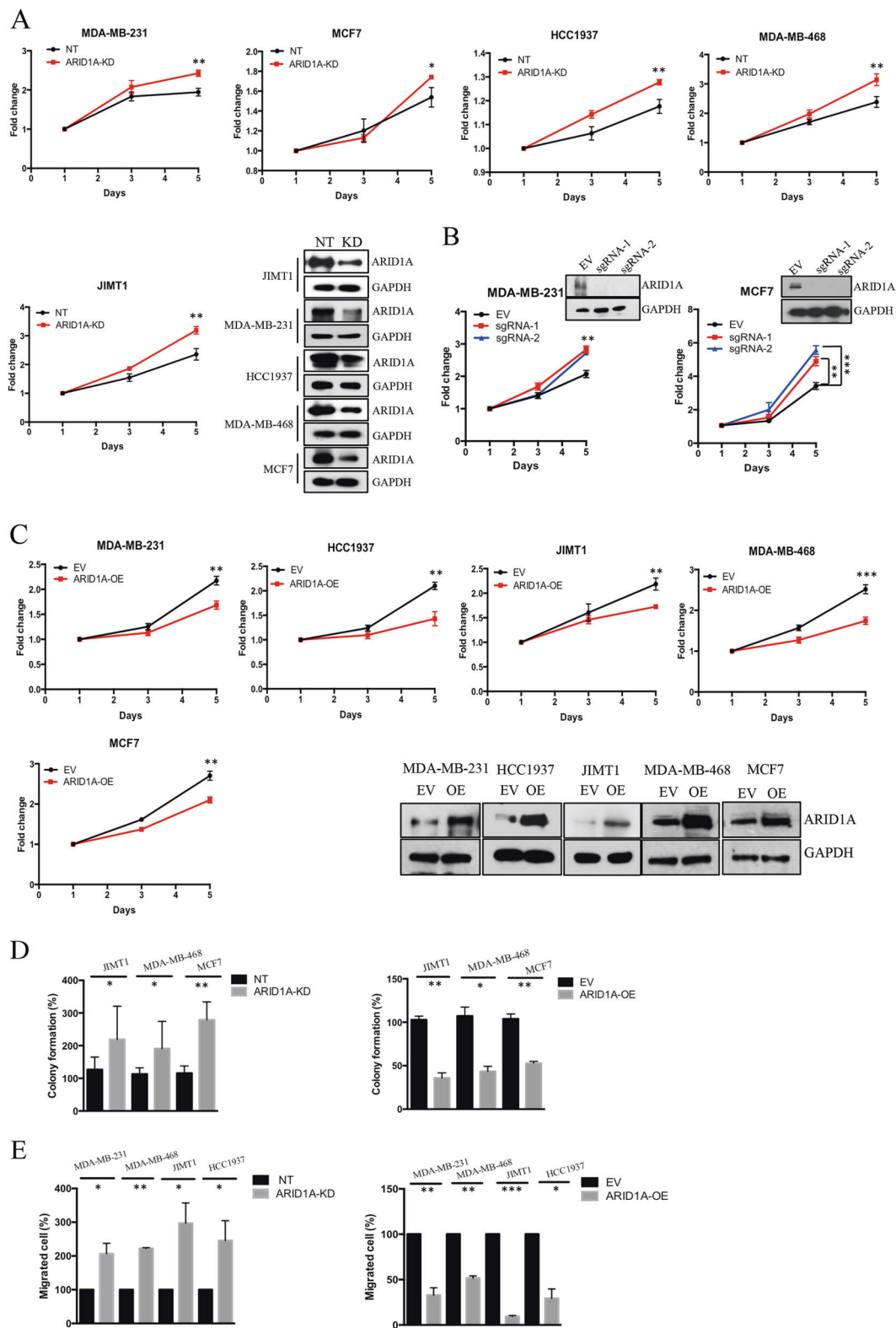
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## Introduction

Globally, breast cancer is the most frequently diagnosed malignancy and the leading cause of cancer-associated death in women. In 2015, 231,840 new cases of invasive breast cancer were diagnosed in America [1]. Therefore, to elucidate its biology and pathogenesis is important for the development of more innovative and effective therapeutic modalities. SWI/SNF complexes regulate gene expression program by remodeling chromatin structure, more specifically, the package of DNA. *ARID1A* is a key component of the multiprotein SWI/SNF complexes, which is critical for differentiation, proliferation, DNA repair, and tumor suppression [2, 3].

Notably, somatic mutations targeting *ARID1A* are found at high frequency in many different cancer types, such as those originating from ovary [4, 5], endometrium [6, 7], cervix [8], and breast cancers [9, 10]. These mutations were associated with loss of ARID1A expression. In addition, cancer patients had reduced survival with loss of ARID1A, suggesting that ARID1A may play a role in the biology of cancer cells [8, 11–13]. The aim of this study was to explore the functions of ARID1A in breast cancer and its mechanisms of action.



**Fig. 1** ARID1A inhibits the proliferation and migration of breast cancer. **a** Short-term proliferation of breast cancer cell lines following ARID1A knockdown by siRNA. Western blots showing the knockdown effect of ARID1A. **b** Short-term proliferation of MDA-MB-231 and MCF7 cell lines following *ARID1A* disruption by CRISPR/Cas9 System. **c** Short-term proliferation of breast cancer cell lines stably

over-expressing ARID1A. Western blots showing the overexpression effect of ARID1A. **d** Soft agar colony formation of breast cancer cell lines following ARID1A knockdown by siRNA and ARID1A overexpression. **e** Migration assays of breast cancer cell lines following ARID1A knockdown by siRNA and ARID1A overexpression. Data show mean  $\pm$  s.d.  $N = 3$ . \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$

## Results

### ARID1A inhibits the proliferation and migration of breast cancer cells

To explore the biological functions of ARID1A in breast cancer, ARID1A was silenced by small interfering RNA (siRNA), and cell proliferation was measured by 3-(4, 5-dimethylthiazol-2-yl) -2,5-diphenyltetrazolium bromide (MTT). ARID1A knockdown promoted proliferation of three triple-negative cell lines (MDA-MB-231, HCC1937, MDA-MB-468), one estrogen receptor-positive cell line (MCF7) and JIMT1, which is HER2-positive, indicating that the biological functions of ARID1A are independent of breast cancer subtypes (Fig. 1a). Consistently, ARID1A-silencing enhanced the growth of colonies in soft agar; conversely, overexpression of ARID1A decreased colony formation in soft agar (Fig. 1d). These loss-of-function results were further confirmed by CRISPR/Cas9-mediated gene editing (Fig. 1b). Moreover, the inhibition of proliferation of ARID1A was evident in gain-of-function assays (Fig. 1c). Loss of ARID1A in HCT116 cells (a colon cancer cell line) promoted cell migration and invasiveness [14]. We thus performed transwell migration assay and found that ARID1A potently inhibited cell migration in multiple breast cancer cell lines (Fig. 1e). These results strongly suggest that *ARID1A* is a tumor-suppressor gene in breast cancer in vitro.

### Transcriptome analysis of ARID1A-regulated genes and cellular processes

The SWI/SNF complexes regulate chromatin remodeling, and thus loss of ARID1A might affect gene transcription program in cancer cells. To gain insights into the molecular mechanisms underlying the tumor-suppressive effect of ARID1A, RNA-seq was performed in isogenic MCF10A cells (a nonmalignant mammary cell line) with intact (wild type (WT)) or truncated (knockout (KO)) ARID1A. This truncation was generated by introducing a biallelic and premature stop codon (Q456\*) through CRISPR/Cas9 approach (See Methods). Loss of full-length protein expression of ARID1A was confirmed by western blot (Supplementary Fig. 1) and led to significant inhibition of the established ARID1A targets (as described in the Results), suggesting that this truncating mutation ablates the function of ARID1A.

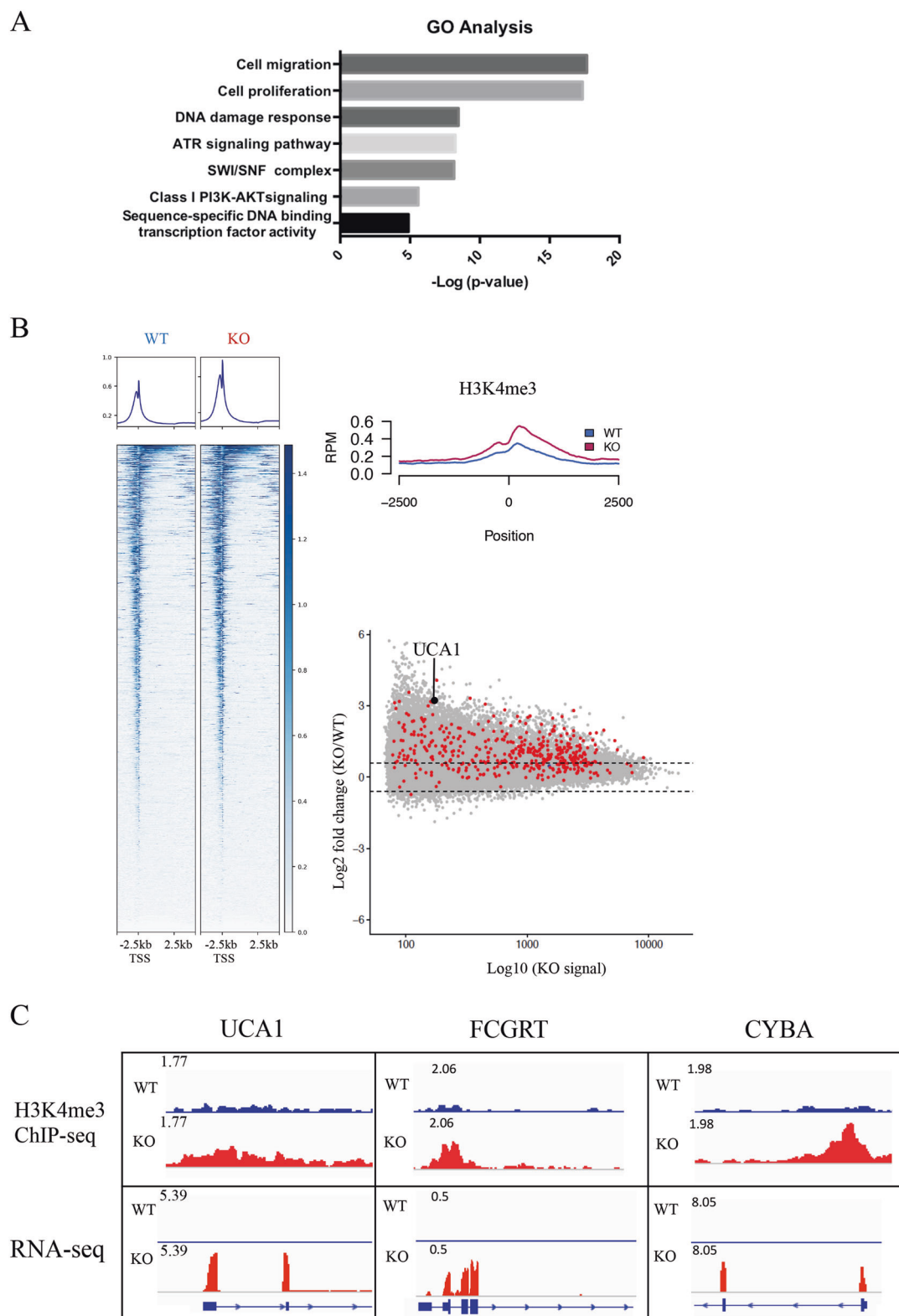
A total of 1542 and 580 transcripts (fold change >2) were upregulated and downregulated upon ARID1A truncation, respectively, indicating that ARID1A loss profoundly altered the transcriptome and that *ARID1A* primarily functioned as a transcriptional repressor. A number of important cancer-driver genes were regulated by ARID1A. For

example, *CDKN1A* and *SMAD3* mRNA levels decreased in MCF10A KO cells, congruent with earlier findings [15, 16]. Other key cancer genes such as *NFKBIA*, *FGFR4*, and *GDF15* were all significantly regulated following ARID1A loss (Supplementary Fig. 2). Gene ontology and pathway analysis suggested that ARID1A loss-of-function affected many important processes in cancer biology, including cell migration, cell proliferation, DNA damage response, and SWI/SNF complexes. In agreement with previous reports, ARID1A regulates ATR signaling pathway [17], phosphoinositide-3 kinase-AKT signaling [18], and transcription factor activity (Fig. 2a).

A recent study in colon cancer suggested that ARID1A-depletion promoted aberrant activation of distal enhancers rather than promoters [14]. To probe the mechanisms underlying the changes in breast transcriptome upon ARID1A truncation, we measured epigenomic changes by performing chromatin immunoprecipitation–sequencing (ChIP-seq) analysis using antibodies against H3K27ac (predicative of active enhancers and promoters) and H3K4me3 (predicative of active promoters). In contrast with the findings in colon cancers, we did not observe significant changes in H3K27ac cistrome upon loss of ARID1A (Supplementary Fig. 3). Instead, compared to WT cells, markedly increased H3K4me3 signals were found in a total of 330 genomic segments, whereas only 91 regions showed decreased H3K4me3 modification (fold change >2) (Fig. 2b). This result was consistent with the above transcriptome analysis showing that upregulated genes outnumbered the downregulated ones and further suggested that ARID1A predominantly operates as a repressor of gene transcription.

We next attempted to identify key downstream targets of ARID1A in breast cancer. In order to pinpoint direct transcriptional target, we focused on the transcripts with consistent changes in mRNA expression and H3K4me3 modification, which revealed a total of 108 candidate downstream targets of *ARID1A*. Three top target genes (*UCA1*, *FCGRT*, *CYBA*) were chosen, whose expression as well as promoter-binding peaks increased in ARID1A KO cell (Fig. 2c).

Based on these criteria, *UCA1* ranked No.1, which is a well-known oncogenic long non-coding RNA (LncRNA) upregulated in several tumor types, including breast cancer [19], acute myeloid leukemia [20], and bladder [21] as well as gastric cancers [22]. Thus we speculate that loss of ARID1A might promote breast cancer through negative regulation of *UCA1*. Real-time polymerase chain reaction (PCR) analysis confirmed that *UCA1* expression level was elevated in ARID1A-depleted cells (Fig. 3a). The other two top-ranked candidate targets, *FCGRT* and *CYBA*, were also inhibited by ARID1A, consistent with the RNA-seq data (Supplementary Fig. 4A).



**Fig. 2** Transcriptome analysis of ARID1A-regulated genes and processes. **a** GO analysis of ARID1A-regulated genes measured by RNA-seq comparing WT and ARID1A KO MCF10A cells. **b** ChIP-seq assays of H3K4me3 for WT and ARID1A KO cells. Heatmap depicting signals of H3K4me3 (left). Composite plot showing the

average levels of H3K4me3 intensity (top right). Red dots highlighting upregulated genes with concomitantly increased H3K4me3 modifications at their TSSs following ARID1A truncation (right bottom). **c** Signal tracks of H3K4me3 and RNA-seq for top 3 ARID1A-regulated candidates

## ARID1A directly binds to *UCA1* promoter and reduces its transcription activity

Given the above results, ability of ARID1A directly to bind to the promoter of *UCA1* was examined. ChIP–quantitative PCR (qPCR) analysis revealed the association of ARID1A with the *UCA1* promoter in different breast cell lines (Fig. 3b). Furthermore, in line with H3K4me3 ChIP-seq data obtained in nonmalignant MCF10A cells, this histone modification in *UCA1* promoter was also significantly stronger in breast cancer cell lines following ARID1A knockdown (Fig. 3c). These data suggest that direct binding of ARID1A to *UCA1* promoter reduces H3K4me3 levels and decreases the transcription activity of *UCA1*.

## ARID1A inhibits breast cancer malignancy via suppressing *UCA1* expression

*UCA1* has been observed to promote growth of MCF7 cells [19]. To confirm the role of this lncRNA in breast cancer and, more importantly, to relate its function with ARID1A, we first silenced *UCA1* in a panel of breast cancer cell lines. Indeed, *UCA1* depletion prominently decreased both proliferation (Fig. 4a and Supplementary Fig. 6A) and migration (Supplementary Fig. 6B) of breast cancer cells. Importantly, enhanced cell proliferation, colony formation, and migration conferred by loss of ARID1A was reversed by silencing of *UCA1* (Fig. 4b–d). On the other hand, reduced cell proliferation upon ectopic expression of ARID1A was rescued by *UCA1* overexpression (Fig. 4e).

To examine further the roles of *UCA1* and ARID1A in breast cancer in vivo, we ectopically expressed *UCA1* and ARID1A either individually or together (Supplementary Fig. 7A) and monitored the growth of the xenografts formed by these stable clones in female nude mice. Strongly corroborating our original in vitro results, overexpression of ARID1A alone significantly reduced tumor growth, while *UCA1* overexpression produced the opposite effect (Fig. 4f). Importantly, *UCA1* overexpression reversed the growth-inhibitory effect produced by ARID1A, again supporting our in vitro data (Supplementary Fig. 7B). Collectively, these results strongly suggest that the tumor-suppressive function of ARID1A is at least partially mediated by *UCA1* in breast cancer.

With respect to the other two candidate targets (FCGRT and CYBA), only a limited number of reports showed their potential relevance in tumor biology [23, 24], and their roles in breast cancer are unknown. We thus next explored their biological functions as well as their relationships with ARID1A in the context of breast cancer. Knockdown of either CYBA or FCGRT potentially inhibited cellular proliferation (Supplementary Fig. 4C), migration (Supplementary Fig. 4D), and colony formation (Supplementary

Fig. 4E). More importantly, depletion of FCGRT and CYBA partially rescued the cellular phenotypical changes in ARID1A-deficient cell lines (Supplementary Fig. 4C,D, E). Collectively, these results identified both CYBA and FCGRT as novel oncogenic factors in breast cancer cells, which are under the transcriptional control of ARID1A.

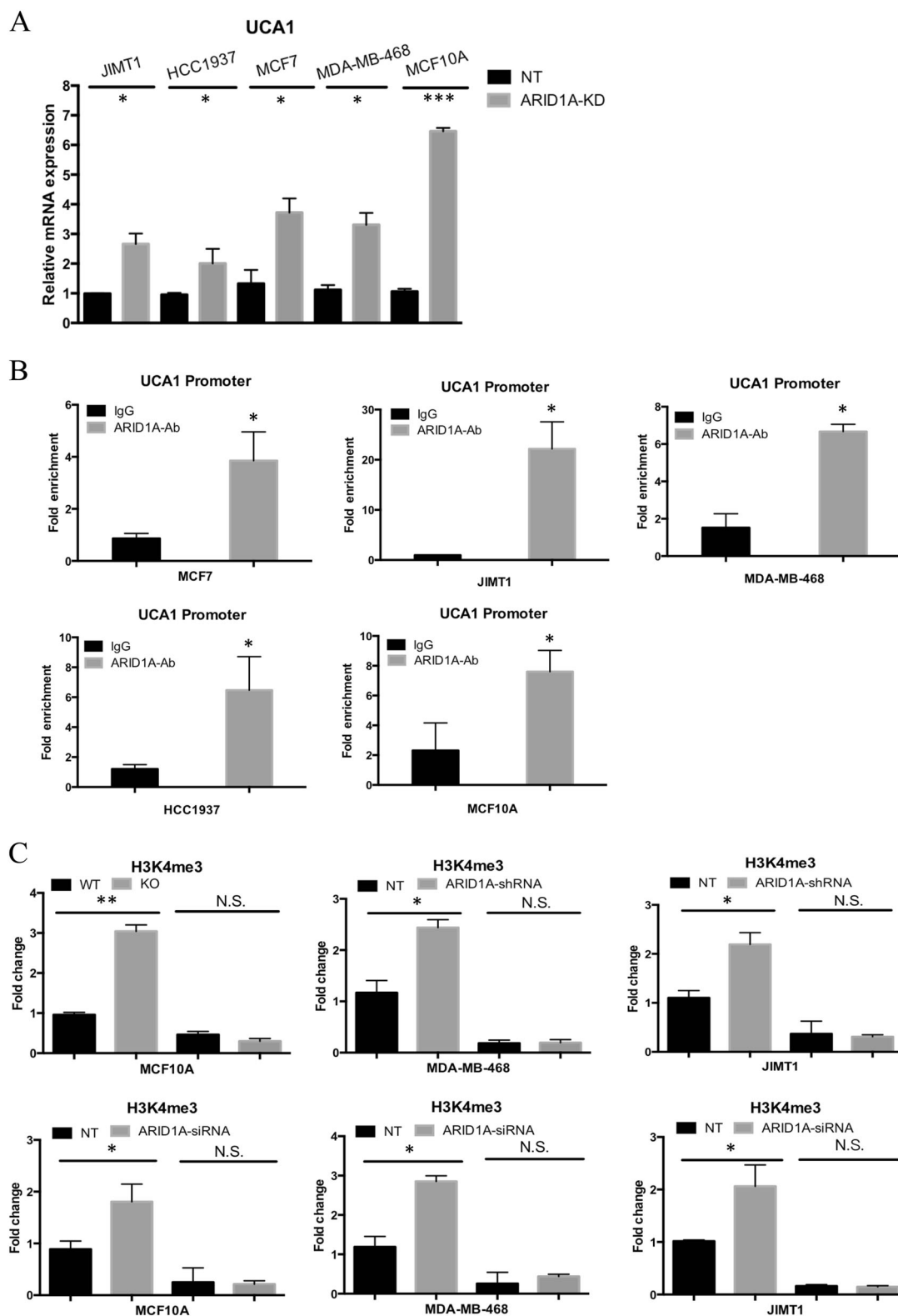
## ARID1A and CEBP $\alpha$ collaboratively suppress the transcription of *UCA1*

SWI/SNF complexes often cooperate with transcription factors in the regulation of gene expression program. To identify such transcription factors collaborating with ARID1A in breast cancer, we first performed gene set enrichment analysis (GSEA) to interrogate the transcriptome changes upon loss of ARID1A. Notably, ARID1A-regulated gene set was strikingly enriched in genes whose expression levels were altered by CEBP $\alpha$  (Fig. 5a), Tp63, and nuclear factor- $\kappa$ B (data not shown). Among these candidate transcription factors, CEBP $\alpha$  was of particular interest, since ARID1A was observed to interact with CEBP $\alpha$  during liver regeneration [25]. In addition, CEBP $\alpha$  was reported to bind the *UCA1* promoter in leukemia cells [20]. Supporting the GSEA results, the direction and degree of alterations of potential transcriptional targets of CEBP $\alpha$  were remarkably correlated in two datasets (Fig. 5b). qPCR results showed that *UCA1* mRNA was enhanced by knockdown of CEBP $\alpha$  in breast cancer cells (Fig. 5c). Importantly, ChIP-seq results of key SWI/SNF factors (SMARCC1 and SMARCA4, often used as surrogates for determination of the genome-wide-binding patterns of SWI/SNF complexes [14]), demonstrated co-occupancy of both the SWI/SNF complexes and CEBP $\alpha$  at the *UCA1* promoter in different cell line models, including a chronic myeloid leukemia line, K562 cells (Fig. 5d). We further performed ChIP-qPCR in K562 cells and identified the binding of ARID1A on the promoter of *UCA1* (Supplementary Fig. 10). These results suggest that this binding pattern is not unique to breast cancer.

Moreover, ChIP-qPCR results showed that silencing of ARID1A reduced the binding of CEBP $\alpha$  to the *UCA1* promoter (Fig. 5e); on the other hand, association of ARID1A with *UCA1* promoter was weakened by CEBP $\alpha$  depletion (Fig. 5f). In addition, we performed either single or double knockdown experiments and found that *UCA1* was not further upregulated by co-silencing of ARID1A and CEBP $\alpha$  (Supplementary Fig. 11). These data together suggest that ARID1A and CEBP $\alpha$  negatively regulate *UCA1* transcription in a collaborative manner, thereby suppressing the proliferation and migration of breast cancer cells.

To probe whether ARID1A and CEBP $\alpha$  could regulate the expression of each other, we performed western blot.

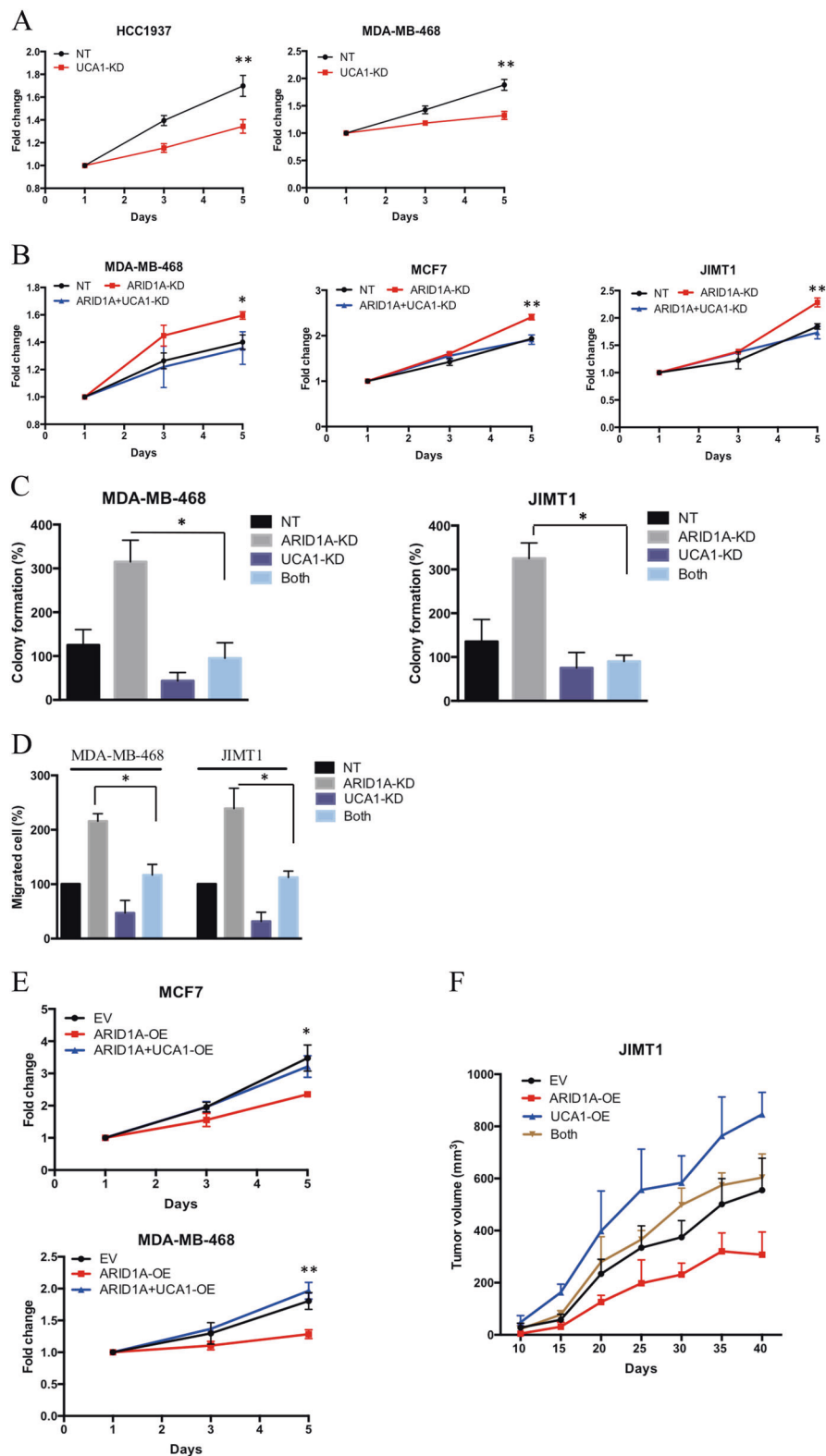




**Fig. 3** ARID1A directly binds to UCA1 promoter and reduces its transcription activity. **a** qRT-PCR analysis of UCA1 mRNA expression in breast cell lines following ARID1A knockdown by siRNA. **b** ChIP-qPCR analysis of ARID1A-binding affinity in UCA1 promoter. This result was confirmed by a second primer set

(Supplementary Fig. 5). **c** ChIP-qPCR analysis of H3K4me3-binding affinity in UCA1 promoter following ARID1A knockdown by shRNA and siRNA. N.S. non-specific primer as negative control. Data show mean  $\pm$  s.d.  $N = 3$ . \* $P < 0.05$ , \*\* $P < 0.01$

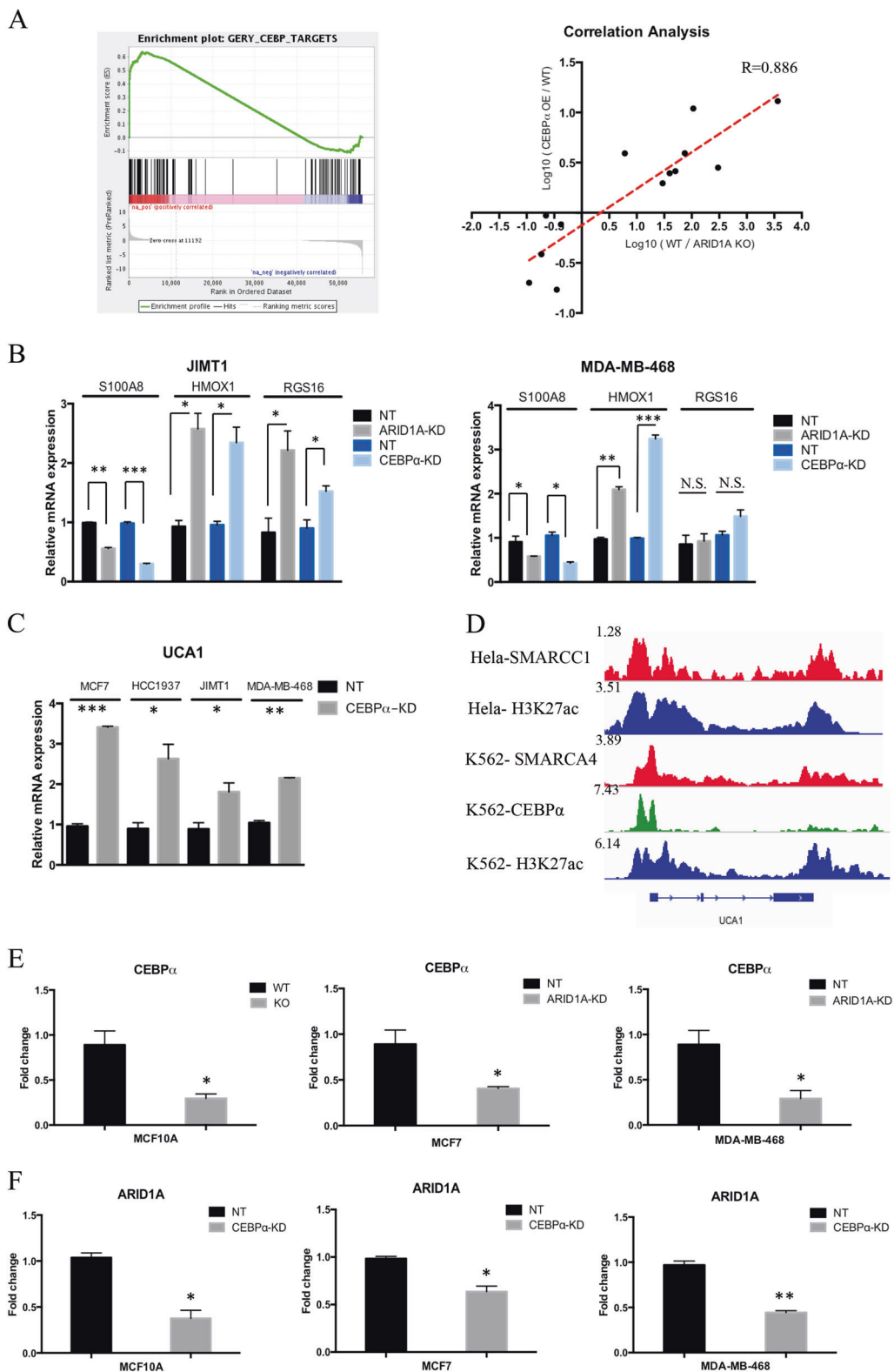
**Fig. 4** ARID1A inhibits breast cancer malignancy via suppressing UCA1 expression. **a** Short-term proliferation assays of breast cancer cell lines following UCA1 knockdown by siRNA. **b** Short-term proliferation assays, **c** colony-formation assays, and **d** migration assays of breast cancer cells following either ARID1A/UCA1 knockdown alone or dual knockdown. **e** Short-term proliferation assay of breast cancer cell lines stably expressing either ARID1A or UCA1 individually or together. **f** JIMT1 cells with overexpression of either ARID1A or UCA1 individually or together were injected into female nude mice. Tumor growth was measured every 5 days beginning 10 days after the injection. Data show mean  $\pm$  s.d.  $N = 3$ . \* $P < 0.05$ , \*\* $P < 0.01$



Depletion of ARID1A did not affect the level of CEBP $\alpha$ , and vice versa. This indicates that the cooperativity between ARID1A and CEBP $\alpha$  was not due to the changes of their protein amount (Supplementary Fig. 12).

### Clinical implications of the expression of ARID1A, CEBP $\alpha$ , and UCA1

Given the above findings, we queried The Cancer Genome Atlas (TCGA) breast cancer dataset ( $n = 1247$ ) to determine



the expression patterns between ARID1A/CEBP $\alpha$  and their downstream target genes. Importantly, in ARID1A-high tumor samples, all three target genes identified in the present study (UCA1, CYBA, and FCGRT) had significantly

lower mRNA abundance compared with ARID1A-low tumors (Fig. 6a, subpanels a–c). Furthermore, CEBP $\alpha$ -high breast tumors expressed lower levels of UCA1



◀ **Fig. 5** ARID1A inactivates UCA1 transcription in cooperation with CEBP $\alpha$ . **a** (Left) GSEA plot showing enrichment of ARID1A induced genes in CEBP $\alpha$  gene signatures. (Right) Expression changes of selected genes upon either CEBP $\alpha$ -overexpression (OE) or ARID1A truncation (PTX3, DCN, CXCL1, S100A8, SH3GL3, CRABP2, RGS2, HMOX1, RGS16, ALDH3A1, SERPINB2, GLRX1, EREG). **b** qRT-PCR analysis of representative genes (S100A8, HMOX1, and RGS16) following knockdown of either ARID1A or CEBP $\alpha$  by siRNA. **c** qRT-PCR analysis of UCA1 mRNA in breast cancer cell lines following CEBP $\alpha$  knockdown by siRNA. **d** ChIP-seq profiles of CEBP $\alpha$ , SMARCC1, SMARCA4, and H3K27ac in UCA1 locus and flanking regions. **e** ChIP-qPCR analysis of CEBP $\alpha$ -binding affinity in UCA1 promoter following ARID1A knockdown by shRNA. **f** ChIP-qPCR analysis of ARID1A-binding affinity in UCA1 promoter following CEBP $\alpha$  knockdown by siRNA. Data show mean  $\pm$  s.d.  $N = 3$ . \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , N.S. not significant

transcript than CEBP $\alpha$ -low samples (Fig. 6a, subpanel d), supporting our *in vitro* findings.

To explore the clinical relevance of altered expression of ARID1A, CEBP $\alpha$ , and UCA1 in terms of survival of breast cancer patients, we re-analyzed the survival data from TCGA and a series of Gene Expression Omnibus datasets. Although we did not see significant differences in TCGA cohorts, the Kaplan–Meier analysis based on GSE42568 ( $n = 104$ ) [26] revealed that low expression of either ARID1A or CEBP $\alpha$  were significantly correlated with poor survival of breast cancer patients (Fig. 6b). High expression of UCA1 was also associated with poor survival of these patients, but it did not reach statistical significance. These results together suggested the clinical significance of these genes and further corroborated our *in vitro* findings of the tumor-suppressive function of ARID1A and CEBP $\alpha$  and oncogenic property of UCA1.

## Discussion

This study demonstrates an inter-dependency between ARID1A and CEBP $\alpha$  in the regulation of lncRNA UCA1 expression, which results in the suppression of the malignant phenotype of breast cancer cells. Recently, data showed that loss of ARID1A expression decreased protein expression of other SWI/SNF family members, reshaping the chromatin landscape that quantitatively restricted access of transcription factors [27]. Thus, in addition to itself, ARID1A loss may affect the transcriptome by de-regulating other interactions between chromatin remodelers and transcription factors. A previous report suggested that the interaction between CEBP $\alpha$  and the SWI/SNF chromatin-remodeling complexes is critical for CEBP $\alpha$ -induced growth arrest in murine cell system [28]. Although we failed to detect direct protein–protein interaction between ARID1A and CEBP $\alpha$  in breast cells (data not

shown), our ChIP-qPCR results suggest a functional cooperation of ARID1A and CEBP $\alpha$  in the regulation of UCA1 transcription.

We attempted but failed to map genome-wide occupancy profile of ARID1A in breast cancer cells, due to the lack of suitable antibodies. Alternatively, we analyzed ChIP-seq data of SMARCC1 and SMARCA4, which can serve as surrogate markers for ARID1A-containing SWI/SNF complexes, as shown by previous studies [14]. We also analyzed publically available CEBP $\alpha$  genome-wide binding data, which strongly supported that CEBP $\alpha$  and ARID1A bind to the promoter region of UCA1. Finally, we mapped key histone modifications including H3K27ac and H3K4me3 on WT and ARID1A KO MCF10A cells. Unlike recent findings in colon cancer cells, we found that ARID1A affected the activation of gene promoters instead of enhancers.

A recent report noted that re-expression of ARID1A in T47D breast cell line inhibited colony formation in soft agar, in line with our current findings [29]. However, ARID1A<sup>-/-</sup> HCT-116 colon cancer cells exhibited unaltered proliferation rate but increased invasiveness [14], indicating cell-type-specific functions of ARID1A. In our *in vitro* models, tumor-suppressive properties of ARID1A were independent of the status of hormone receptors or HER2, which was compatible with a study showing that ARID1A expression was similar across different subtypes of breast cancers [30].

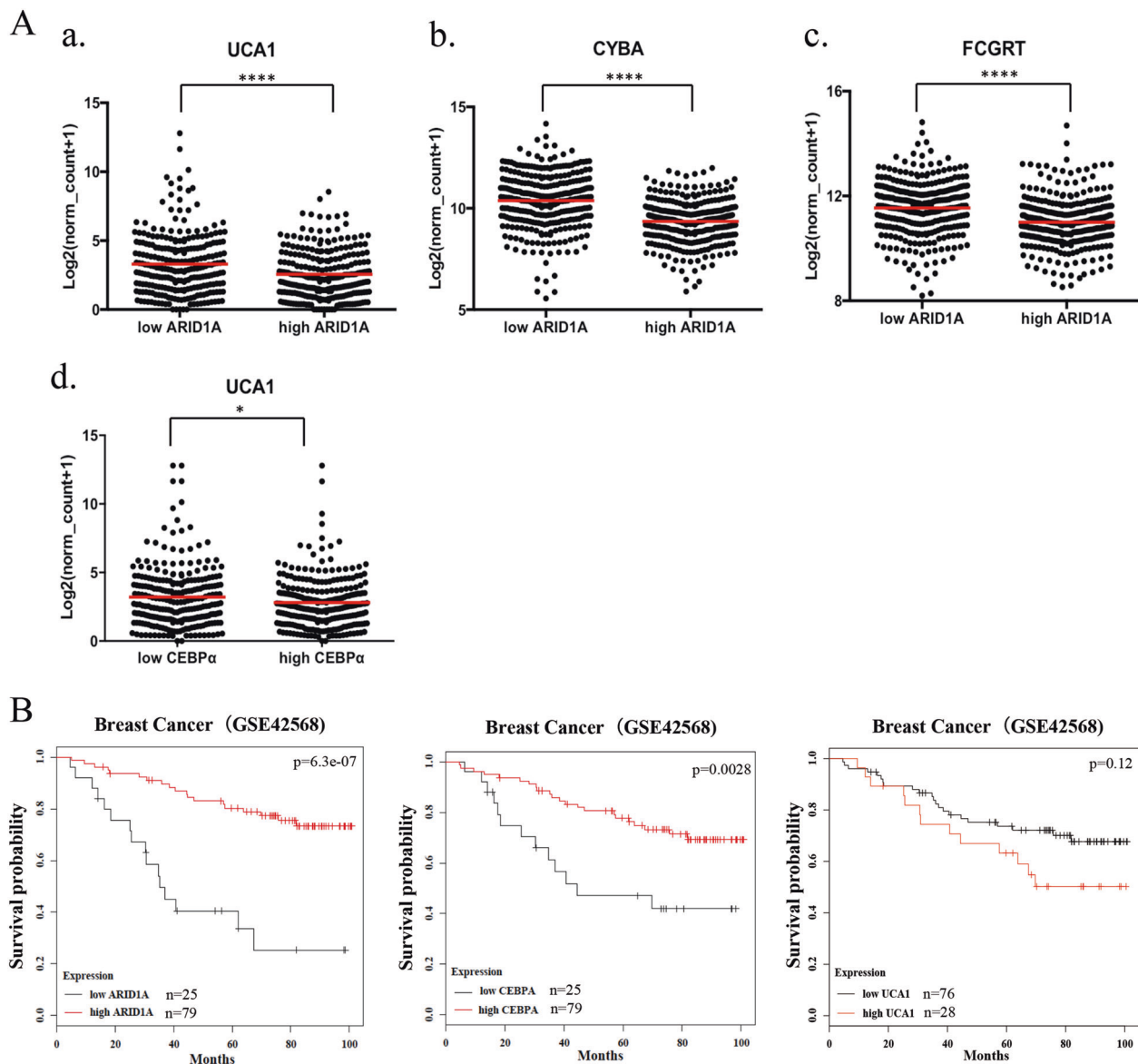
Furthermore, we observed prognostic value of ARID1A, CEBP $\alpha$ , and UCA1 levels in breast cancer patients (Fig. 6b). UCA1 has been reported to enhance chemoresistance in a broad range of human tumors [31–33], including tamoxifen resistance in MCF7 cell lines [34]. Thus UCA1 appears more than just a prognostic factor but is capable of mediating chemotherapeutic response, ultimately leading to a worse outcome.

In summary, the present study demonstrates that ARID1A inhibits the growth of breast cancer cells *in vitro* and *in vivo*. Through a series of mechanistic studies, UCA1, FCGRT, and CYBA were identified as functional downstream factors of ARID1A. These results shed light on the understanding of the transcriptional dysregulation and chromatin remodeling in this malignancy, with potential clinical implications given the prognostic value of ARID1A, CEBP $\alpha$ , and UCA1.

## Materials and methods

### Reagents and antibodies

For details, please refer Supplementary information Table 1.



**Fig. 6** The clinical implications of the expression of ARID1A, CEBP $\alpha$ , and UCA1. **a** Dot plots showing negative correlation between ARID1A and UCA1 (a), CYBA (b), and FCGRT (c), as well as CEBP $\alpha$  and UCA1 (d). Gene expression levels (log<sub>2</sub>) were retrieved

from RNA-seq data from TCGA. **b** Survival analysis of indicated breast cancer cohorts based on the expression levels of ARID1A, CEBP $\alpha$ , and UCA1

## Cell culture

Isogenic WT and ARID1A truncated (KO) MCF10A cell line (contains biallelic stop-gain mutations, Q456\*/Q456\*, targeting *ARID1A*) were purchased from Horizon Discovery (HD 101-022), and they were cultured in Dulbecco's modified Eagle's medium (DMEM)/Ham's F12 mixture supplemented with 5% horse serum, epidermal growth factor (20 ng/mL), hydrocortisone (0.5 mg/mL), insulin (10  $\mu$ g/mL), and cholera toxin (100 ng/mL) at 37 °C in 5% CO<sub>2</sub> atmosphere. Breast cancer cell lines MDA-MB-231, JIMT1, and MDA-MB-468 were cultured in DMEM; HCC1937 and MCF7 were cultured in RPMI-1640.

## Cell proliferation assay

Cells were seeded into a 96-well plate at 3000 cells/well and cultured in 1% fetal bovine serum (FBS) medium for the indicated time courses. Cell viability was assessed using the MTT method as we have described previously [35]. In brief, 10  $\mu$ L of 5 mg/mL MTT solution was added into each well followed by 4 h incubation in a humidified chamber, which was stopped by adding 100  $\mu$ L of 10% sodium dodecyl sulfate (SDS). Samples were mixed thoroughly until the formazan was dissolved and then read absorbance at 570 nm.

## Transfections, viral particle production, and infection

DNA and siRNA transfections were performed using BioT (Bioland Scientific, Paramount, CA, USA) and Lipofectamine RNAiMAX (Invitrogen, Carlsbad, CA, USA), respectively. Lentiviral particles were produced with the MISSION Lentiviral Packaging System (Sigma-Aldrich, St. Louis, MO, USA). Cells were transfected with the lentiviral particles in the presence of 8  $\mu$ g/mL polybrene (Sigma-Aldrich) for 48 h. Two days after infection, puromycin (1  $\mu$ g/mL) was added for 48–72 h to eliminate uninfected cells.

## Immunoblotting

Protein lysates were extracted using M-PER Mammalian Cell Lysis Reagent (Thermo Scientific, Waltham, MA, USA), and protein concentrations were determined by BCA assay (Thermo Scientific). Protein lysates were resolved by SDS-polyacrylamide gel electrophoresis, transferred to polyvinylidene difluoride membrane (Merck Millipore, Temecula, CA, USA), and followed by immunoblotting procedures as previously described [36].

## ChIP assay

ChIP was performed as described previously [37]. Briefly, formaldehyde was used to cross-link proteins to DNA. Cells were lysed with lysis buffer (NaCl 8.766 g, 0.5 M EDTA pH 7.5 10 mL, 1 M Tris pH 7.5 50 mL, NP-40 5 mL, H<sub>2</sub>O up to 1 L), followed by centrifuge at 12,000  $\times g \times 1$  min to aspirate the supernatant. In all, 120  $\mu$ L of cold buffer (20% SDS 50 mL, 0.5 M EDTA pH 8.0 20 mL, 1 M Tris pH 8.0 50 mL, H<sub>2</sub>O up to 1 L) was added to the nuclear pellet for shearing in microTUBE (Covaris, Woburn, MA, USA). Immunoprecipitation was performed using the indicated antibodies. Five microgram of primary antibody per 10 million cells was incubated with precleared chromatin for 16 h, followed by the addition of Dynabeads protein G (Life Technology, Grand Island, NY, USA). Immunoprecipitates were washed with increasing stringency in Lysis buffer and TE buffer. Bound DNA was eluted by the buffer containing 1% SDS and 0.1 M NaHCO<sub>3</sub>, reverse-crosslinked overnight at 65  $^{\circ}$ C, and purified using a QIAquick PCR Purification Kit (Qiagen, Valencia, CA, USA).

## Transwell cell migration assay

The lower chambers were filled with 500  $\mu$ L of DMEM medium containing 10% FBS. The mixture of indicated cells in 200  $\mu$ L of migration medium was added into each

top chamber, which is a 6.5-mm Transwell with an 8.0- $\mu$ m pore polycarbonate membrane insert (Corning, Kennebunk, ME, USA). After incubation for 16–24 h, the non-migrating cells that remained on the upper surface were removed with a cotton swab. The migrated cells on the lower surface were fixed with 4% paraformaldehyde for 30 min and stained with 0.1% crystal violet for 5 min, followed by washing with phosphate-buffered saline for 3 times for a total of 30 min. The experiments were repeated three times independently.

## Animal work

Female nude (nu/nu) mice (4–5 week old) were purchased from GuangDong Medical Experiments Animal Center. Animal experiments were performed in accordance with protocols approved by the Ethics Committees of Sun Yat-Sen University Cancer Center. JIMT1 cells were transfected with solo- or co-overexpression of ARID1A and UCA1, respectively. The cells at their exponential proliferation stage were harvested and were then mixed with 50% matrigel (BD Biosciences, San Jose, CA, USA). In all, 3  $\times 10^6$  cells were injected into both flanks. Tumor volumes were measured with calipers every 5 days beginning 10 days after the injection and calculated using the following formula: volume (mm<sup>3</sup>) = [width(mm)]<sup>2</sup>  $\times$  length (mm)/2.

## Statistical analysis

All data were analyzed using Graph Pad Prism version 6. Data were expressed as the mean  $\pm$  standard error of the mean, and *T* test (non-parametric) was applied for statistical comparison. Each group contained two or three repeats. All experiments subjected to statistical analysis were repeated at least three times. Kaplan–Meier survival curves were established by KM plotter (<http://kmplot.com>) to analyze correlations between the overall survival of breast cancer patients and the expression of ARID1A, CEBP $\alpha$ , and UCA1. Auto-selected best cutoff was chosen in the analysis. Log-rank *P* value was calculated.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

## References

1. Alteri R, Bertaut T, Brinton LA, Fedewa S, Freedman RA, Gansler T, et al. Breast Cancer Facts and Figures 2015-2016. Atlanta: American Cancer Society, Inc.; 2015.
2. Wilson BG, Roberts CW. SWI/SNF nucleosome remodelers and cancer. *Nat Rev Cancer*. 2011;11:481–92.
3. Cho HD, Lee JE, Jung HY, Oh MH, Lee JH, Jang SH, et al. Loss of tumor suppressor ARID1A protein expression correlates with poor prognosis in patients with primary breast cancer. *J Breast Cancer*. 2015;18:339–46.
4. Wiegand KC, Shah SP, Al-Agha OM, Zhao Y, Tse K, Zeng T, et al. ARID1A mutations in endometriosis-associated ovarian carcinomas. *N Engl J Med*. 2010;363:1532–43.
5. Jones S, Wang TL, Shih IEM, Mao TL, Nakayama K, Roden R, et al. Frequent mutations of chromatin remodeling gene ARID1A in ovarian clear cell carcinoma. *Science*. 2010;330:228–31.
6. Wiegand KC, Lee AF, Al-Agha OM, Chow C, Kalloger SE, Scott DW, et al. Loss of BAF250a (ARID1A) is frequent in high-grade endometrial carcinomas. *J Pathol*. 2011;224:328–33.
7. Takeda T, Banno K, Okawa R, Yanokura M, Iijima M, Irie-Kunitomi H, et al. ARID1A gene mutation in ovarian and endometrial cancers (Review). *Oncol Rep*. 2016;35:607–13.
8. Cho H, Kim JS, Chung H, Perry C, Lee H, Kim JH, et al. Loss of ARID1A/BAF250a expression is linked to tumor progression and adverse prognosis in cervical cancer. *Hum Pathol*. 2013;44:1365–74.
9. Cornen S, Adelaide J, Bertucci F, Finetti P, Guille A, Birnbaum DJ, et al. Mutations and deletions of ARID1A in breast tumors. *Oncogene*. 2012;31:4255–6.
10. Wu JN, Roberts CW. ARID1A mutations in cancer: another epigenetic tumor suppressor? *Cancer Discov*. 2013;3:35–43.
11. Xiao W, Awadallah A, Xin W. Loss of ARID1A/BAF250a expression in ovarian endometriosis and clear cell carcinoma. *Int J Clin Exp Pathol*. 2012;5:642–50.
12. Zhang X, Zhang Y, Yang Y, Niu M, Sun S, Ji H, et al. Frequent low expression of chromatin remodeling gene ARID1A in breast cancer and its clinical significance. *Cancer Epidemiol*. 2012;36:288–93.
13. Zhao J, Liu C, Zhao Z. ARID1A: a potential prognostic factor for breast cancer. *Tumour Biol*. 2014;35:4813–9.
14. Mathur R, Alver BH, San Roman AK, Wilson BG, Wang X, Agoston AT, et al. ARID1A loss impairs enhancer-mediated gene regulation and drives colon cancer in mice. *Nat Genet*. 2017;49:296–302.
15. Guan B, Gao M, Wu CH, Wang TL, Shih IEM. Functional analysis of in-frame indel ARID1A mutations reveals new regulatory mechanisms of its tumor suppressor functions. *Neoplasia*. 2012;14:986–93.
16. Guan B, Wang TL, Shih IEM. ARID1A, a factor that promotes formation of SWI/SNF-mediated chromatin remodeling, is a tumor suppressor in gynecologic cancers. *Cancer Res*. 2011;71:6718–27.
17. Shen J, Peng Y, Wei L, Zhang W, Yang L, Lan L, et al. ARID1A deficiency impairs the DNA damage checkpoint and sensitizes cells to PARP inhibitors. *Cancer Discov*. 2015;5:752–67.
18. Samartzis EP, Gutsche K, Dedes KJ, Fink D, Stucki M, Imesch P. Loss of ARID1A expression sensitizes cancer cells to PI3K- and AKT- inhibition. *Oncotarget*. 2014;5:5295–303.
19. Huang J, Zhou N, Watabe K, Lu Z, Wu F, Xu M, et al. Long non-coding RNA UCA1 promotes breast tumor growth by suppression of p27 (Kip1). *Cell Death Dis*. 2014;5:e1008.
20. Hughes JM, Legnini I, Salvatori B, Masciarelli S, Marchioni M, Fazi F, et al. C/EBP $\alpha$ -p30 protein induces expression of the oncogenic long non-coding RNA UCA1 in acute myeloid leukemia. *Oncotarget*. 2015;6:18534–44.
21. Wang Z, Wang X, Zhang D, Yu Y, Cai L, Zhang C. Long non-coding RNA urothelial carcinoma-associated 1 as a tumor biomarker for the diagnosis of urinary bladder cancer. *Tumour Biol*. 2017;39:1010428317709990.
22. Wang ZQ, Cai Q, Hu L, He CY, Li JF, Quan ZW, et al. Long noncoding RNA UCA1 induced by SP1 promotes cell proliferation via recruiting EZH2 and activating AKT pathway in gastric cancer. *Cell Death Dis*. 2017;8:e2839.
23. Block K, Gorin Y, New DD, Eid A, Chelmicki T, Reed A, et al. The NADPH oxidase subunit p22<sup>phox</sup> inhibits the function of the tumor suppressor protein tuberlin. *Am J Pathol*. 2010;176:2447–55.
24. Baker K, Rath T, Flak MB, Arthur JC, Chen Z, Glickman JN, et al. Neonatal Fc receptor expression in dendritic cells mediates protective immunity against colorectal cancer. *Immunity*. 2013;39:1095–107.
25. Sun X, Chuang JC, Kanchwala M, Wu L, Celen C, Li L, et al. Suppression of the SWI/SNF component ARID1A promotes mammalian regeneration. *Cell Stem Cell*. 2016;18:456–66.
26. Clarke C, Madden SF, Doolan P, Aherne ST, Joyce H, O'Driscoll L, et al. Correlating transcriptional networks to breast cancer survival: a large-scale co-expression analysis. *Carcinogenesis*. 2013;34:2300–8.
27. Priam P, Krasteva V, Rousseau P, D'Angelo G, Gaboury L, Sauvageau G, et al. SMARCD2 subunit of SWI/SNF chromatin-remodeling complexes mediates granulopoiesis through a CEBP $\epsilon$  dependent mechanism. *Nat Genet*. 2017;49:753–64.
28. Müller C, Calkhoven CF, Sha X, Leutz A. The CCAAT enhancer-binding protein alpha (C/EBP $\alpha$ ) requires a SWI/SNF complex for proliferation arrest. *J Biol Chem*. 2004;279:7353–8.
29. Mamo A, Cavallone L, Tuzmen S, Chabot C, Ferrario C, Hassan S, et al. An integrated genomic approach identifies ARID1A as a candidate tumor-suppressor gene in breast cancer. *Oncogene*. 2012;31:2090–100.
30. Takao C, Morikawa A, Ohkubo H, Kito Y, Saigo C, Sakuratani T, et al. Downregulation of ARID1A, a component of the SWI/SNF chromatin remodeling complex, in breast cancer. *J Cancer*. 2017;8:1–8.
31. Zhang L, Cao X, Zhang L, Zhang X, Sheng H, Tao K. UCA1 overexpression predicts clinical outcome of patients with ovarian cancer receiving adjuvant chemotherapy. *Cancer Chemother Pharmacol*. 2016;77:629–34.
32. Fan Y, Shen B, Tan M, Mu X, Qin Y, Zhang F, et al. Long non-coding RNA UCA1 increases chemoresistance of bladder cancer cells by regulating Wnt signaling. *FEBS J*. 2014;281:1750–8.
33. Wang H, Guan Z, He K, Qian J, Cao J, Teng L. Lnc RNA UCA1 in anti-cancer drug resistance. *Oncotarget*. 2017;8:64638–50.
34. Li X, Wu Y, Liu A, Tang X. Long non-coding RNA UCA1 enhances tamoxifen resistance in breast cancer cells through a miR-18a-HIF1 $\alpha$  feedback regulatory loop. *Tumour Biol*. 2016;37:14733–43.

35. Yuan J, Jiang YY, Mayakonda A, Huang M, Ding LW, Lin H, et al. Super-enhancers promoter transcriptional dysregulation in nasopharyngeal carcinoma. *Cancer Res.* 2017;77:6614–26.
36. Guo X, Dong Y, Yin S, Zhao C, Huo Y, Fan L, et al. Patulin induces pro-survival functions via autophagy inhibition and p62 accumulation. *Cell Death Dis.* 2013;4:e822.
37. Lin DC, Dinh HQ, Xie JJ, Mayakonda A, Silva TC, Jiang YY, et al. Identification of distinct mutational patterns and new driver genes in oesophageal squamous cell carcinomas and adenocarcinomas. *Gut.* 2017. <https://doi.org/10.1136/gutjnl-2017-314607>.