



# Targeting Pin1 by All-Trans Retinoic Acid (ATRA) Overcomes Tamoxifen Resistance in Breast Cancer via Multifactorial Mechanisms

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Breast cancer is the most prevalent tumor in women worldwide and about 70% patients are estrogen receptor positive. In these cancer patients, resistance to the anticancer estrogen receptor antagonist tamoxifen emerges to be a major clinical obstacle. Peptidyl-prolyl isomerase Pin1 is prominently overexpressed in breast cancer and involves in tamoxifen-resistance. Here, we explore the mechanism and effect of targeting Pin1 using its chemical inhibitor all-trans retinoic acid (ATRA) in the treatment of tamoxifen-resistant breast cancer. We found that Pin1 was up-regulated in tamoxifen-resistant human breast cancer cell lines and tumor tissues from relapsed patients. Pin1 overexpression increased the phosphorylation of ER $\alpha$  on S118 and stabilized ER $\alpha$  protein. ATRA treatment, resembling the effect of Pin1 knockdown, promoted ER $\alpha$  degradation in tamoxifen-resistant cells. Moreover, ATRA or Pin1 knockdown decreased the activation of ERK1/2 and AKT pathways. ATRA also reduced the nuclear expression and transcriptional activity of ER $\alpha$ . Importantly, ATRA inhibited cell viability and proliferation of tamoxifen-resistant human breast cancer cells *in vitro*. Slow-releasing ATRA tablets reduced the growth of tamoxifen-resistant human breast cancer xenografts *in vivo*. In conclusion, ATRA-induced Pin1 ablation inhibits tamoxifen-resistant breast cancer growth by suppressing multifactorial mechanisms of tamoxifen resistance simultaneously, which demonstrates an attractive strategy for treating aggressive and endocrine-resistant tumors.

**Keywords:** ATRA, Pin1, breast cancer, tamoxifen, ER $\alpha$

## INTRODUCTION

Breast cancer is a leading cause of cancer-related death in female (Chen et al., 2016). In all breast cancer patients, approximately 70% patients are estrogen receptor positive (Deroo and Korach, 2006; Yager and Davidson, 2006; Nilsson et al., 2011). Although selective estrogen receptor modulator such as tamoxifen are effective for ER positive patient, approximately 30% of patients

are not sensitive to tamoxifen treatment at the beginning, and over 50% of initial effective patients finally suffer from tamoxifen-resistance (TAMR) (Osborne and Schiff, 2011). The mechanism of TAMR is still not completely known. The possible molecular mechanisms include, but not limited to, the alteration of estrogen receptor transcriptional co-regulatory proteins (Shao et al., 2004; Girault et al., 2006), cross-talk between receptor tyrosine kinase signaling and estrogen receptor (Stenoien et al., 2001), non-canonical transcriptional activation of estrogen receptor (Anbalagan and Rowan, 2015), the expression of specific microRNAs (Miller et al., 2008), etc. Given that many studies have demonstrated that estrogen receptors play a central role in TAMR (Wijayarathne and McDonnell, 2001; Marsh et al., 2017; Zhang et al., 2017), blocking estrogen receptor related pathways is an attractive strategy to treat TAMR breast cancer.

Pin1 is a peptidyl-prolyl cis/trans isomerase (PPIase), which specifically recognizes pSer/Thr-Pro motifs of proteins and catalyzes their *trans-cis* conformational change (Lu and Zhou, 2007). Pin1 plays a vital role in cancer development by regulating more than 40 oncoproteins and over 20 tumor suppressors, therefore promoting cancer growth and cancer stem cell tumorigenesis (Zhou and Lu, 2016). Pin1 has been found to be up-regulated in tamoxifen-resistant breast cancer (Stanya et al., 2008; Namgoong et al., 2010; Khanal et al., 2012). Overexpression of Pin1 reduces the protein stability of estrogen receptor transcriptional co-regulatory protein SMRT (Stanya et al., 2008), as well as regulates the transcription function of ER $\alpha$  (Rajbhandari et al., 2012, 2015). Knockdown of Pin1 by siRNA inhibits the viability of TAMR breast cancer cells (Namgoong et al., 2010), indicating that Pin1 might be a promising therapeutic target for tamoxifen-resistant breast cancer. However, due to the lack of appropriate Pin1 inhibitors, it is challenging to evaluate the effect of targeting Pin1 on overcoming TAMR. Recently, Wei et al. has discovered all-trans retinoic acid (ATRA) as a specific Pin1 chemical inhibitor (Wei et al., 2015). ATRA has been used to induce differentiation and treat acute promyelocytic leukemia (APL). In APL, ATRA facilitates PML-RAR- $\alpha$  degradation, thereby suppresses APL stem cells (Huang et al., 1988; de The and Chen, 2010; Sanz and Lo-Coco, 2011). Wei et al. (2015) has found that besides RAR, Pin1 is a key target of ATRA in APL and breast cancer. ATRA directly and selectively binds to and degrades active Pin1, thereby inhibiting multiple Pin1-regulated cancer driving pathways.

In the current study, we explored the effects of ATRA in inhibiting Pin1 and treating tamoxifen-resistant breast cancer *in vitro* and *in vivo*. Our experiments showed that Pin1 was up-regulated in tamoxifen-resistant cells and increased ER $\alpha$  protein stability. ATRA treatment accelerated ER $\alpha$  protein turnover, reduced ER $\alpha$  transcriptional activity, and decreased the phosphorylation of AKT and ERK1/2 simultaneously, which further inhibited ER $\alpha$  activation. Thus, ATRA induced the degradation of Pin1 and suppressed cell viability and proliferation of tamoxifen-resistant breast cancer cells. More importantly, slow-releasing ATRA tablets showed remarkable anti-tumor effects in the tamoxifen-resistant xenograft model. Therefore, targeting Pin1 by ATRA promised a new potential approach to treat tamoxifen-resistant breast cancer.

## MATERIALS AND METHODS

### Cell Culture

The human breast cancer cell lines MCF7 and T47D were purchased from American Type Culture Collection (Manassas, VA, United States) and cultured in DMEM (Thermo Fisher Scientific, Waltham, MA, United States) supplemented with 10% fetal bovine serum (FBS, GBICO). Tamoxifen resistant cell lines (MCF-7R and T47DR) were kindly provided by Dr. Qiang Liu as gift, and were cultured in no-phenol red 1640 medium (Life Technologies, United States) supplemented with 10% charcoal-stripped FBS (cFBS) (HyClone, United States) and 1  $\mu$ M 4-hydroxytamoxifen (Sigma-Aldrich, St. Louis, MO, United States). Cells were maintained at 37°C in a humidified atmosphere containing 5% CO<sub>2</sub>.

### Antibodies, Reagents, and Sequences

Antibodies of ER $\alpha$  was from cell signaling technology (8644) and Abcam (16660). Antibodies of Pin1 was from R&D (MAB2294) and Abnova (MAB12340). Phospho-ER $\alpha$  S118 antibody was from Abcam (ab32396). Phospho-ER $\alpha$  S167 antibody was from cell signaling technology (64508). ATRA was from Sigma (R2625). Tamoxifen was from Sigma (H6278). Pin1 shRNA targeting sequence: CCACCGTCACACAGTATTTAT; Pin1 siRNA-1 targeting sequence: TCAGGCCGAGTGACTACT; Pin1 siRNA-2 targeting sequence: GCTCAGGCCGAGTGACTACT; RAR $\alpha$  siRNA-1 targeting sequence: CCAGCTCACAGAAGCTGCTT; RAR $\alpha$  siRNA-2 targeting sequence: TTCCGCACGTAGACCTT TAGC; ER $\alpha$  siRNA-1 targeting sequence: CAGGCCAAATTCA GATAAT; ER $\alpha$  siRNA-2 targeting sequence: GGTCCAC CTTCTAGAATGT.

### Colony-Forming Assays

Six-well plates were seeded 2000 cells per well. Cells were treated with vehicle (DMSO), 10  $\mu$ M ATRA, 10  $\mu$ M tamoxifen (TAM) or ATRA plus TAM, and medium were changed every 3 days. 14 days later cells were fixed with methanol, stained with 0.5% crystal violet.

### Cell Viability Assays

Cell viability was measured using the Cell Titer Glo reagent (Promega). The cells were plated in 96-well plates at 1  $\times$  10<sup>3</sup> cells per well and maintained at 37°C. At the indicated time points, cell viability was measured according to the manufacturer's instructions.

### Quantitative RT-PCR

Total RNA was extracted from cells using Trizol (Life, United States). MMLV kit (Life, United States) was used to generate cDNA. Real time PCR were performed using Toyobo SYBR GREEN. The primers used were as follows: Pin1 (forward, 5'-AGCTCAGGCCGAGTGACTACTA-3'; reverse, 5'-CCTTGGTCCGGGTGATCTTC-3'); growth regulation by estrogen in breast cancer 1 (GREB1) (forward, 5'-GTGGT AGCCGAGTGGACAAT-3'; reverse, 5'-ATTTG TTTCCAGCC CTCTT-3'); progesterone receptor (PGR) (forward, 5'-GG

CATGGTCCTTGGAGGT -3'; reverse, 5'-CCACTGGCTGTGG GAGAG-3'); c-Myc (forward, 5'- TACAACACCC GAGC AAGGAC-3'; reverse, 5'-GAGGCTGCTGGTTTTTCCACT-3');  $\beta$ -actin (forward, 5'-GGAAGGGGACGGGGACAGC-3'; reverse, 5'- GGAGGAGCAAG GAGCGGGAG-3').

## Immunoblot Analysis

Cells were lysed with RIPA buffer containing 0.1% protease inhibitors or phosphatase inhibitors (Life, United States). The supernatant of lysate was separated by electrophoresis and blotted onto a PVDF membrane, then blocked with 5% skim milk at room temperature for 1 h. The blots were incubated with the following antibody at 4°C overnight: ER $\alpha$  (1:1000, CST, #8644); Pin1 (1:1000, R&D, #MAB2294); phospho-ER $\alpha$  S118 (1:1000, CST, #ab32396); AKT (1:1000, CST); Flag-tag (1:5000, Sigma, United States); phospho-AKT (1:1000, CST); phospho-c-Raf (1:1000, CST); phospho-MEK1/2 (1:1000, CST); ERK1/2 (1:1000, CST); phospho-ERK1/2 (1:1000, CST); phospho-ER $\alpha$ S167 (1:1000, CST, #64508);  $\beta$ -Actin (1:2000, CST); GAPDH (1:2000, proteintech). After incubation with HRP-conjugated secondary antibodies at room temperature for 1 h, all blots were detected by an enhanced chemiluminescence (ECL) and were scanned using ChemiDoc<sup>TM</sup> XRS + imaging system (Bio-Rad, Hercules, CA, United States).

## Immunofluorescence

MCF-7R and T47DR cells were fixed in 4% polyoxymethylene at 4°C for 20 min, washed with PBS and permeabilized in 0.1% Triton X-100 at room temperature for 10 min. Cells were then blocked in 10% goat serum at room temperature for 30 min, and incubated with ER $\alpha$  antibody (1:100, Abcam, #16660) in 10% goat serum at 4°C overnight. Cells were washed, incubated with secondary antibodies at room temperature for 1 h, washed, incubated with DAPI at room temperature for 15 min. Slides were then covered with fluorescently quencher 30  $\mu$ l, sealed and photographed with an Olympus confocal microscope.

## Animal Experiments

Nude mice were purchased from Laboratory Animal Service Center, Sun Yat-sen University. The experiment protocol was approved by the Animal Care and Use Committee of Sun Yat-sen University.  $2 \times 10^6$  MCF-7R cells were mixed with an equal volume of matrigel (Corning) and injected into the mammary fat pads of 4 week-old female BALB/c nude mice. One week later, when tumor size reached  $\sim 100$  mm<sup>3</sup>, the tumor-bearing mice were randomized into treatment groups. 21-days ATRA tablets were implanted under neck skin. Tamoxifen was injected at 4 mg/kg per day. Tumor volume was measured every 3 days.

## Patients and Immunohistochemistry

Tumor samples were obtained from patients with ER positive breast cancer who underwent tamoxifen therapy in Sun Yat-sen Memorial Hospital. All samples were collected from patients with informed consent, and all related procedures were performed with the approval of the internal review and ethics boards of Sun Yat-sen Memorial Hospital. Immunohistochemistry staining

for Pin1 and ER $\alpha$  was performed as described previously (Luo et al., 2015; Zhang et al., 2016). Briefly, sodium citrate was used to repair tissue antigen. Incubation of primary antibodies (Pin1, 1:50, Abnova, #MAB12340; ER $\alpha$ , 1:50, CST, #16660) was carried out at 4°C overnight. The slides were incubated with HRP-conjugated secondary antibodies at room temperature for 1 h, washed, visualized with DAB solution, followed by staining with hematoxylin. Immunostaining results was analyzed by ImageJ software.

## Statistical Analyses

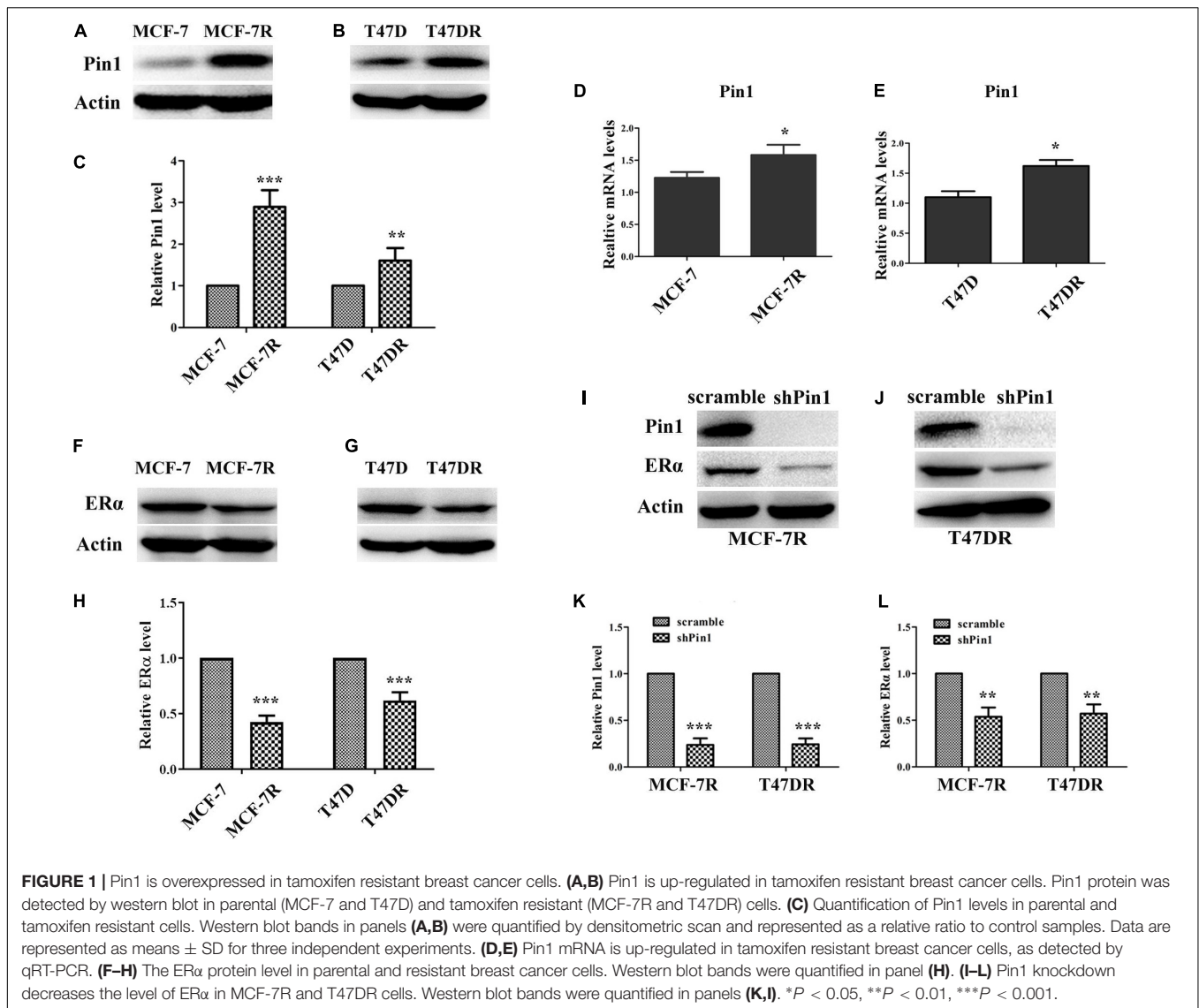
All data are presented as the means  $\pm$  SD. Student's *t*-test was used to analysis the significance between two experimental groups, and ANOVA test was used to analysis among three or more groups. *P* < 0.05 was considered significant. All the statistical analyses were performed using SPSS20.

## RESULTS

### Pin1 Is Up-Regulated in Tamoxifen-Resistant Breast Cancer and Correlates With ER $\alpha$ Expression in Human Breast Cancer Cell Lines and Cancer Tissues

We established tamoxifen-resistant human breast cancer cell lines MCF-7 and T47D by long-term exposure to tamoxifen (Herman and Katzenellenbogen, 1996; Knowlden et al., 2003; Chu et al., 2015). We confirmed the resistance of these cells by showing that the viability of resistance cells was significantly higher than parental cells and apoptosis were remarkable lower in the presence of 1  $\mu$ M tamoxifen (Chu et al., 2015). We found that both Pin1 protein and mRNA were up-regulated in tamoxifen-resistant MCF-7 (MCF-7R) and T47D (T47DR) cells, comparing to parental cells (**Figures 1A–E** and **Supplementary Figure S5**), which was consistent with previous reports that Pin1 was overexpressed in TAMR human breast cancer tissues (Namgoong et al., 2010; Khanal et al., 2012).

Although ER $\alpha$  was not so indispensable for TAMR cells as for parental cells, depleting ER $\alpha$  still further limited the growth of TAMR cells (Xiong et al., 2017). Indeed, through a variety of mechanisms, TAMR breast cancer cells made full use of remaining ER $\alpha$  to escape from the impact of tamoxifen (Osborne and Schiff, 2005; Johnston, 2010; Marsh et al., 2017). Here we examined the ER $\alpha$  level in TAMR cells, and found that ER $\alpha$  protein was down-regulated in TAMR cells (**Figures 1F–H** and **Supplementary Figure S5**), as shown previously (Stone et al., 2013; Lu et al., 2016). Given that ER $\alpha$  was a known Pin1 substrate which was positively regulated by Pin1 (Rajbhandari et al., 2012, 2015). We asked why Pin1 level was high while ER $\alpha$  level was low in TAMR cells. We found that Pin1 knockdown further decreased ER $\alpha$  level in TAMR cell lines (**Figures 1I–L** and **Supplementary Figure S5**). These results suggest that Pin1 is up-regulated and helps maintain ER $\alpha$  levels in TAMR cells even although ER $\alpha$  levels in these cells are low.



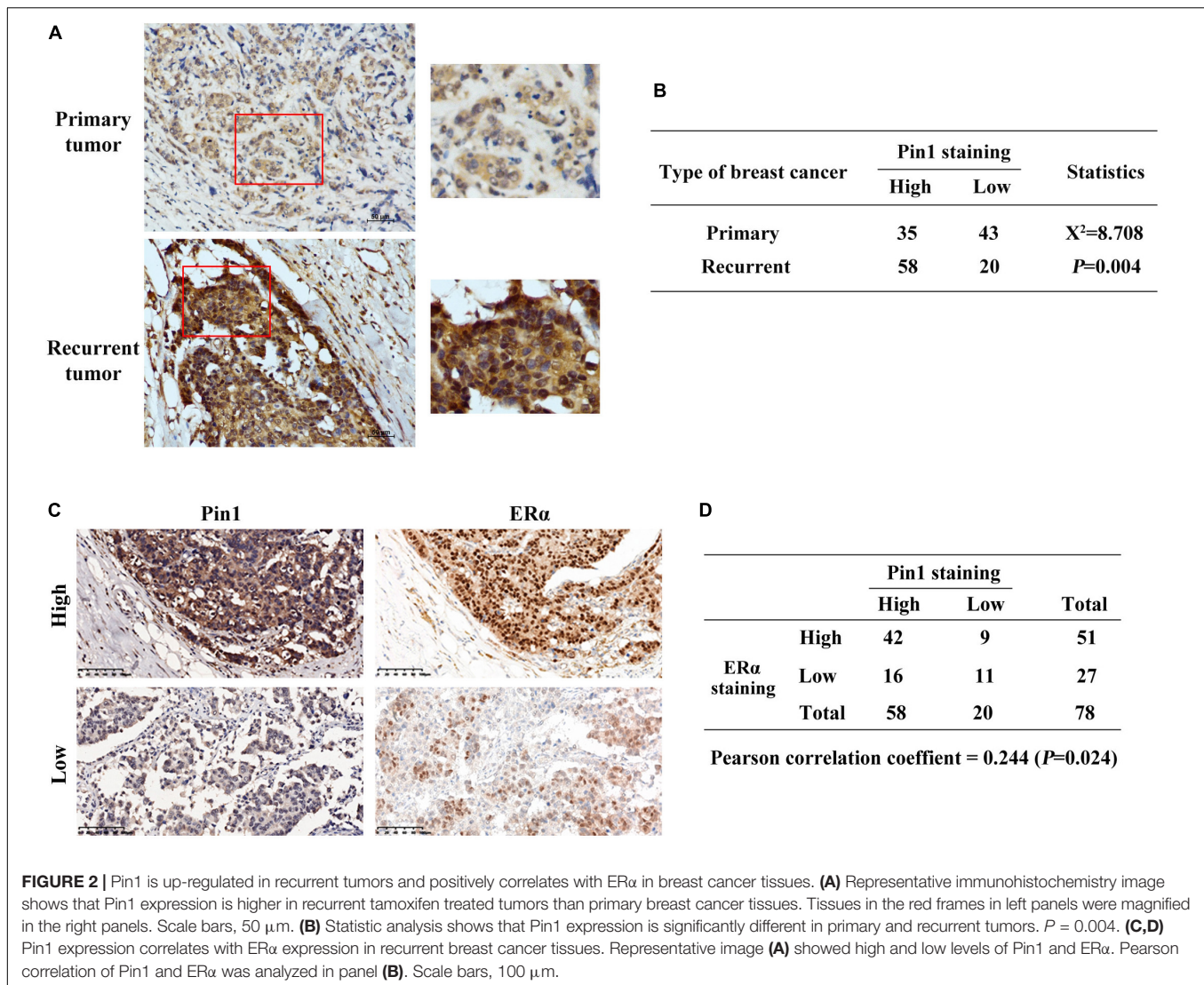
Next, we detected Pin1 and ER $\alpha$  protein levels in tumor tissues of recurrent ER positive breast cancer patients, who have received tamoxifen treatment. Pin1 protein level was significantly higher in recurrent tumors comparing with primary tumors ( $P = 0.004$ ) (**Figures 2A,B**). More importantly, the expression level of Pin1 was associated with ER $\alpha$  in these tissues (**Figures 2C,D**). Together, Pin1 was up-regulated in both TAMR human breast cancer cell lines and relapsed tumor tissues, which positively correlated with ER $\alpha$  expression.

## ATRA-Induced Pin1 Ablation Promotes ER $\alpha$ Protein Degradation

To explore the effect of Pin1 on regulating ER $\alpha$ , and more importantly to test whether ATRA was effective in inhibiting Pin1's function on ER $\alpha$ , we first examined whether overexpressing Pin1 affected ER $\alpha$  protein level. As expected, estradiol (E2) could induce down-regulation of ER $\alpha$  protein

(**Figures 3A,B** and **Supplementary Figure S6**), which was due to ligand-dependent degradation (Wijayarathne and McDonnell, 2001; Nonclercq et al., 2004). We found that not only enforced Pin1 expression (Flag-Pin1) rescued the ER $\alpha$  expression, but the Pin1 inhibitor ATRA reversed this effect in both MCF-7 and MCF-7R cells (**Figures 3A,B** and **Supplementary Figures S1A,B**), suggesting that ATRA specifically inhibiting Pin1 from protecting the degradation of ER $\alpha$ .

Next, to confirm the effect of ATRA on ER $\alpha$  protein degradation, MCF-7 and MCF-7R cells stably knocking down Pin1 with shRNA were treated with or without proteasome inhibitor MG132. Contrary to overexpression experiments, Pin1 knockdown promoted E2-induced ER $\alpha$  degradation (**Figures 3C,D** and **Supplementary Figures S1C,D**). Notably, ATRA had the same effects as shPin1 both in MCF-7 and MCF-7R cells (**Figures 3C,D** and **Supplementary Figures S1C,D**). One of the vital regulatory element governing ER $\alpha$  protein turnover is Ser118 phosphorylation of the N-terminus, which is



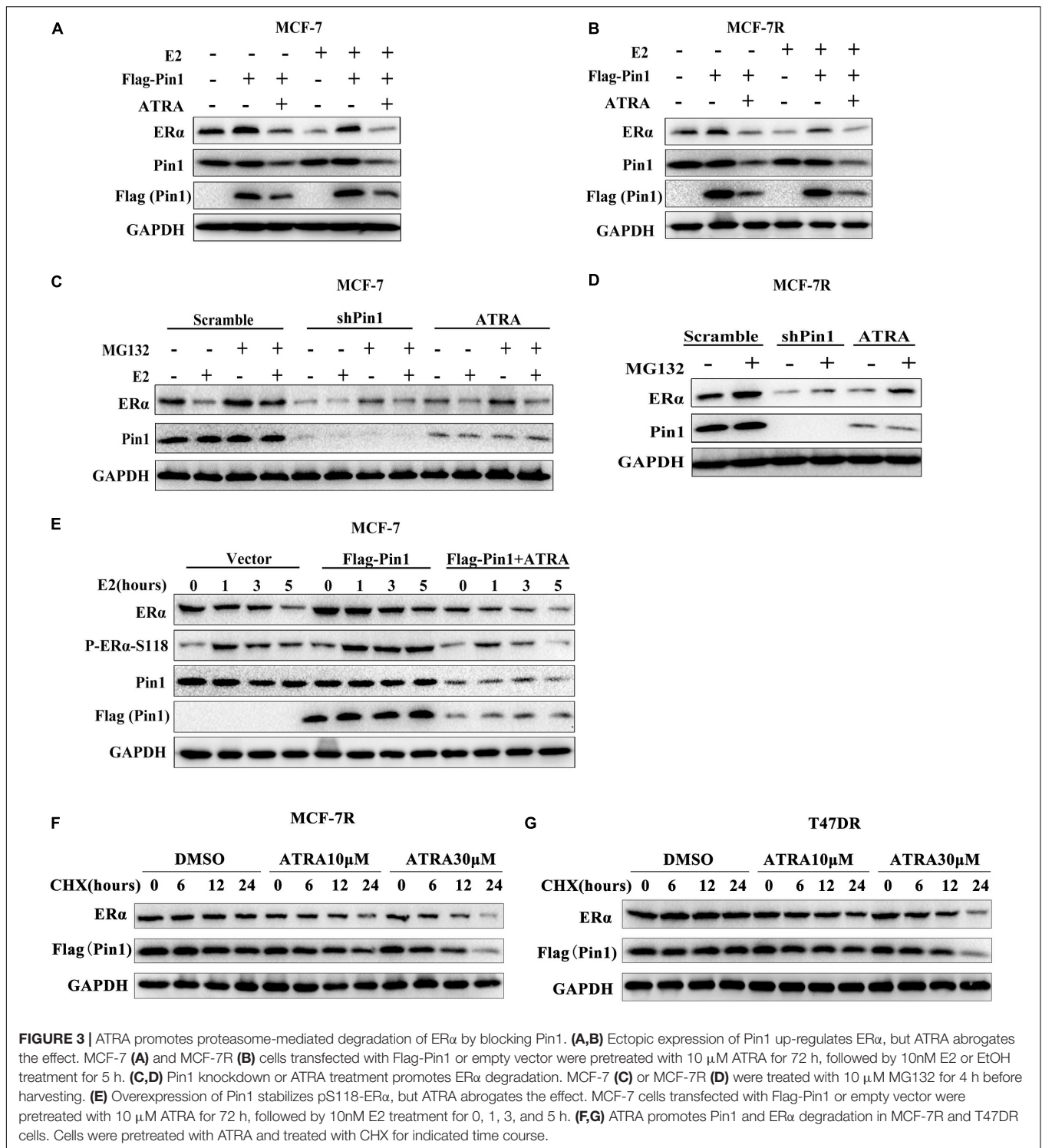
phosphorylated by ERK1/2 as well as other kinases, and regulated by Pin1 (Rajbhandari et al., 2012, 2014, 2015). Therefore we speculated that ATRA promoted ER $\alpha$  protein turnover through ER $\alpha$ -pS118. Indeed, overexpressing Pin1 increased the phosphorylation of S118 as well as total ER $\alpha$  level, suggesting that Pin1 prevented the turnover of pER $\alpha$ , whereas ATRA reversed Pin1's effect (Figure 3E and Supplementary Figures S1E,F).

To directly examine whether ATRA could promote the degradation of Pin1 and ER $\alpha$  in tamoxifen-resistant cells, we treated MCF-7R and T47DR cells with ATRA, followed by cycloheximide (CHX) and detected the protein levels at different time points. Our data showed that ATRA promoted the degradation of both Pin1 and ER $\alpha$  in TAMR breast cancer cells in a dose dependent manner (Figures 3E,G and Supplementary Figures S1G–J). In addition, we treated MCF-7R and T47DR cells with increasing doses of ATRA for different length of time, and found that both Pin1 and ER $\alpha$  protein levels indeed reduced (Supplementary Figures S2A,B, S9). Together, these data demonstrate that overexpressing Pin1 in breast cancer cells

protects the ER $\alpha$  protein from degradation. ATRA blocks the up-regulated Pin1 in tamoxifen-resistant cells, thereby promoting the degradation of remaining ER $\alpha$  in tamoxifen-resistant cells, which suggests that ATRA may be able to overcome TAMR by eradicate ER $\alpha$ .

### ATRA Blocks ERK1/2 and AKT Pathways in TAMR Breast Cancer Cells

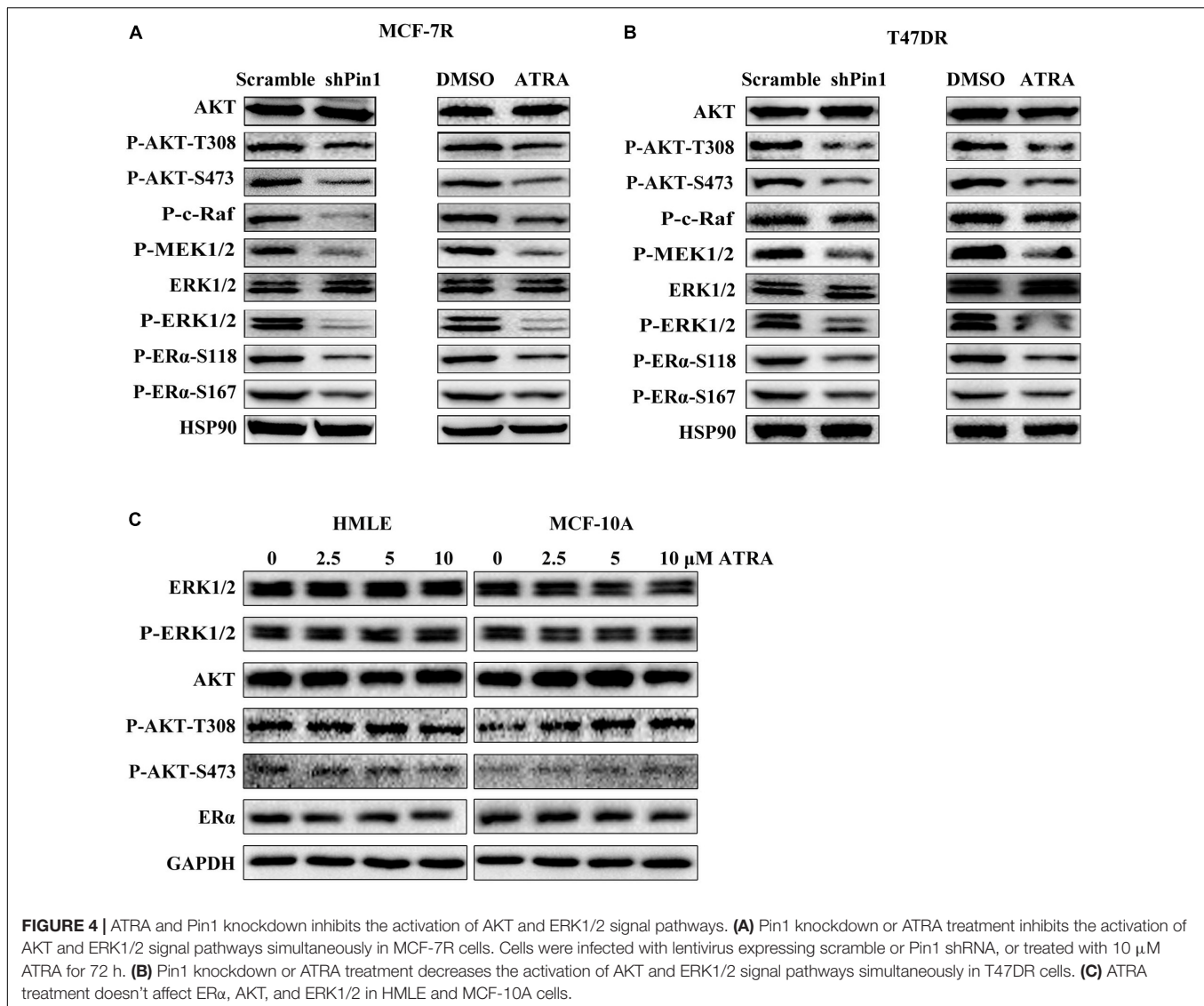
Several kinase pathways have been reported to involve in the growth of TAMR breast cancer cells, including AKT and ERK1/2 (Svensson et al., 2005; Garcia-Becerra et al., 2012). AKT phosphorylates ER $\alpha$  on S167 (Sun et al., 2001), and ERK1/2 phosphorylates ER $\alpha$  on S104/S106, S167, and S118 (Ali et al., 1993; Arnold et al., 1995; Endoh et al., 1999; Sun et al., 2001; Sheeler et al., 2003). Notably, phosphorylation of S118 and S167 induces estrogen-independent activation of ER $\alpha$  (Garcia-Becerra et al., 2012). Moreover, AKT and ERK1/2 activity are also regulated by Pin1 (Liao et al., 2009; Luo et al., 2015). Thus,



we explored the effects of ATRA in inhibiting these pathways in MCF-7R and T47DR cells. ATRA treatment didn't alter the total expression of AKT or ERK1/2, but reduced the level of phosphorylated AKT, MEK1/2, ERK1/2 and Raf (Figures 4A,B). In consistence with decreased activity of these pathways, phosphorylation of ER $\alpha$  on S167 and S118 were also inhibited,

resembling the effect of Pin1 knockdown (Figures 4A,B and Supplementary Figure S7).

To assess the effect of ATRA on ER signaling in normal cells, we treated immortalized mammary epithelial cells MCF-10A and HMLE with different doses of ATRA. These two cell lines expressed very low level of Pin1, comparing to breast cancer cell



lines (Wei et al., 2015). We found that ATRA almost had no effect on the protein level of ER $\alpha$ , or P-ERK1/2 and P-AKT (**Figure 4C**). Hence, ATRA had the unique potential to simultaneously block multiple signal pathways in TAMR breast cancer cells.

In addition, RAR $\alpha$ , another ATRA target, has been indicated to play a role in tamoxifen resistance of breast cancer (Johansson et al., 2013). Using siRNAs, we knocked down either Pin1 or RAR $\alpha$  in MCF-7R cells with or without ATRA treatment (**Supplementary Figures S3A,B**). The total and phosphorylated levels of ER $\alpha$  only decreased in Pin1-silencing, but not RAR $\alpha$ -silencing cells (**Supplementary Figures S3C, S10**). As ATRA can still target other proteins to regulate ER $\alpha$ , our data indicate that ATRA may mainly act on Pin1 to regulate ER $\alpha$ .

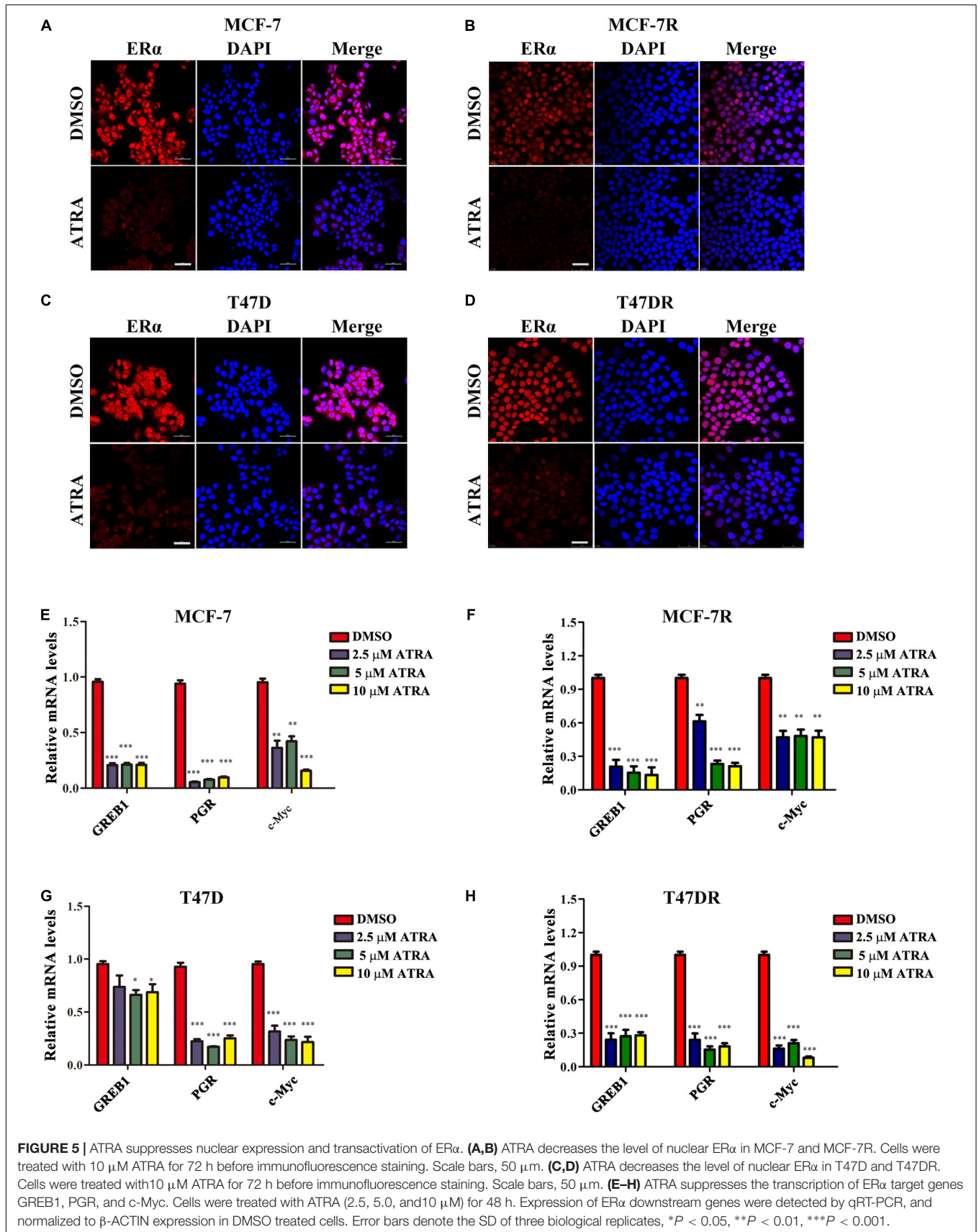
### ATRA Inhibits ER $\alpha$ Transcriptional Activity

To determine whether ATRA affected the transcriptional function of ER $\alpha$  in tamoxifen-resistant cells, we first examined

ER $\alpha$  subcellular expression by immunofluorescence. Parental or resistant MCF-7 and T47D cells were treated with 10  $\mu$ M ATRA for 72 h. The nuclear staining of ER $\alpha$  was dramatically reduced by ATRA treatment in all cell lines (**Figures 5A–D**), indicating a decreased transcriptional activity of ER $\alpha$ . Next, we detected the transcription of three known ER $\alpha$  regulatory genes, including PGR, GREB1, and c-Myc (Lee and Gorski, 1996; Bosch et al., 2015; Wu et al., 2018). The mRNA levels of these three genes were decreased after ATRA treatment (**Figures 5E–H**). These data suggest that ATRA suppresses ER $\alpha$  transcriptional function *in vitro*.

### ATRA Inhibits the Viability and Proliferation of Parental and Tamoxifen-Resistant Breast Cancer Cells

Although our data demonstrated that ATRA targeted Pin1 to promote ER $\alpha$  protein degradation, decrease ER $\alpha$  transcriptional



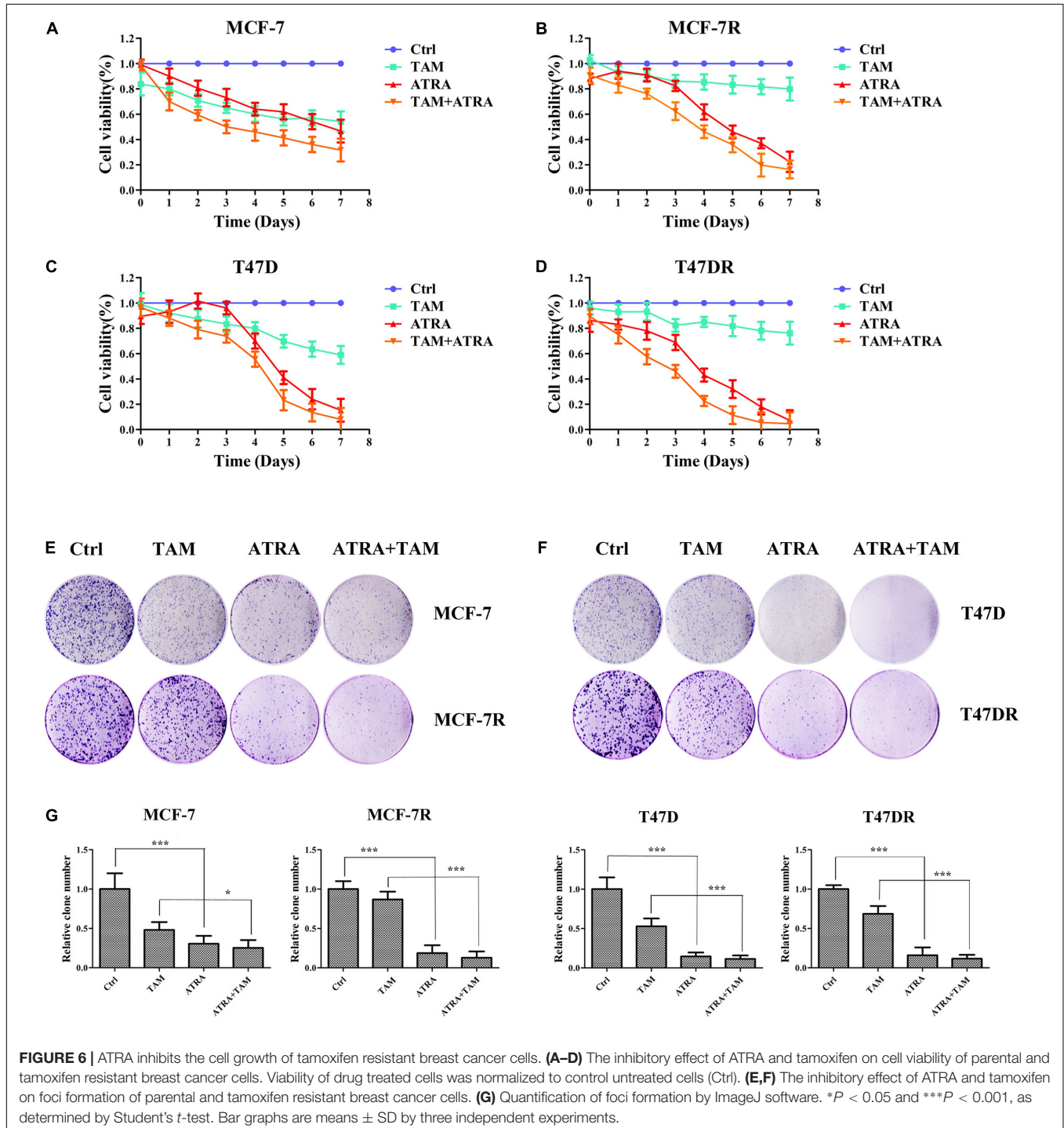
**FIGURE 5 |** ATRA suppresses nuclear expression and transactivation of ERα. **(A,B)** ATRA decreases the level of nuclear ERα in MCF-7 and MCF-7R. Cells were treated with 10 μM ATRA for 72 h before immunofluorescence staining. Scale bars, 50 μm. **(C,D)** ATRA decreases the level of nuclear ERα in T47D and T47DR. Cells were treated with 10 μM ATRA for 72 h before immunofluorescence staining. Scale bars, 50 μm. **(E-H)** ATRA suppresses the transcription of ERα target genes GREB1, PGR, and c-Myc. Cells were treated with ATRA (2.5, 5.0, and 10 μM) for 48 h. Expression of ERα downstream genes were detected by qRT-PCR, and normalized to β-ACTIN expression in DMSO treated cells. Error bars denote the SD of three biological replicates, \**P* < 0.05, \*\**P* < 0.01, \*\*\**P* < 0.001.

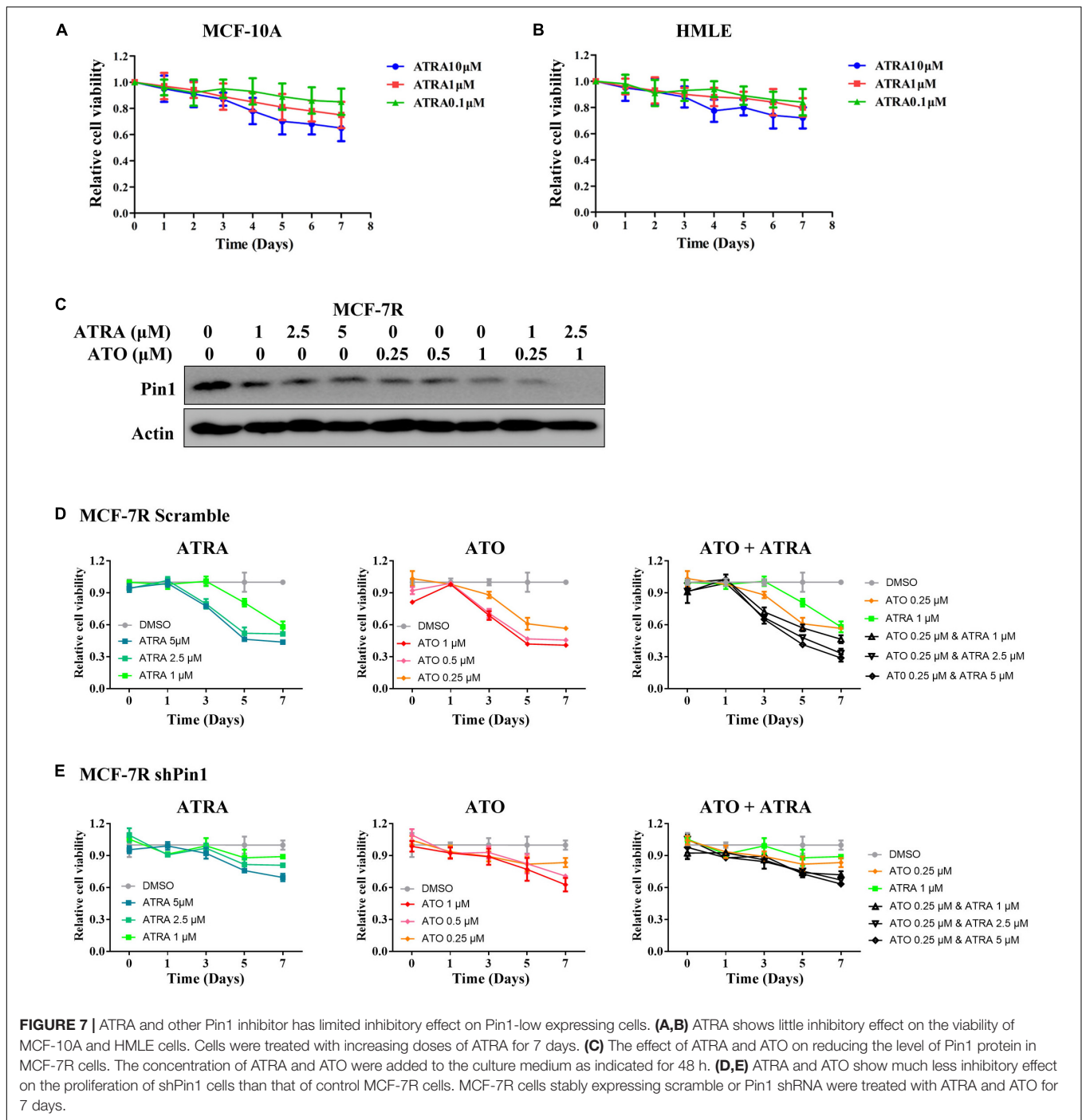


activity, and inhibit AKT and ERK1/2 pathway, the therapeutic potential of ATRA in treating tamoxifen-resistant breast cancer was still not clear. We thus evaluated the effects of ATRA on cell viability and foci formation of parental and TAMR cells. As expected, tamoxifen treatment reduced the growth of parental cells, but not the TAMR cells, whereas ATRA suppressed the proliferation of both parental and TAMR cells (Figures 6A–D). Moreover, ATRA potentiated tamoxifen therapeutic effect in

both parental and TAMR cells (Figures 6A–D). In the colony formation experiments, ATRA showed similar effects as in the proliferation assay (Figures 6E–G).

We have shown that ATRA-induced Pin1 degradation reduces the protein expression of ER $\alpha$  in tamoxifen-resistant breast cancer cells. To confirm that ER $\alpha$  contributes to tamoxifen resistance in our TAMR cell model, we used siRNAs to knock down ER $\alpha$  in MCF-7R and T47DR. ER $\alpha$  siRNAs dramatically





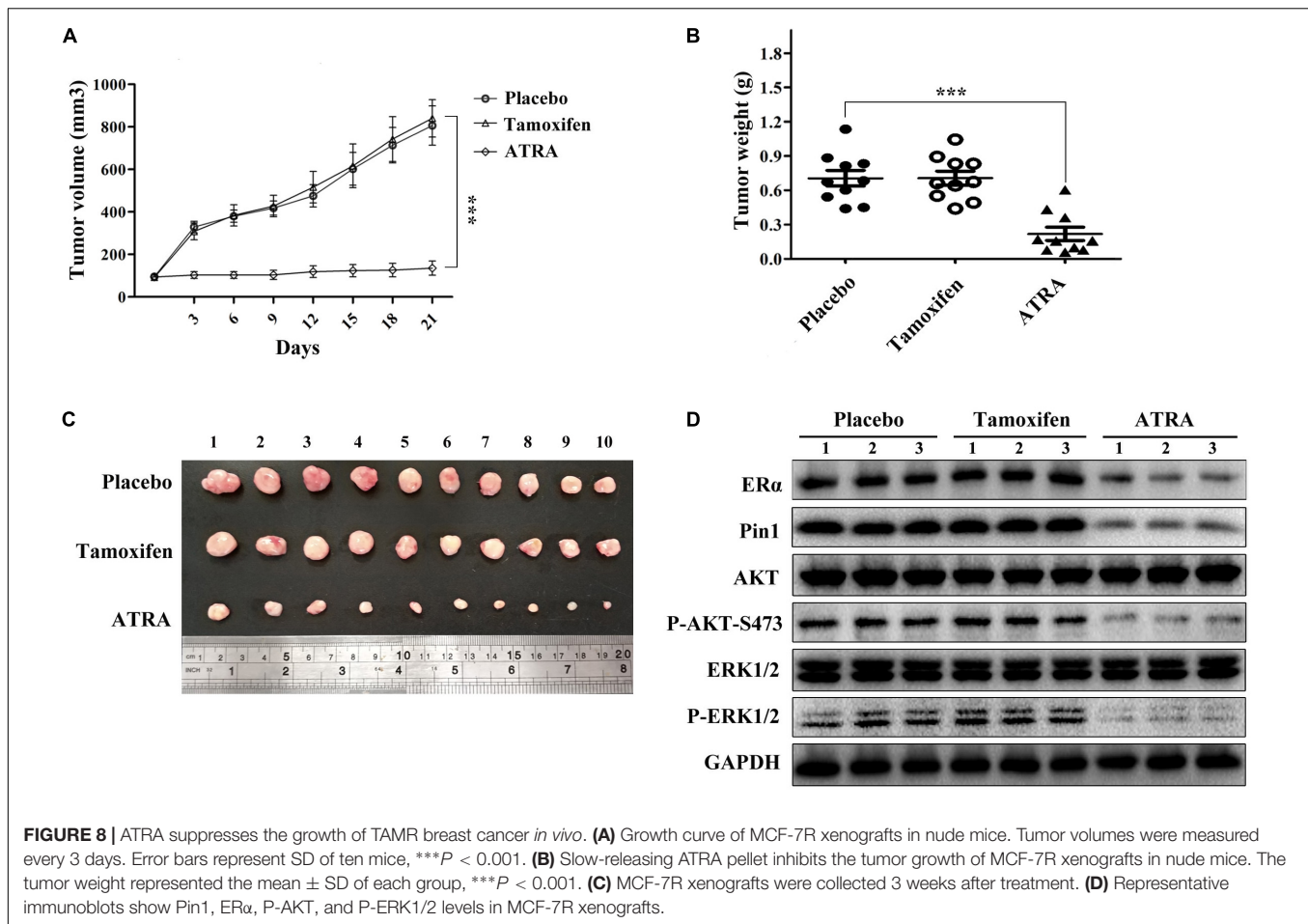
**FIGURE 7 |** ATRA and other Pin1 inhibitor has limited inhibitory effect on Pin1-low expressing cells. **(A,B)** ATRA shows little inhibitory effect on the viability of MCF-10A and HMLE cells. Cells were treated with increasing doses of ATRA for 7 days. **(C)** The effect of ATRA and ATO on reducing the level of Pin1 protein in MCF-7R cells. The concentration of ATRA and ATO were added to the culture medium as indicated for 48 h. **(D,E)** ATRA and ATO show much less inhibitory effect on the proliferation of shPin1 cells than that of control MCF-7R cells. MCF-7R cells stably expressing scramble or Pin1 shRNA were treated with ATRA and ATO for 7 days.

suppressed the proliferation of these cells upon tamoxifen treatment (**Supplementary Figures S4A,B, S10**), suggesting that ERα indeed contributed to TAMR in these cells.

To investigate the effects of ATRA on Pin1-low cells, we treated MCF-10A and HMLE with different doses of ATRA. ATRA exhibited very limited inhibitory effects on cell viability of these epithelial cells (**Figures 7A,B**), likely because ATRA selectively targets active Pin1 in cancer cells, but not in normal cells with low Pin1 levels (Wei et al., 2015). Thus these results

demonstrated that ATRA inhibits cell growth of TAMR breast cancer, with little effects on normal cells.

We also treated MCF-7R and T47DR cells that had knocked down Pin1 with ATRA and ATO, a newly identified Pin1 inhibitor (Kozono et al., 2018). Both ATRA and ATO showed much less inhibitory effect on the proliferation of shPin1 cells than that of control MCF-7R cells (**Figures 7C–E** and **Supplementary Figure S8**). Notably, although either ATRA or ATO could inhibit the proliferation of TAMR cells, the



combination of ATRA + ATO effectively suppressed the cell viability in low dose (Figures 7D,E).

## ATRA Suppresses the Growth of TAMR Breast Cancer *in vivo*

Given the remarkable effects of ATRA on inhibiting ER $\alpha$ , AKT, and ERK1/2, as well as cell proliferation in tamoxifen-resistant breast cancer *in vitro*, we next asked whether ATRA had therapeutic effect against TAMR breast tumors *in vivo*. We established MCF-7R xenografts and implanted 21-day slow-releasing ATRA tablets in nude mice. Tamoxifen showed no therapeutic effect on TAMR xenografts, whereas ATRA remarkably inhibited the growth of TAMR breast cancer cells *in vivo* (Figures 8A–C). In addition, ATRA significantly suppressed Pin1, ER $\alpha$ , as well as the phosphorylation of AKT and ERK1/2 in the xenografts (Figure 8D and Supplementary Figure S8). Therefore, ATRA is effective in overcoming tamoxifen resistance *in vivo*.

## DISCUSSION

Tamoxifen resistance is one of the major hurdles in treating breast cancer. A large body of evidence suggests that modulation

of ER $\alpha$  pathway and activation of pro-survival pathways are important factors of tamoxifen resistance (Cui et al., 2015; Ferraiuolo et al., 2017; Marsh et al., 2017; Zhang et al., 2017). Our study demonstrated that Pin1 was up-regulated in tamoxifen-resistant breast cancer cells and relapsed breast cancer tissues. ATRA-induced Pin1 degradation decreased the protein stability and transcription activity of ER $\alpha$ , as well as reduced the phosphorylation of pro-survival kinases AKT and ERK1/2 in tamoxifen-resistant breast cancer cells. Moreover, targeting Pin1 by ATRA inhibited cell growth *in vitro*, and exhibited anti-tumor effects *in vivo* against tamoxifen-resistant breast cancer. Our data suggest that ATRA is a potent drug in treating tamoxifen-resistant breast cancer via suppressing multifactorial mechanisms of tamoxifen resistance.

Compelling evidence has demonstrated that decreased ER $\alpha$  expression and function contributes to intrinsic and acquired tamoxifen resistance (Cui et al., 2015; Ferraiuolo et al., 2017; Marsh et al., 2017; Zhang et al., 2017). Various clinical and experimental models suggest that tumor cells with acquired resistance to tamoxifen express low level of ER $\alpha$  (Stone et al., 2013; Lu et al., 2016). In the presence of tamoxifen, the resistant cells can still activate ER $\alpha$ , but through a ligand-independent way (He et al., 2018), or rely on non-ER $\alpha$  growth-promoting pathways for survival (Hur et al., 2004; Cannings et al., 2007; Mohseni et al.,

2014). Thus the low level of ER $\alpha$  is one of the key resources of growth signal that are available for the resistant cells to utilize. Previous study showed that Pin1 inhibited phosphorylation-dependent ubiquitination and degradation of ER $\alpha$  in breast cancer cells (Rajbhandari et al., 2014). Here we found that Pin1 was up-regulated in tamoxifen-resistant breast cancer cells. This up-regulated Pin1 prevented ER $\alpha$  from degradation, which substantially enhanced the ER $\alpha$  level in tamoxifen-resistant cells. Although ER $\alpha$  level was low in these resistant cells, it would be even lower if Pin1 was not up-regulated. In our clinical samples, ER $\alpha$  expression was high in more than 60% of recurrent breast cancer tissues. This may be because Pin1 is frequently highly expressed in recurrent tumors, therefore preventing ER $\alpha$  from degradation, which substantially enhances the ER $\alpha$  level in relapsed tumors. Notably, this increased ER $\alpha$ , just as the low level of ER $\alpha$  in the resistant cells, is very likely activated via ligand independent way. This is supported by the evidence that phosphorylation of key serine residues of ER $\alpha$ , in particular serine 118 and 167, promotes re-activation of ER $\alpha$  in a ligand-independent manner (Garcia-Becerra et al., 2012). Pin1 has been reported to bind specifically to pS118 ER $\alpha$  to isomerize the serine118-proline119 bond (Rajbhandari et al., 2012). Therefore, Pin1 overexpression promotes the growth of tamoxifen-resistant breast cancer cells by up-regulating the ligand-independent ER $\alpha$  activity.

In addition to the effects on ER $\alpha$  stabilization, isomerization of phosphorylated ER $\alpha$  by Pin1 directly increases endogenous ER $\alpha$  DNA binding activity (Rajbhandari et al., 2015). Our study showed that inhibiting Pin1 by ATRA suppressed nuclear ER $\alpha$  expression and the transcription of ER $\alpha$  target genes. Moreover, previous data suggest that besides affecting ER $\alpha$ , Pin1 may promote tamoxifen resistance of breast cancer by activating growth-promoting pathways (Khanal et al., 2012), down-regulating SMRT (Stanya et al., 2008) and cyclin dependent kinase (Khanal et al., 2012), facilitating tumor angiogenesis (Kim et al., 2009b, 2012) and epithelial–mesenchymal transition (Kim et al., 2009a). However, few studies assess the potential of Pin1 inhibitor in treating TAMR breast cancer *in vitro* and *in vivo*. Our study showed that ATRA decreased ER $\alpha$  level both *in vitro* and *in vivo*. Moreover, ATRA down-regulated phosphorylation of ER $\alpha$  at S118 and two important pro-survival kinases ERK1/2 and AKT. Thus, ATRA inhibits Pin1 to overcome tamoxifen resistance in breast cancer cells at least at three levels: (1) promotes the degradation of ligand independent ER $\alpha$ , (2) suppresses the transactivation of ER $\alpha$ , (3) inhibits alternative growth pathways. Indeed, our results showed that ATRA exhibited potent anti-tumor activity against tamoxifen-resistant breast cancer *in vitro* and *in vivo*.

All-Trans Retinoic Acid has been used to treat APL for a long period of time. Recently Wei et al. (2015) has discovered that ATRA is a Pin1 inhibitor which binds to Pin1's active site and accelerated its degradation. These findings make it possible to expand the application of ATRA to treat more types of cancer, especially solid tumors, because Pin1 is overexpressed in a wide range of human cancers and regulates multiple cancer-driving pathways (Lu and Hunter, 2014; Zhou and Lu, 2016). Besides Wei et al. demonstrated that ATRA-induced Pin1 ablation inhibits

triple-negative breast cancer cell growth (Wei et al., 2015), Liao et al. also reported the anti-tumor effect of ATRA in hepatocellular carcinoma (HCC) *in vitro* and *in vivo* (Liao et al., 2017; Yang et al., 2018). New Pin1 inhibitors have also been discovered to suppress the growth of cancer cells (Campaner et al., 2017; Kozono et al., 2018), even the tumor-initiating cells, as Pin1 promotes the self-renewal of these stem-like cancer cells (Luo et al., 2014; Rustighi et al., 2014, 2017). Our data showed that ATRA suppressed the cell proliferation of tamoxifen-resistant breast cancer cells, and effectively reduced the tumor growth of tamoxifen-resistant xenografts by promoting ER $\alpha$  degradation, decreasing ER $\alpha$  transactivation, and inhibits the activation of ERK1/2 and AKT. Given that multiple survival pathways and factors contribute to tamoxifen resistance, blocking a single pathway may be ineffective to overcome the resistance. Therefore, ATRA may have the advantage of suppressing multifactorial mechanisms of tamoxifen resistance simultaneously.

Currently there are still obstacles of using ATRA to treat solid tumors in human. Regular ATRA has a half-life of only 45 min in humans. Although slow-releasing pellets can be implanted subcutaneously in mice, the formulation of ATRA pellet is different from that used for oral administration or intravenous injection in human therapies, and can't be applied to human yet. Novel controlled releasing formulation of ATRA for effective cancer therapy are being developed actively (Westervelt et al., 2002; Tsimberidou et al., 2006; Yang et al., 2018). In addition, ATRA concentration is high in treating solid tumors. Similar to the previous report that the combination of ATRA + ATO inhibited tumor-initiating cells (Kozono et al., 2018), we found that this combination could reduce ATRA concentration and effectively inhibited the growth of TAMR cells. Thus, studies are ongoing to increase the efficacy of ATRA by improving its formulation, or using ATRA as part of combination therapies.

In summary, our data have shown for the first time that targeting Pin1 by ATRA effectively inhibits the growth of tamoxifen resistant breast cancer. This new approach represents a potential therapeutic strategy for intrinsic tamoxifen-resistant patients and relapsed ER $\alpha$ -positive breast cancer patients. Our findings shed new light on the molecular mechanism of ATRA in overcoming tamoxifen resistance and warrant future preclinical and clinical studies of ATRA in treating the tamoxifen resistant breast cancers.

## DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/**Supplementary Material**.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Internal review and ethics boards of Sun Yat-sen Memorial Hospital. The patients/participants provided their written informed consent to participate in this study. The animal study was reviewed and approved by the Animal Care and Use Committee of Sun Yat-sen University.

## AUTHOR CONTRIBUTIONS

M-LL and SH: conception and design. YL and YZ: development of methodology. YC, Z-ML, N-NL, and DL: acquisition of data. YC and SH: analysis and interpretation of data. M-LL and YY: writing, review, and/or revision of the manuscript. XZ and KL: technical and material support. M-LL and YY: study supervision.

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## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fcell.2019.00322/full#supplementary-material>

## REFERENCES

- Ali, S., Metzger, D., Bornert, J. M., and Chambon, P. (1993). Modulation of transcriptional activation by ligand-dependent phosphorylation of the human oestrogen receptor A/B region. *EMBO J.* 12, 1153–1160. doi: 10.1002/j.1460-2075.1993.tb05756.x
- Anbalagan, M., and Rowan, B. G. (2015). Estrogen receptor alpha phosphorylation and its functional impact in human breast cancer. *Mol. Cell Endocrinol.* 418(Pt 3), 264–272. doi: 10.1016/j.mce.2015.01.016
- Arnold, S. F., Obourn, J. D., Jaffe, H., and Notides, A. C. (1995). Phosphorylation of the human estrogen receptor by mitogen-activated protein kinase and casein kinase II: consequence on DNA binding. *J. Steroid Biochem. Mol. Biol.* 55, 163–172. doi: 10.1016/0960-0760(95)00177-2
- Bosch, A., Li, Z., Bergamaschi, A., Ellis, H., Toska, E., Prat, A., et al. (2015). PI3K inhibition results in enhanced estrogen receptor function and dependence in hormone receptor-positive breast cancer. *Sci. Transl. Med.* 7:283ra51. doi: 10.1126/scitranslmed.aaa4442
- Campaner, E., Rustighi, A., Zannini, A., Cristiani, A., Piazza, S., Ciani, Y., et al. (2017). A covalent PIN1 inhibitor selectively targets cancer cells by a dual mechanism of action. *Nat. Commun.* 8:15772. doi: 10.1038/ncomms15772
- Cannings, E., Kirkegaard, T., Tovey, S. M., Dunne, B., Cooke, T. G., and Bartlett, J. M. (2007). Bad expression predicts outcome in patients treated with tamoxifen. *Breast Cancer Res. Treat.* 102, 173–179. doi: 10.1007/s10549-006-9323-8
- Chen, W., Zheng, R., Baade, P. D., Zhang, S., Zeng, H., Bray, F., et al. (2016). Cancer statistics in China, 2015. *CA Cancer J. Clin.* 66, 115–132. doi: 10.3322/caac.21338
- Chu, J., Zhu, Y., Liu, Y., Sun, L., Lv, X., Wu, Y., et al. (2015). E2F7 overexpression leads to tamoxifen resistance in breast cancer cells by competing with E2F1 at miR-15a/16 promoter. *Oncotarget* 6, 31944–31957. doi: 10.18632/oncotarget.5128
- Cui, J., Yang, Y., Li, H., Leng, Y., Qian, K., Huang, Q., et al. (2015). MiR-873 regulates ERalpha transcriptional activity and tamoxifen resistance via targeting CDK3 in breast cancer cells. *Oncogene* 34, 3895–3907. doi: 10.1038/nc.2014.430
- de The, H., and Chen, Z. (2010). Acute promyelocytic leukaemia: novel insights into the mechanisms of cure. *Nat. Rev. Cancer* 10, 775–783. doi: 10.1038/nrc2943
- Deroo, B. J., and Korach, K. S. (2006). Estrogen receptors and human disease. *J. Clin. Invest.* 116, 561–570. doi: 10.1172/jci27987
- Endoh, H., Maruyama, K., Masuhiro, Y., Kobayashi, Y., Goto, M., Tai, H., et al. (1999). Purification and identification of p68 RNA helicase acting as a transcriptional coactivator specific for the activation function 1 of human estrogen receptor alpha. *Mol. Cell Biol.* 19, 5363–5372. doi: 10.1128/mcb.19.8.5363
- Ferraiuolo, R. M., Tubman, J., Sinha, I., Hamm, C., and Porter, L. A. (2017). The cyclin-like protein, SPY1, regulates the ERalpha and ERK1/2 pathways promoting tamoxifen resistance. *Oncotarget* 8, 23337–23352. doi: 10.18632/oncotarget.15578
- Garcia-Becerra, R., Santos, N., Diaz, L., and Camacho, J. (2012). Mechanisms of resistance to endocrine therapy in breast cancer: focus on signaling pathways, miRNAs and genetically based resistance. *Int. J. Mol. Sci.* 14, 108–145. doi: 10.3390/ijms14010108
- Girault, I., Bieche, I., and Lidereau, R. (2006). Role of estrogen receptor alpha transcriptional coregulators in tamoxifen resistance in breast cancer. *Maturitas* 54, 342–351. doi: 10.1016/j.maturitas.2006.06.003
- He, H., Sinha, I., Fan, R., Haldosen, L. A., Yan, F., Zhao, C., et al. (2018). c-Jun/AP-1 overexpression reprograms ERalpha signaling related to tamoxifen response in ERalpha-positive breast cancer. *Oncogene* 37, 2586–2600. doi: 10.1038/s41388-018-0165-8
- Herman, M. E., and Katzenellenbogen, B. S. (1996). Response-specific antiestrogen resistance in a newly characterized MCF-7 human breast cancer cell line resulting from long-term exposure to trans-hydroxytamoxifen. *J. Steroid Biochem. Mol. Biol.* 59, 121–134. doi: 10.1016/s0960-0760(96)00114-8
- Huang, M. E., Ye, Y. C., Chen, S. R., Chai, J. R., Lu, J. X., Zhao, L., et al. (1988). Use of all-trans retinoic acid in the treatment of acute promyelocytic leukemia. *Blood* 72, 567–572. doi: 10.1182/blood.v72.2.567.567
- Hur, J., Chesnes, J., Coser, K. R., Lee, R. S., Geck, P., Isselbacher, K. J., et al. (2004). The Bik BH3-only protein is induced in estrogen-starved and antiestrogen-exposed breast cancer cells and provokes apoptosis. *Proc. Natl. Acad. Sci. U.S.A.* 101, 2351–2356. doi: 10.1073/pnas.0307337101
- Johansson, H. J., Sanchez, B. C., Mundt, F., Forshed, J., Kovacs, A., Panizza, E., et al. (2013). Retinoic acid receptor alpha is associated with tamoxifen resistance in breast cancer. *Nat. Commun.* 4:2175. doi: 10.1038/ncomms3175
- Johnston, S. R. (2010). New strategies in estrogen receptor-positive breast cancer. *Clin. Cancer Res.* 16, 1979–1987. doi: 10.1158/1078-0432.CCR-09-1823
- Khanal, P., Yun, H. J., Lim, S. C., Ahn, S. G., Yoon, H. E., Kang, K. W., et al. (2012). Proyl isomerase Pin1 facilitates ubiquitin-mediated degradation of cyclin-dependent kinase 10 to induce tamoxifen resistance in breast cancer cells. *Oncogene* 31, 3845–3856. doi: 10.1038/nc.2011.548
- Kim, J. A., Kim, M. R., Kim, O., Phuong, N. T., Yun, J., Oh, W. K., et al. (2012). Amurensin G inhibits angiogenesis and tumor growth of tamoxifen-resistant breast cancer via Pin1 inhibition. *Food Chem. Toxicol.* 50, 3625–3634. doi: 10.1016/j.fct.2012.07.027
- Kim, M. R., Choi, H. K., Cho, K. B., Kim, H. S., and Kang, K. W. (2009a). Involvement of Pin1 induction in epithelial-mesenchymal transition of tamoxifen-resistant breast cancer cells. *Cancer Sci.* 100, 1834–1841. doi: 10.1111/j.1349-7006.2009.01260.x
- Kim, M. R., Choi, H. S., Yang, J. W., Park, B. C., Kim, J. A., and Kang, K. W. (2009b). Enhancement of vascular endothelial growth factor-mediated angiogenesis in tamoxifen-resistant breast cancer cells: role of Pin1 overexpression. *Mol. Cancer Ther.* 8, 2163–2171. doi: 10.1158/1535-7163.MCT-08-1061
- Knowlden, J. M., Hutcheson, I. R., Jones, H. E., Madden, T., Gee, J. M., Harper, M. E., et al. (2003). Elevated levels of epidermal growth factor receptor/c-erbB2 heterodimers mediate an autocrine growth regulatory pathway in tamoxifen-resistant MCF-7 cells. *Endocrinology* 144, 1032–1044. doi: 10.1210/en.2002-220620

- Kozono, S., Lin, Y. M., Seo, H. S., Pinch, B., Lian, X., Qiu, C., et al. (2018). Arsenic targets Pin1 and cooperates with retinoic acid to inhibit cancer-driving pathways and tumor-initiating cells. *Nat. Commun.* 9:3069. doi: 10.1038/s41467-018-05402-2
- Lee, Y. J., and Gorski, J. (1996). Estrogen-induced transcription of the progesterone receptor gene does not parallel estrogen receptor occupancy. *Proc. Natl. Acad. Sci. U.S.A.* 93, 15180–15184. doi: 10.1073/pnas.93.26.15180
- Liao, X. H., Zhang, A. L., Zheng, M., Li, M. Q., Chen, C. P., Xu, H., et al. (2017). Chemical or genetic Pin1 inhibition exerts potent anticancer activity against hepatocellular carcinoma by blocking multiple cancer-driving pathways. *Sci. Rep.* 7:43639. doi: 10.1038/srep43639
- Liao, Y., Wei, Y., Zhou, X., Yang, J. Y., Dai, C., Chen, Y. J., et al. (2009). Peptidyl-prolyl cis/trans isomerase Pin1 is critical for the regulation of PKB/Akt stability and activation phosphorylation. *Oncogene* 28, 2436–2445. doi: 10.1038/onc.2009.98
- Lu, K. P., and Zhou, X. Z. (2007). The prolyl isomerase PIN1: a pivotal new twist in phosphorylation signalling and disease. *Nat. Rev. Mol. Cell Biol.* 8, 904–916. doi: 10.1038/nrm2261
- Lu, R., Hu, X., Zhou, J., Sun, J., Zhu, A. Z., Xu, X., et al. (2016). COPS5 amplification and overexpression confers tamoxifen-resistance in ERalpha-positive breast cancer by degradation of NCoR. *Nat. Commun.* 7:12044. doi: 10.1038/ncomms12044
- Lu, Z., and Hunter, T. (2014). Prolyl isomerase Pin1 in cancer. *Cell Res.* 24, 1033–1049. doi: 10.1038/cr.2014.109
- Luo, M. L., Gong, C., Chen, C. H., Hu, H., Huang, P., Zheng, M., et al. (2015). The Rab2A GTPase promotes breast cancer stem cells and tumorigenesis via Erk signaling activation. *Cell Rep.* 11, 111–124. doi: 10.1016/j.celrep.2015.03.002
- Luo, M. L., Gong, C., Chen, C. H., Lee, D. Y., Hu, H., Huang, P., et al. (2014). Prolyl isomerase Pin1 acts downstream of miR200c to promote cancer stem-like cell traits in breast cancer. *Cancer Res.* 74, 3603–3616. doi: 10.1158/0008-5472.CAN-13-2785
- Marsh, L. A., Carrera, S., Shandilya, J., Heesom, K. J., Davidson, A. D., Medler, K. F., et al. (2017). BASP1 interacts with oestrogen receptor alpha and modifies the tamoxifen response. *Cell Death Dis.* 8:e2771. doi: 10.1038/cddis.2017.179
- Miller, T. E., Ghoshal, K., Ramaswamy, B., Roy, S., Datta, J., Shapiro, C. L., et al. (2008). MicroRNA-221/222 confers tamoxifen resistance in breast cancer by targeting p27Kip1. *J. Biol. Chem.* 283, 29897–29903. doi: 10.1074/jbc.M804612200
- Mohseni, M., Cidado, J., Croessmann, S., Cravero, K., Cimino-Mathews, A., Wong, H. Y., et al. (2014). MACROD2 overexpression mediates estrogen independent growth and tamoxifen resistance in breast cancers. *Proc. Natl. Acad. Sci. U.S.A.* 111, 17606–17611. doi: 10.1073/pnas.1408650111
- Namgoong, G. M., Khanal, P., Cho, H. G., Lim, S. C., Oh, Y. K., Kang, B. S., et al. (2010). The prolyl isomerase Pin1 induces LC-3 expression and mediates tamoxifen resistance in breast cancer. *J. Biol. Chem.* 285, 23829–23841. doi: 10.1074/jbc.M109.092874
- Nilsson, S., Koehler, K. F., and Gustafsson, J. A. (2011). Development of subtype-selective oestrogen receptor-based therapeutics. *Nat. Rev. Drug Discov.* 10, 778–792. doi: 10.1038/nrd3551
- Nonclercq, D., Journe, F., Body, J. J., Leclercq, G., and Laurent, G. (2004). Ligand-independent and agonist-mediated degradation of estrogen receptor-alpha in breast carcinoma cells: evidence for distinct degradative pathways. *Mol. Cell Endocrinol.* 227, 53–65. doi: 10.1016/j.mce.2004.07.003
- Osborne, C. K., and Schiff, R. (2005). Estrogen-receptor biology: continuing progress and therapeutic implications. *J. Clin. Oncol.* 23, 1616–1622. doi: 10.1200/jco.2005.10.036
- Osborne, C. K., and Schiff, R. (2011). Mechanisms of endocrine resistance in breast cancer. *Annu. Rev. Med.* 62, 233–247.
- Rajbhandari, P., Finn, G., Solodin, N. M., Singarapu, K. K., Sahu, S. C., Markley, J. L., et al. (2012). Regulation of estrogen receptor alpha N-terminus conformation and function by peptidyl prolyl isomerase Pin1. *Mol. Cell Biol.* 32, 445–457. doi: 10.1128/MCB.06073-11
- Rajbhandari, P., Ozers, M. S., Solodin, N. M., Warren, C. L., and Alarid, E. T. (2015). Peptidylprolyl isomerase Pin1 directly enhances the DNA binding functions of estrogen receptor alpha. *J. Biol. Chem.* 290, 13749–13762. doi: 10.1074/jbc.M114.621698
- Rajbhandari, P., Schalper, K. A., Solodin, N. M., Ellison-Zelski, S. J., Ping Lu, K., Rimm, D. L., et al. (2014). Pin1 modulates ERalpha levels in breast cancer through inhibition of phosphorylation-dependent ubiquitination and degradation. *Oncogene* 33, 1438–1447. doi: 10.1038/onc.2013.78
- Rustighi, A., Zannini, A., Campaner, E., Ciani, Y., Piazza, S., and Del Sal, G. (2017). PIN1 in breast development and cancer: a clinical perspective. *Cell Death Dif.* 24, 200–211. doi: 10.1038/cdd.2016.122
- Rustighi, A., Zannini, A., Tiberi, L., Sommaggio, R., Piazza, S., Sorrentino, G., et al. (2014). Prolyl-isomerase Pin1 controls normal and cancer stem cells of the breast. *EMBO Mol. Med.* 6, 99–119. doi: 10.1002/emmm.201302909
- Sanz, M. A., and Lo-Coco, F. (2011). Modern approaches to treating acute promyelocytic leukemia. *J. Clin. Oncol.* 29, 495–503. doi: 10.1200/jco.2010.32.1067
- Shao, W., Keeton, E. K., McDonnell, D. P., and Brown, M. (2004). Coactivator AIB1 links estrogen receptor transcriptional activity and stability. *Proc. Natl. Acad. Sci. U.S.A.* 101, 11599–11604. doi: 10.1073/pnas.0402997101
- Sheeler, C. Q., Singleton, D. W., and Khan, S. A. (2003). Mutation of serines 104, 106, and 118 inhibits dimerization of the human estrogen receptor in yeast. *Endocr. Res.* 29, 237–255. doi: 10.1081/erc-120022321
- Stanya, K. J., Liu, Y., Means, A. R., and Kao, H. Y. (2008). Cdk2 and Pin1 negatively regulate the transcriptional corepressor SMRT. *J. Cell Biol.* 183, 49–61. doi: 10.1083/jcb.200806172
- Stenoien, D. L., Patel, K., Mancini, M. G., Dutertre, M., Smith, C. L., O'Malley, B. W., et al. (2001). FRAP reveals that mobility of oestrogen receptor-alpha is ligand- and proteasome-dependent. *Nat. Cell Biol.* 3, 15–23. doi: 10.1038/35050515
- Stone, A., Cowley, M. J., Valdes-Mora, F., McCloy, R. A., Sergio, C. M., Gallego-Ortega, D., et al. (2013). BCL-2 hypermethylation is a potential biomarker of sensitivity to antimetabolic chemotherapy in endocrine-resistant breast cancer. *Mol. Cancer Ther.* 12, 1874–1885. doi: 10.1158/1535-7163.MCT-13-0012
- Sun, M., Paciga, J. E., Feldman, R. I., Yuan, Z., Coppola, D., Lu, Y. Y., et al. (2001). Phosphatidylinositol-3-OH Kinase (PI3K)/AKT2, activated in breast cancer, regulates and is induced by estrogen receptor alpha (ERalpha) via interaction between ERalpha and PI3K. *Cancer Res.* 61, 5985–5991.
- Svensson, S., Jirstrom, K., Ryden, L., Roos, G., Emdin, S., Ostrowski, M. C., et al. (2005). ERK phosphorylation is linked to VEGFR2 expression and Ets-2 phosphorylation in breast cancer and is associated with tamoxifen treatment resistance and small tumours with good prognosis. *Oncogene* 24, 4370–4379. doi: 10.1038/sj.onc.1208626
- Tsimberidou, A. M., Tirado-Gomez, M., Andreeff, M., O'Brien, S., Kantarjian, H., Keating, M., et al. (2006). Single-agent liposomal all-trans retinoic acid can cure some patients with untreated acute promyelocytic leukemia: an update of The University of Texas M. D. Anderson Cancer Center series. *Leuk. Lymphoma* 47, 1062–1068. doi: 10.1080/10428190500463932
- Wei, S., Kozono, S., Kats, L., Nechama, M., Li, W., Guarnerio, J., et al. (2015). Active Pin1 is a key target of all-trans retinoic acid in acute promyelocytic leukemia and breast cancer. *Nat. Med.* 21, 457–466. doi: 10.1038/nm.3839
- Westervelt, P., Pollock, J. L., Oldfather, K. M., Walter, M. J., Ma, M. K., Williams, A., et al. (2002). Adaptive immunity cooperates with liposomal all-trans-retinoic acid (ATRA) to facilitate long-term molecular remissions in mice with acute promyelocytic leukemia. *Proc. Natl. Acad. Sci. U.S.A.* 99, 9468–9473. doi: 10.1073/pnas.132657799
- Wijayarathne, A. L., and McDonnell, D. P. (2001). The human estrogen receptor-alpha is a ubiquitinated protein whose stability is affected differentially by agonists, antagonists, and selective estrogen receptor modulators. *J. Biol. Chem.* 276, 35684–35692. doi: 10.1074/jbc.M101097200
- Wu, Y., Zhang, Z., Cenciari, M. E., Proietti, C. J., Amasino, M., Hong, T., et al. (2018). Tamoxifen resistance in breast cancer is regulated by the EZH2-ERalpha-GREB1 transcriptional axis. *Cancer Res.* 78, 671–684. doi: 10.1158/0008-5472.CAN-17-1327
- Xiong, R., Zhao, J., Gutgesell, L. M., Wang, Y., Lee, S., Karumudi, B., et al. (2017). Novel Selective Estrogen Receptor Downregulators (SERDs) developed against treatment-resistant breast cancer. *J. Med. Chem.* 60, 1325–1342. doi: 10.1021/acs.jmedchem.6b01355
- Yager, J. D., and Davidson, N. E. (2006). Estrogen carcinogenesis in breast cancer. *N. Engl. J. Med.* 354, 270–282.
- Yang, D., Luo, W., Wang, J., Zheng, M., Liao, X. H., Zhang, N., et al. (2018). A novel controlled release formulation of the Pin1 inhibitor ATRA to improve liver cancer therapy by simultaneously blocking multiple cancer pathways. *J. Control Release* 269, 405–422. doi: 10.1016/j.jconrel.2017.11.031

- Zhang, J., Zhou, C., Jiang, H., Liang, L., Shi, W., Zhang, Q., et al. (2017). ZEB1 induces ER-alpha promoter hypermethylation and confers antiestrogen resistance in breast cancer. *Cell Death Dis.* 8, e2732. doi: 10.1038/cddis.2017.154
- Zhang, X., Peng, D., Xi, Y., Yuan, C., Sagum, C. A., Klein, B. J., et al. (2016). G9a-mediated methylation of ERalpha links the PHF20/MOF histone acetyltransferase complex to hormonal gene expression. *Nat. Commun.* 7:10810. doi: 10.1038/ncomms10810
- Zhou, X. Z., and Lu, K. P. (2016). The isomerase PIN1 controls numerous cancer-driving pathways and is a unique drug target. *Nat. Rev. Cancer.* 16, 463–478. doi: 10.1038/nrc.2016.49

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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