iScience, Volume *23*

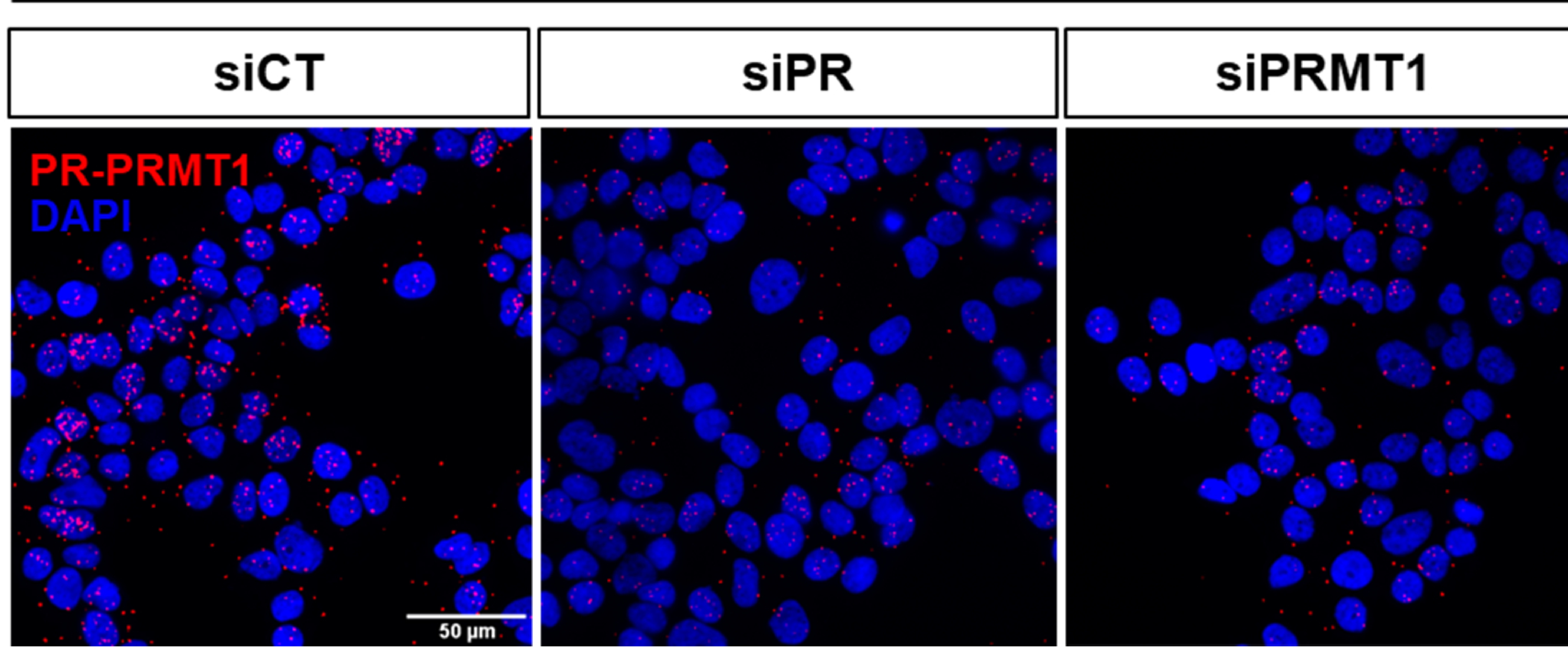
Supplemental Information

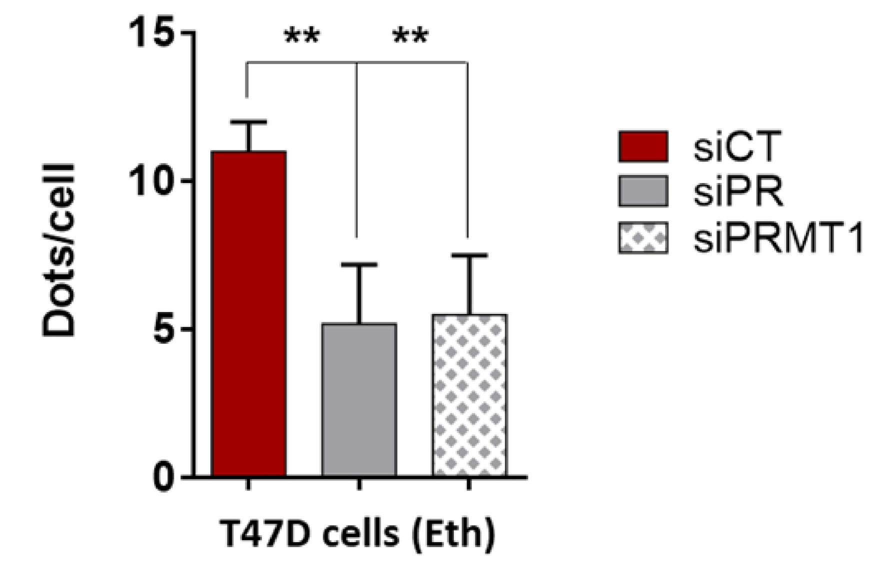
PRMT1 Is Critical for the Transcriptional Activity and the Stability of the Progesterone Receptor

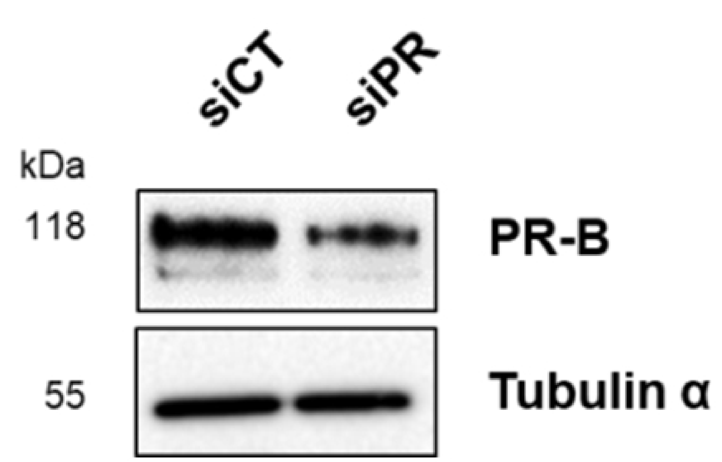
Lucie Malbeteau, Coralie Poulard, Cécile Languilaire, Ivan Mikaelian, Frédéric Flamant, Muriel Le Romancer, and Laura Corbo

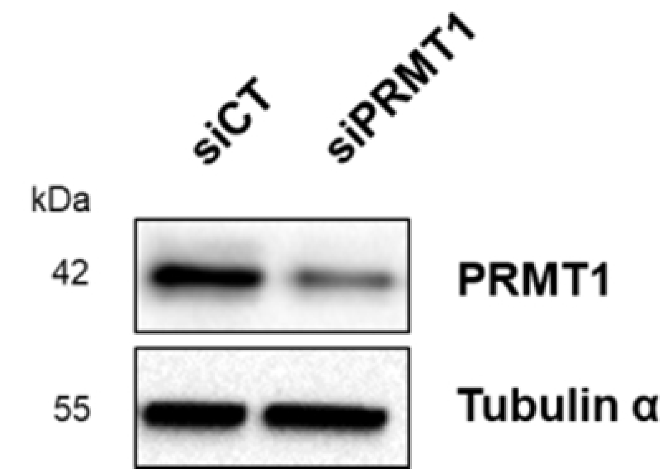
A.

PR - PRMT1



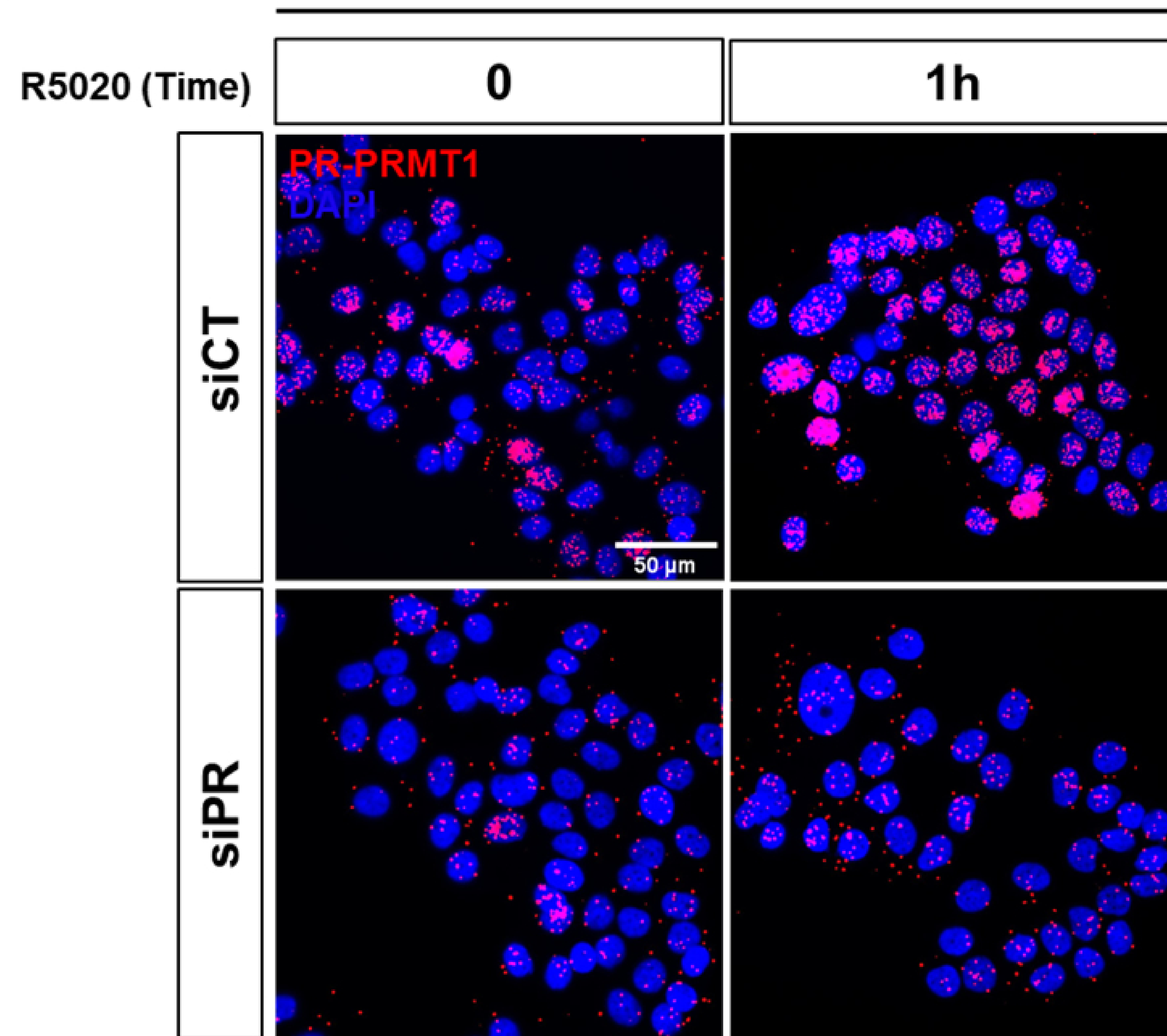


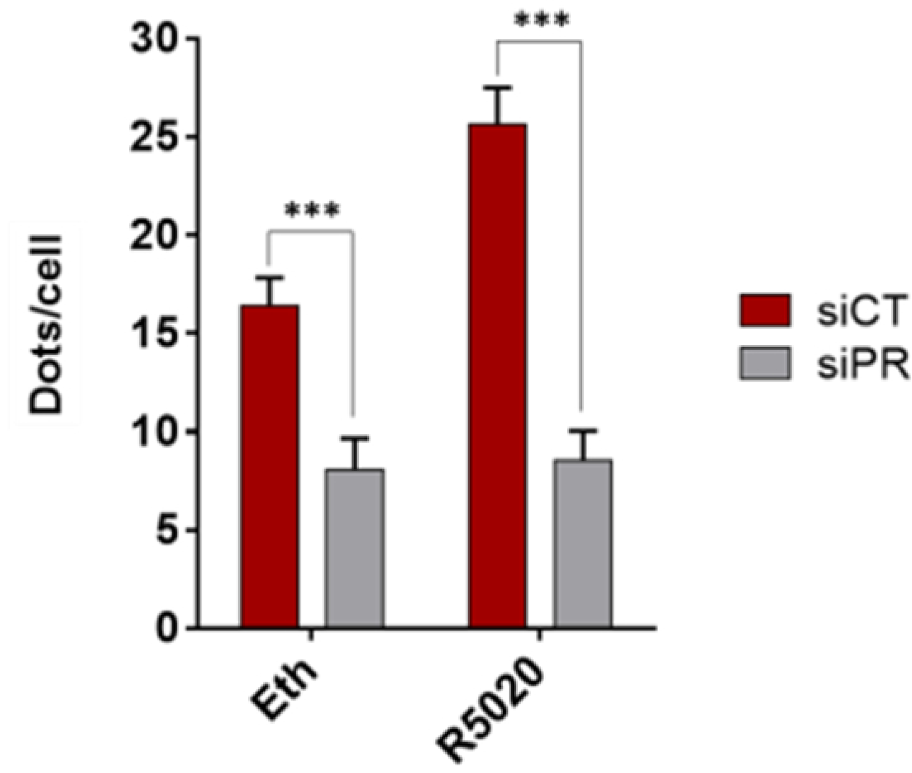




B.

PR - PRMT1





C.

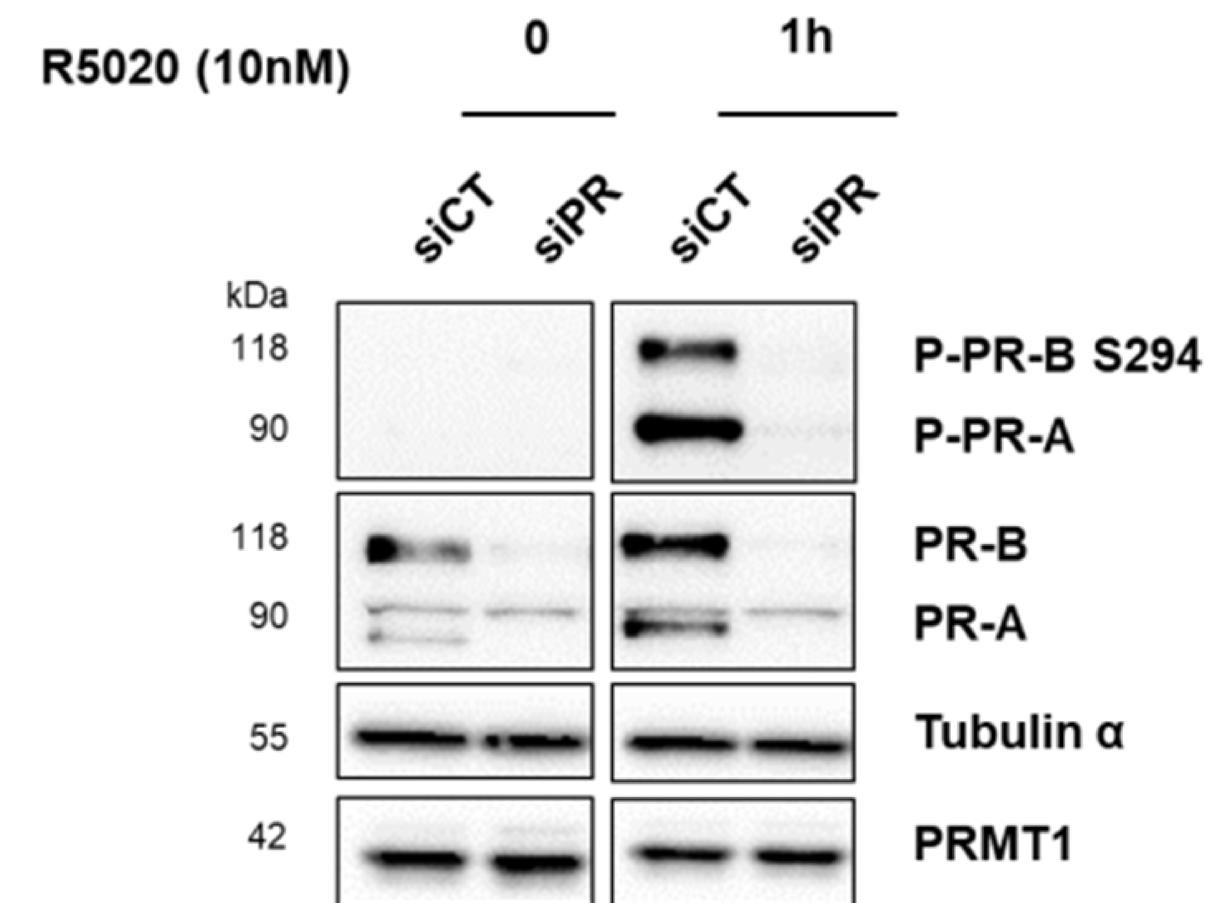
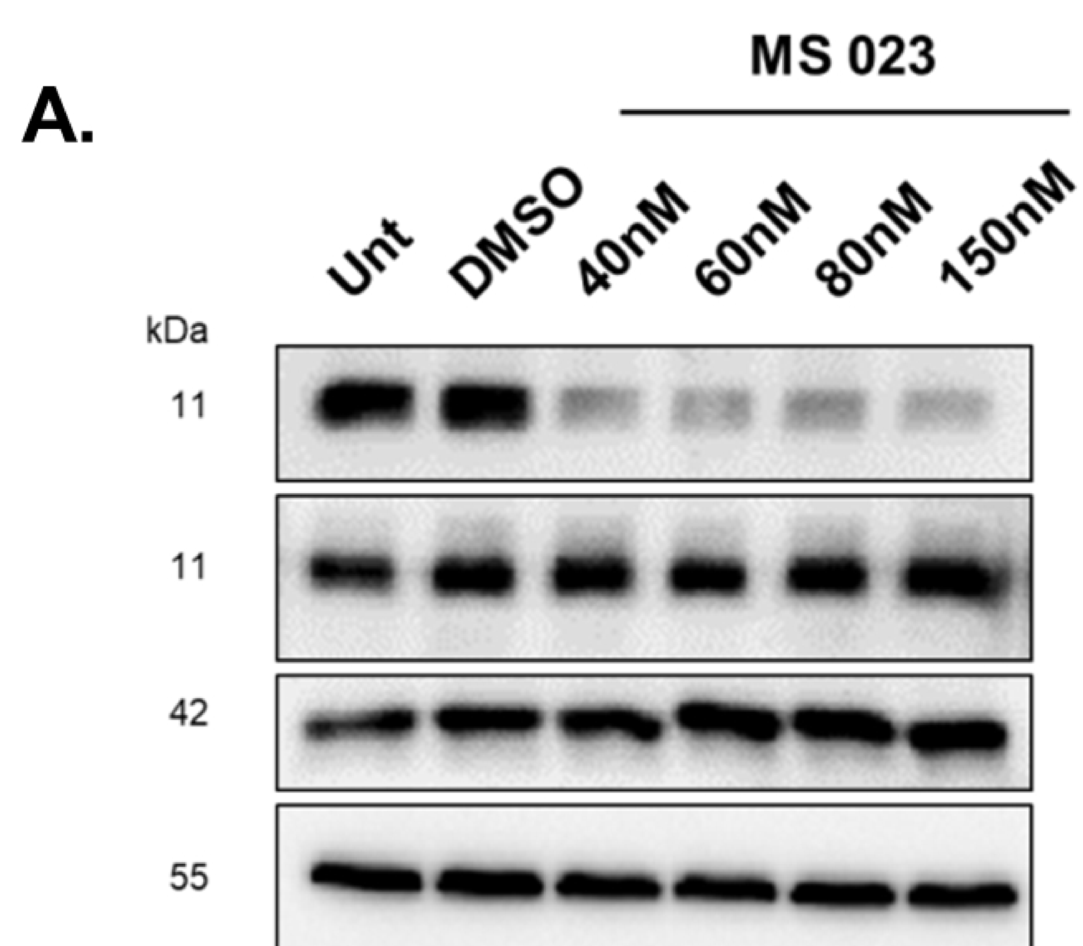
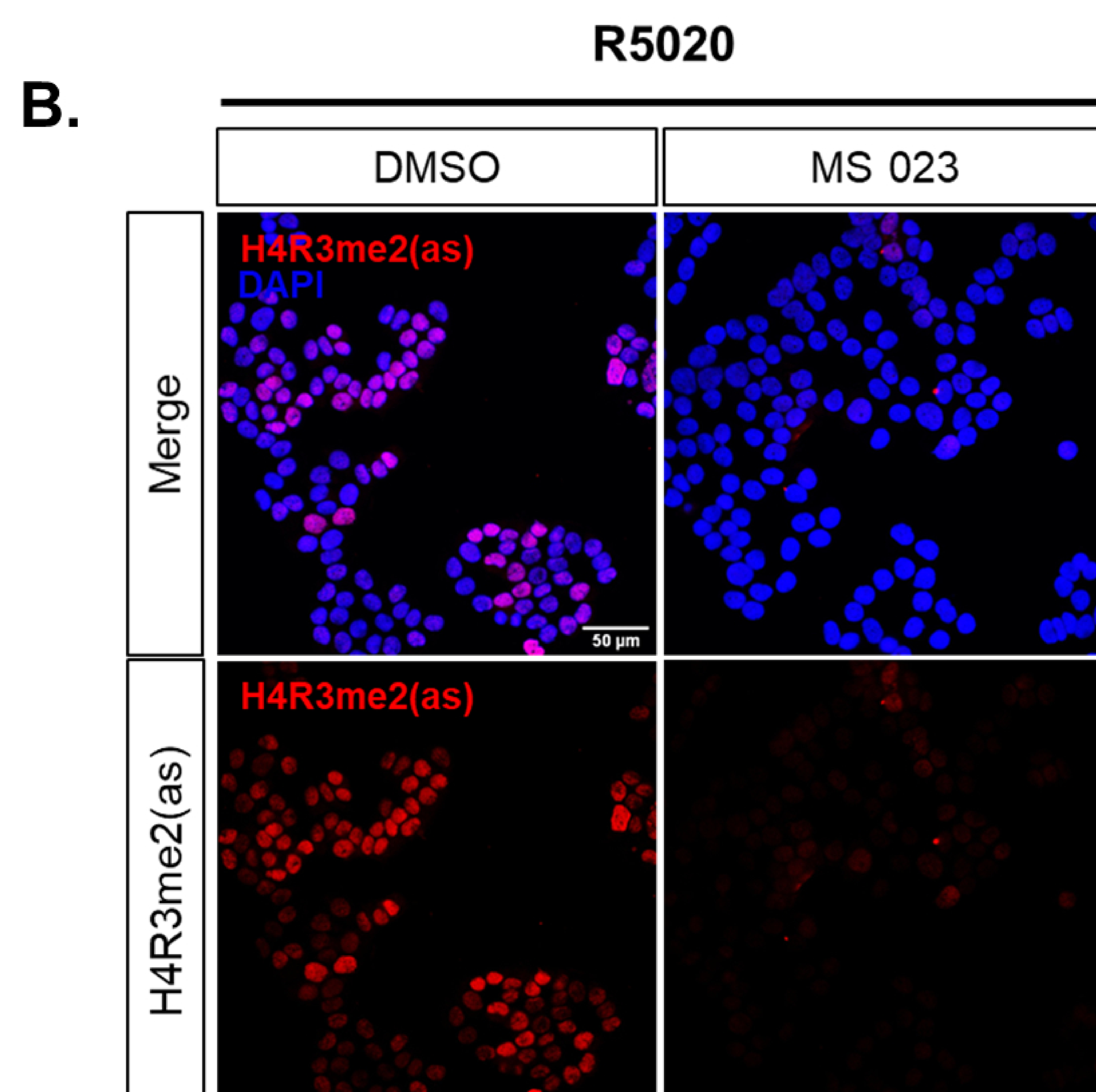
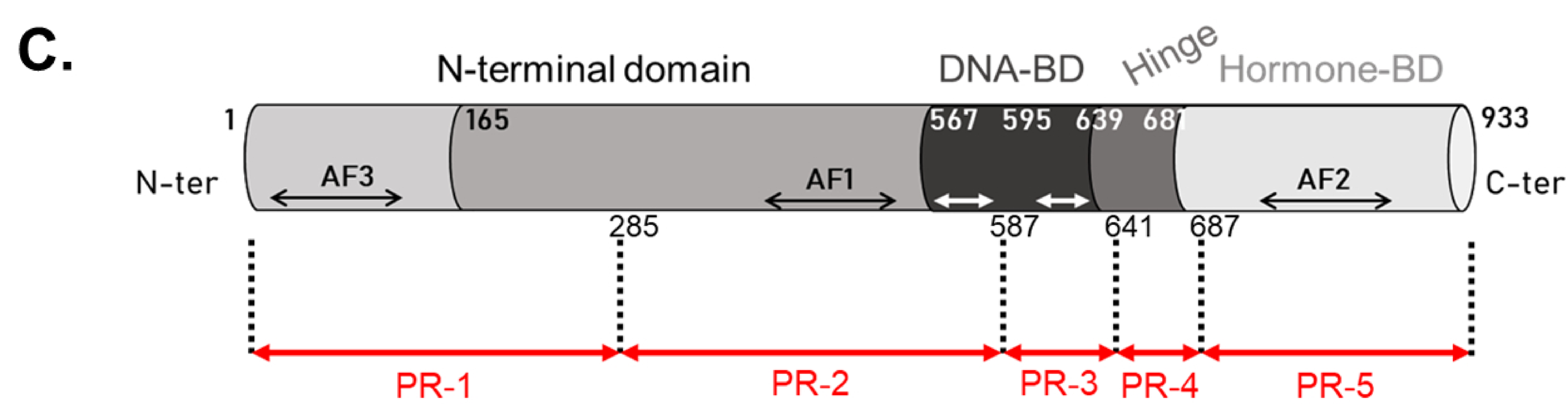
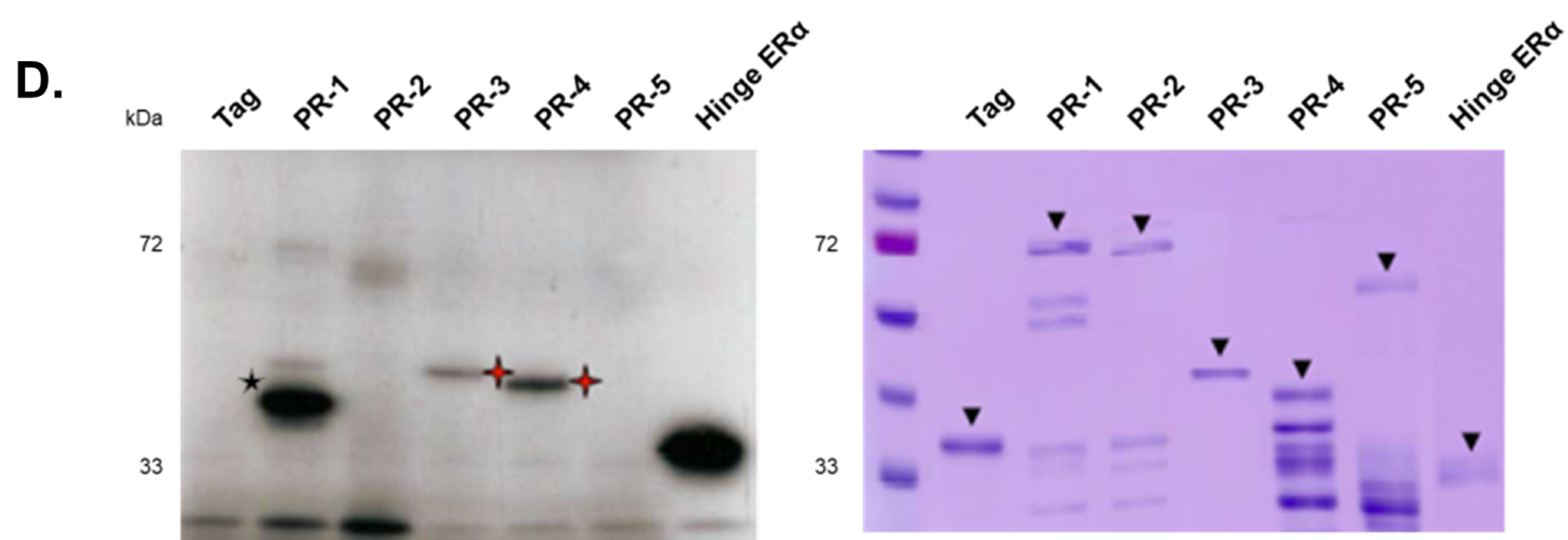


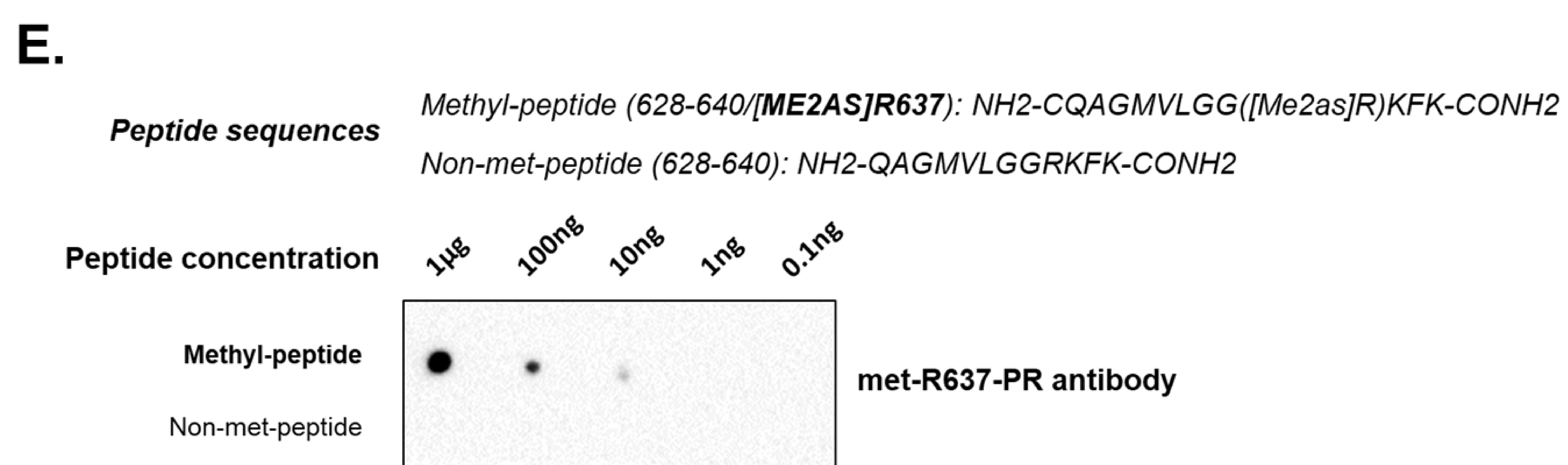
Figure S1, related to Fig. 1 | PRMT1 and PR interact in the nucleus of T47D breast cancer cells. Validation of the specificity of PRMT1-PR association detected by Ligation Assay (PLA). T47D were grown on coverslips in 12-well plates and transfected with siRNAs control (siCT) or against PR (siPR) or PRMT1 (siPRMT1). PLA was used to detect the cellular interaction of endogenous PRMT1 and PR **A.** in unstimulated cells or **B.** after R5020 treatment (1h). The interactions are represented by red dots. The nuclei were counterstained with DAPI (blue) (Obj: X60). Quantification of the number of signals per cell was performed by computer-assisted analysis, as reported in the Transparent Methods section, and is shown in the right panels. The mean ± SD of one experiment representative of three experiments is shown. The *p*-value was determined using the Student's t-test. ** indicates a $p \leq 0.01$ and *** indicates a $p \leq 0.001$. **C.** The efficacy of PRMT1- and PR-siRNAs treatments were analyzed by immunoblot.


A.
MS 023
Unt
DMSO
40nM
60nM
80nM
150nM
kDa
11
11
42
55
H4R3me2(as)
Total H4
PRMT1
Tubulin α


B.
R5020
DMSO
MS 023
Merge
H4R3me2(as)
DAPI
50 µm
H4R3me2(as)
H4R3me2(as)


C.
N-terminal domain
DNA-BD
Hinge
Hormone-BD
1
165
567
595
639
687
933
N-ter
AF3
AF1
AF2
C-ter
285
587
641
687
PR-1
PR-2
PR-3
PR-4
PR-5


D.
kDa
Tag
PR-1
PR-2
PR-3
PR-4
PR-5
Hinge ERα
72
33
Tag
PR-1
PR-2
PR-3
PR-4
PR-5
Hinge ERα
72
33


E.
Peptide sequences
Methyl-peptide (628-640/[ME2AS]R637): NH2-CQAGMVLGG([Me2as]R)KFK-CONH2
Non-met-peptide (628-640): NH2-QAGMVLGGRKFK-CONH2
Peptide concentration
1µg
100ng
10ng
1ng
0.1ng
Methyl-peptide
Non-met-peptide
met-R637-PR antibody

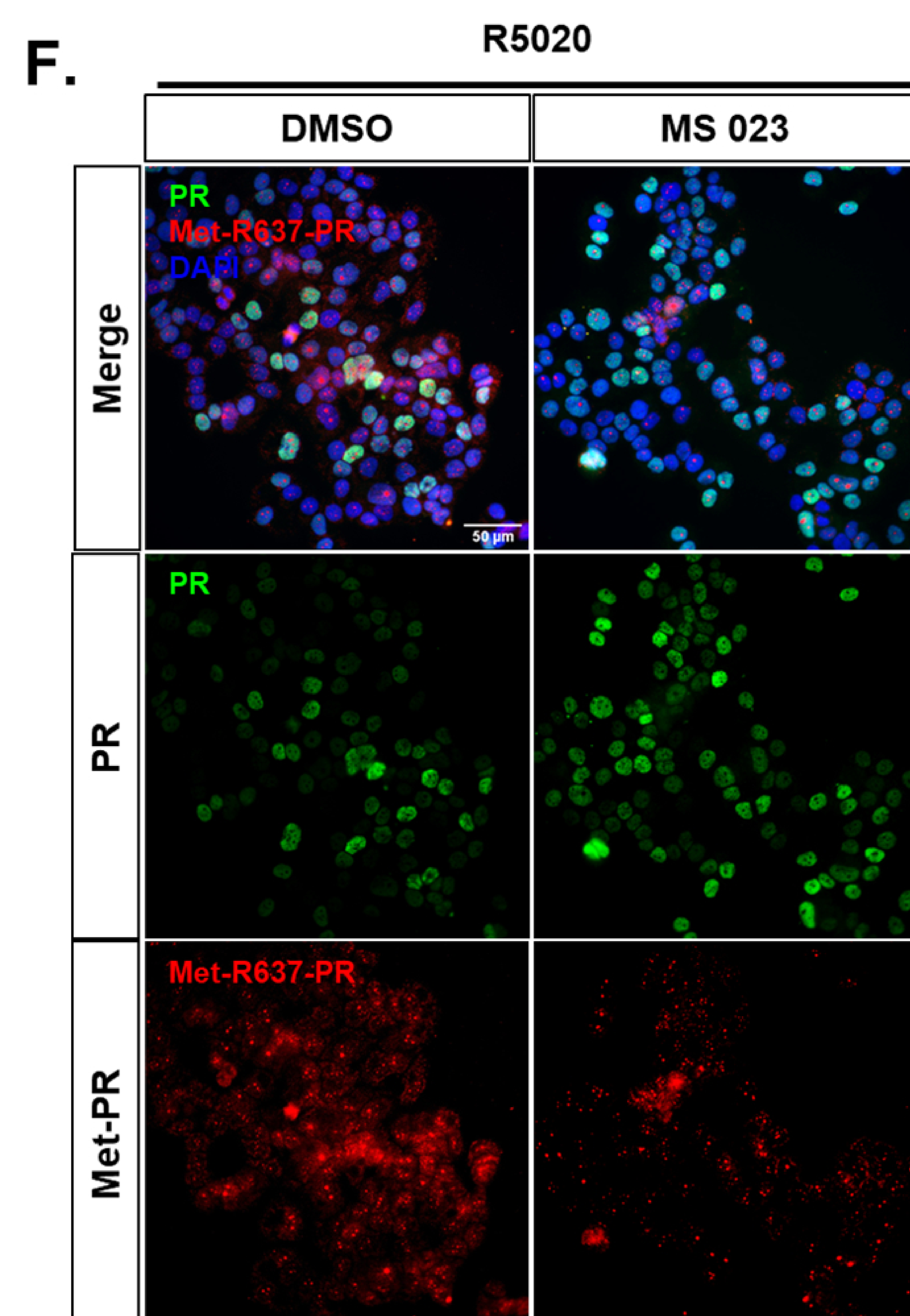
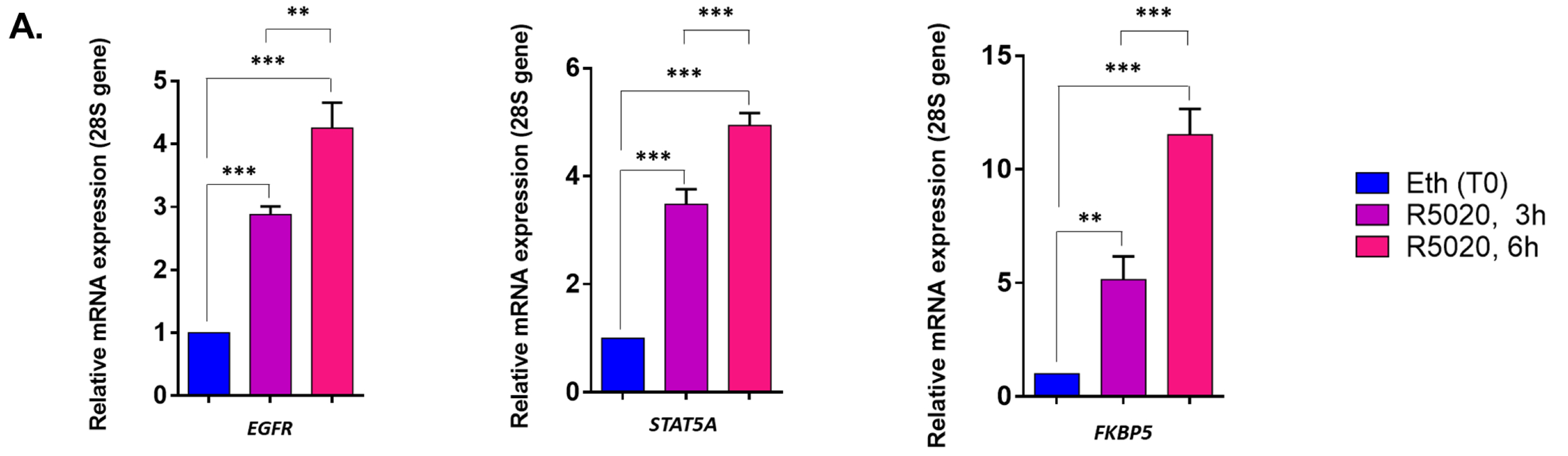
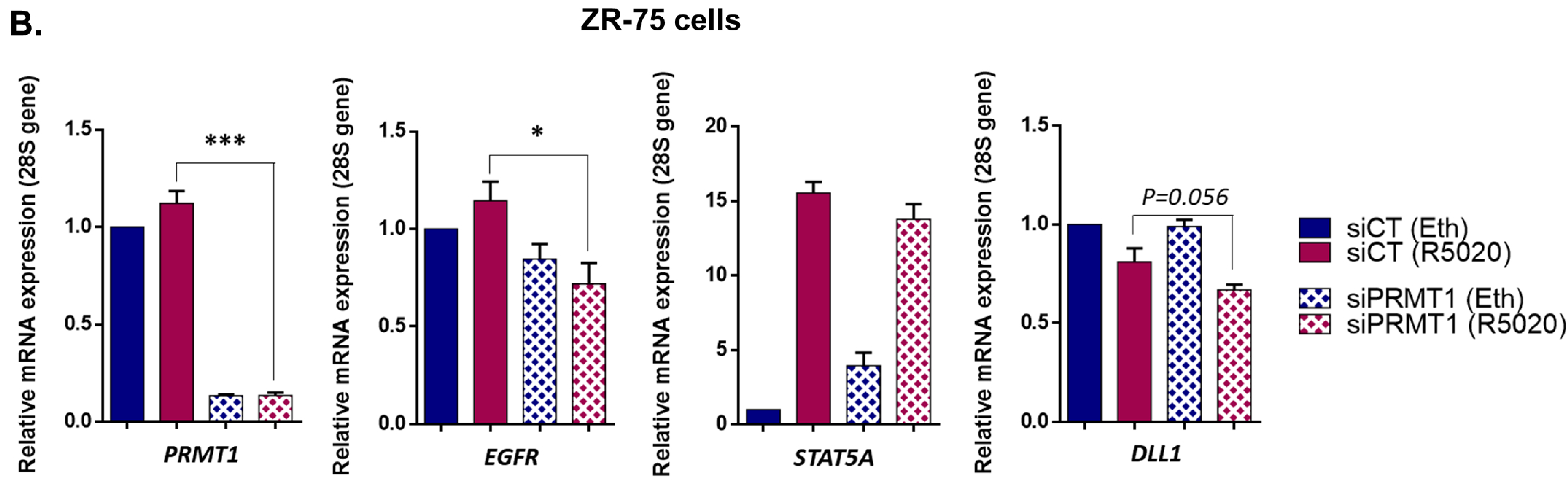

F.
R5020
DMSO
MS 023
Merge
PR
Met-R637-PR
DAPI
50 µm
PR
PR
Met-PR
Met-R637-PR

Figure S2, related to Fig. 2 | PR is methylated on arginine residues in R5020-stimulated T47D breast cancer cells. A-B. Analysis of MS 023 inhibitor specificity. **A**. Immunoblot of R5020-stimulated T47D cells (1h), treated or not (DMSO) with different quantities of MS 023. Expression of indicated proteins were analyzed. **B.** Immunofluorescence of T47D cells, treated with 60 nM of MS 023 (or DMSO) and then stimulated 1h with 10 nM of R5020, using anti-H4R3me2(as) primary antibody. The nuclei were counterstained with DAPI (blue) (Obj: X40). **C.** Schematic representation of human GST-tagged PR fragments used for the *in vitro* methylation assays. **D.** An *in vitro* methylation assay was conducted by incubating different recombinant GST-PR fragments, GST-ER hinge used as a positive control and GST (tag) as a negative control, with recombinant GST-PRMT1, in the presence of [methyl-3H] SAM. Reaction products were analyzed by SDS-PAGE followed by fluorography. The migration and the quality of recombinant GST-fragments used as substrates were verified by a Coomassie-stained SDS-PAGE gel, shown in the right panel. The methylated proteins were visualized by autoradiography. Red stars indicate the methylated fragments of PR. Black star indicates a bacteria associated contaminant, as the signal did not correspond to any detectable fragment by Coomassie blue staining gel, shown in the right panel. **E.** Dot-blot was performed using increasing amounts of the indicated peptides, asymmetrically methylated or not on the arginine 637, and immunoblotted with the met-R637-PR antibody. Peptide sequences were shown in the upper panel. **F.** Immunofluorescence assay performed on T47D cells treated with 60 nM of MS 023 inhibitor (or DMSO) and stimulated with 10 nM of R5020 for 1h using the met-R637-PR and anti-PR antibodies. The nuclei were counterstained with DAPI (blue) (Obj: X40).


A.
Relative mRNA expression (28S gene)
EGFR
STAT5A
FKBP5
Eth (T0)
R5020, 3h
R5020, 6h


B.
ZR-75 cells
Relative mRNA expression (28S gene)
PRMT1
EGFR
STAT5A
DLL1
P=0.056
siCT (Eth)
siCT (R5020)
siPRMT1 (Eth)
siPRMT1 (R5020)

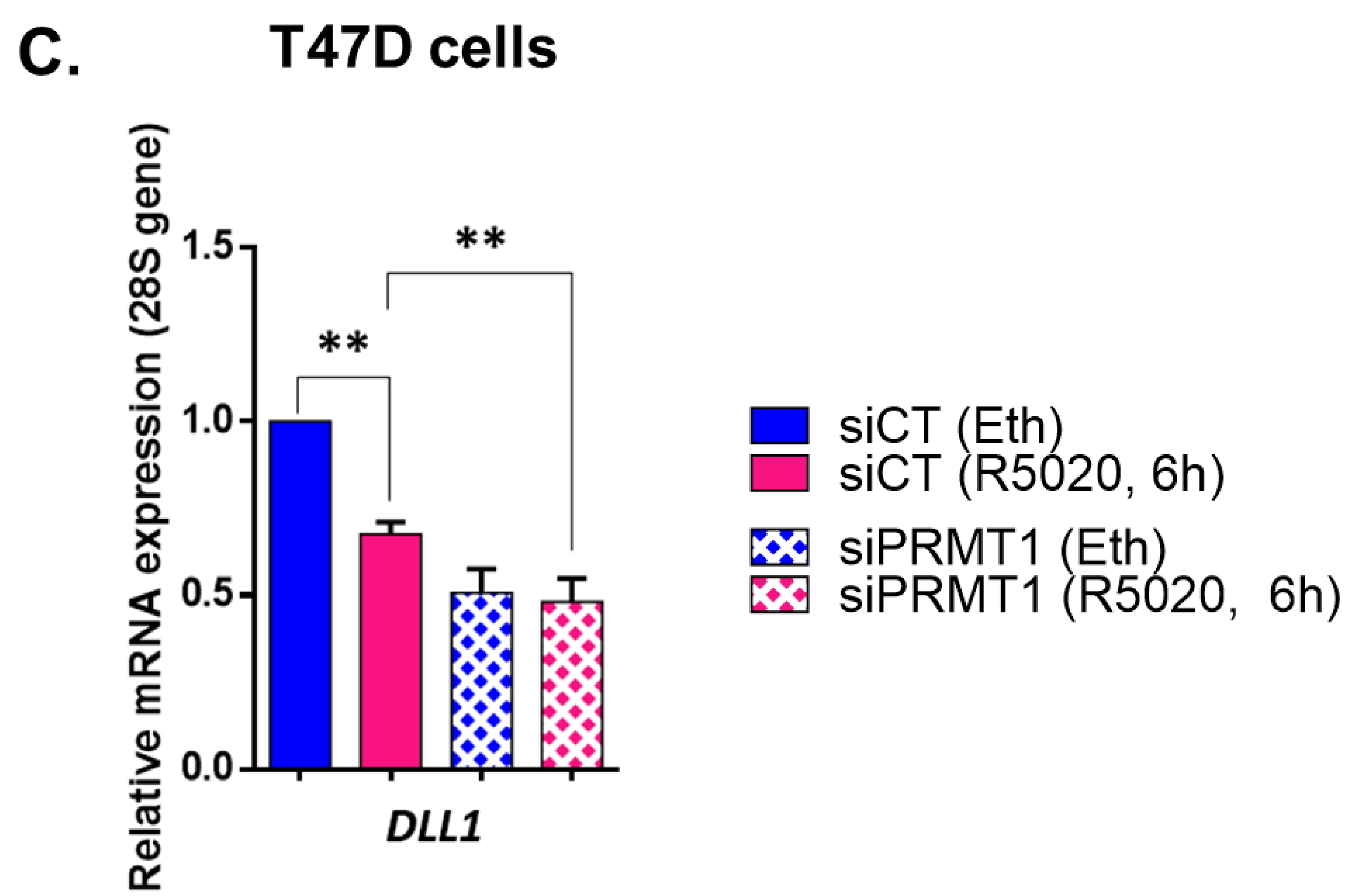
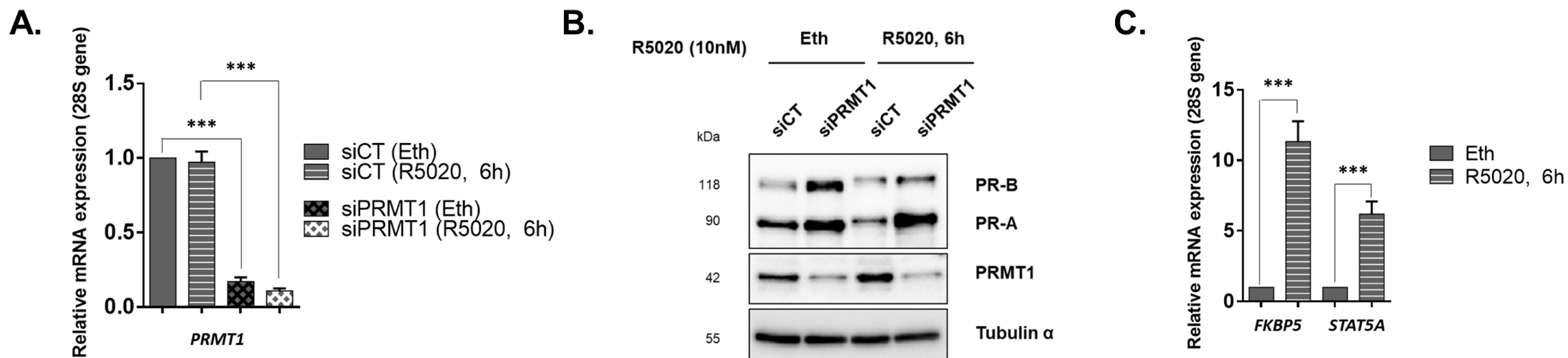
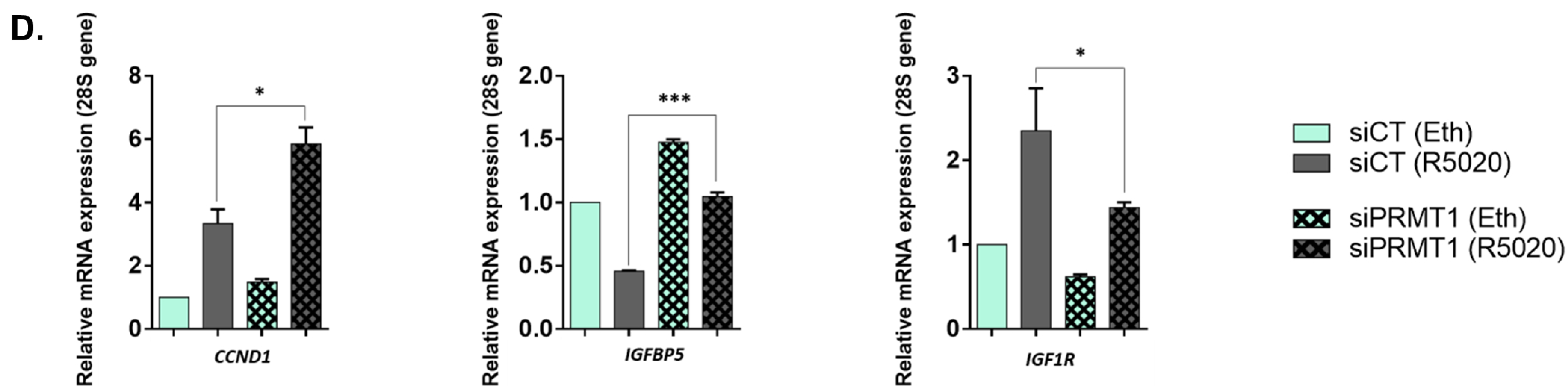
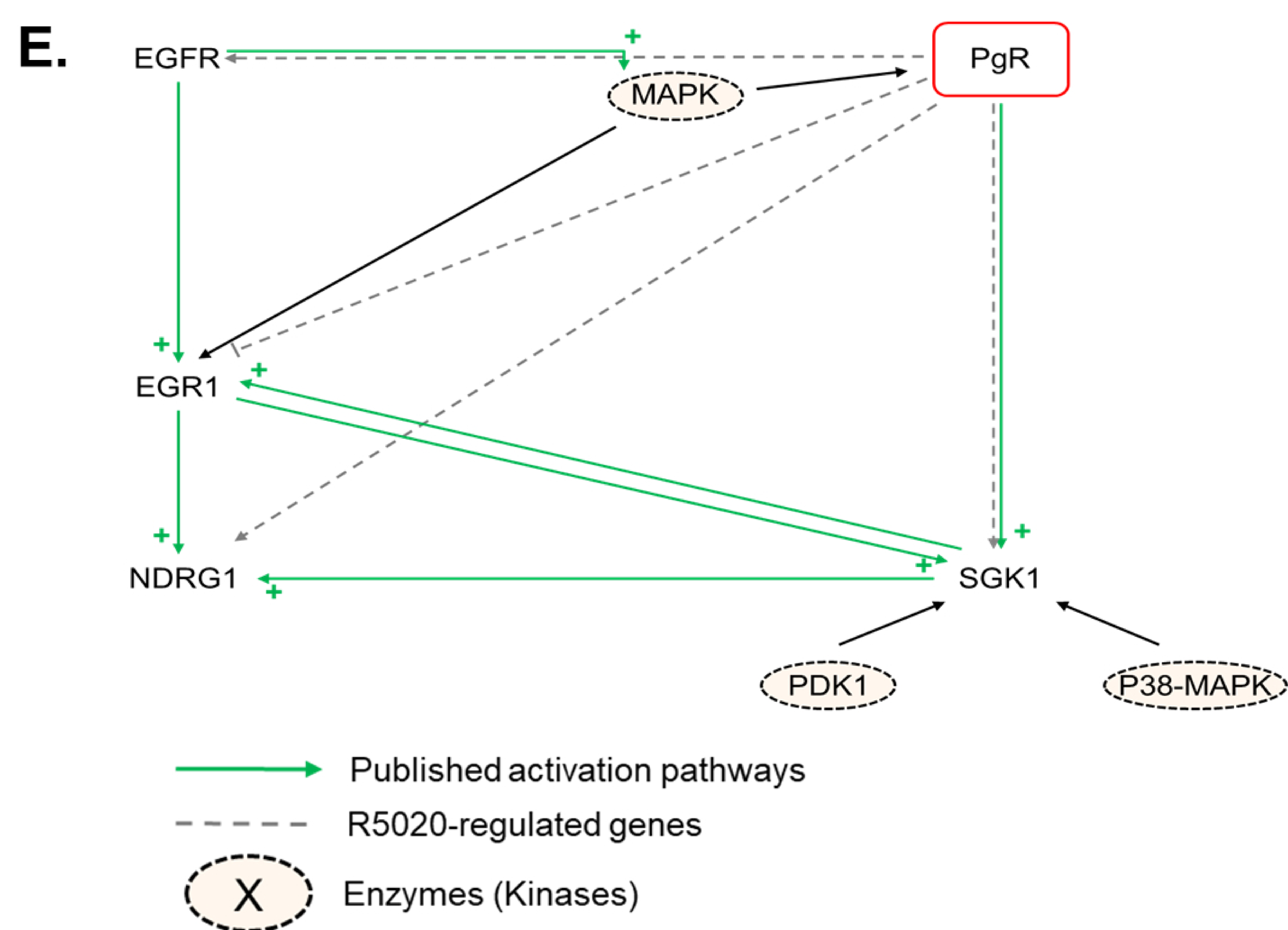
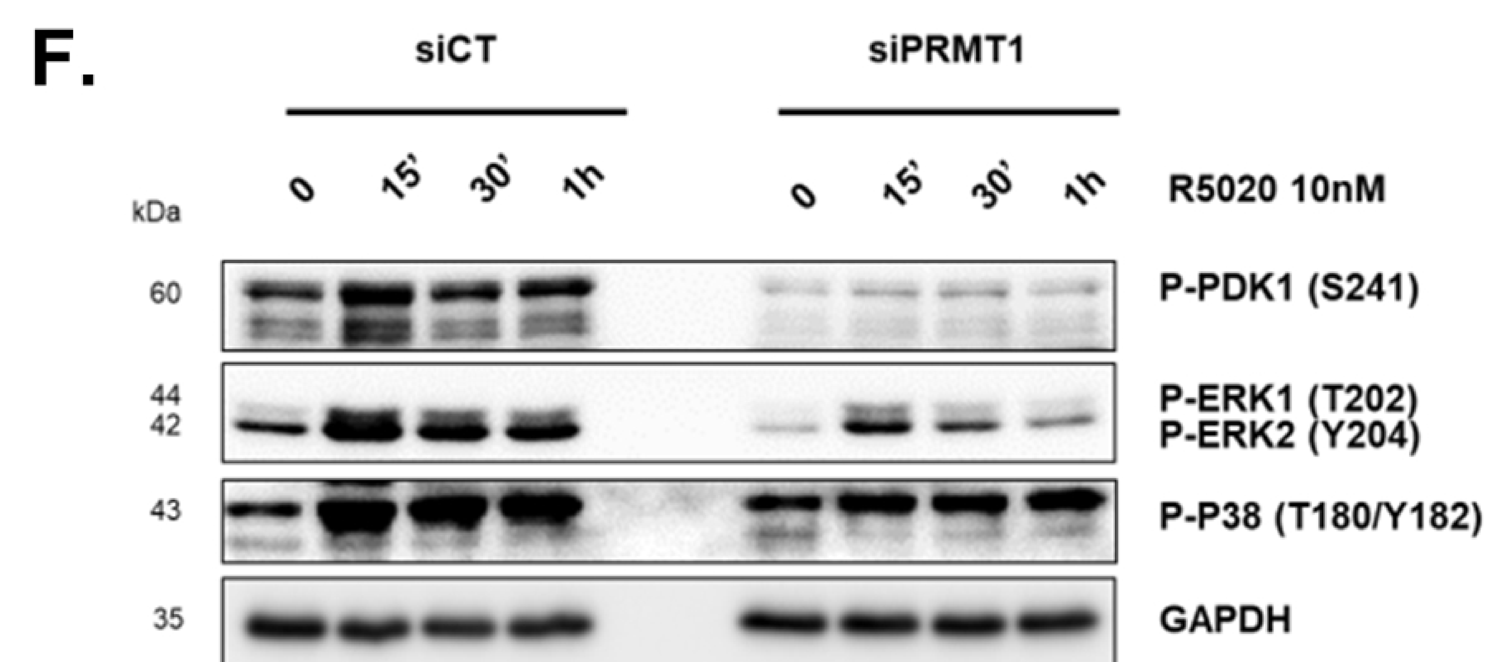

C.
T47D cells
Relative mRNA expression (28S gene)
DLL1
siCT (Eth)
siCT (R5020, 6h)
siPRMT1 (Eth)
siPRMT1 (R5020, 6h)

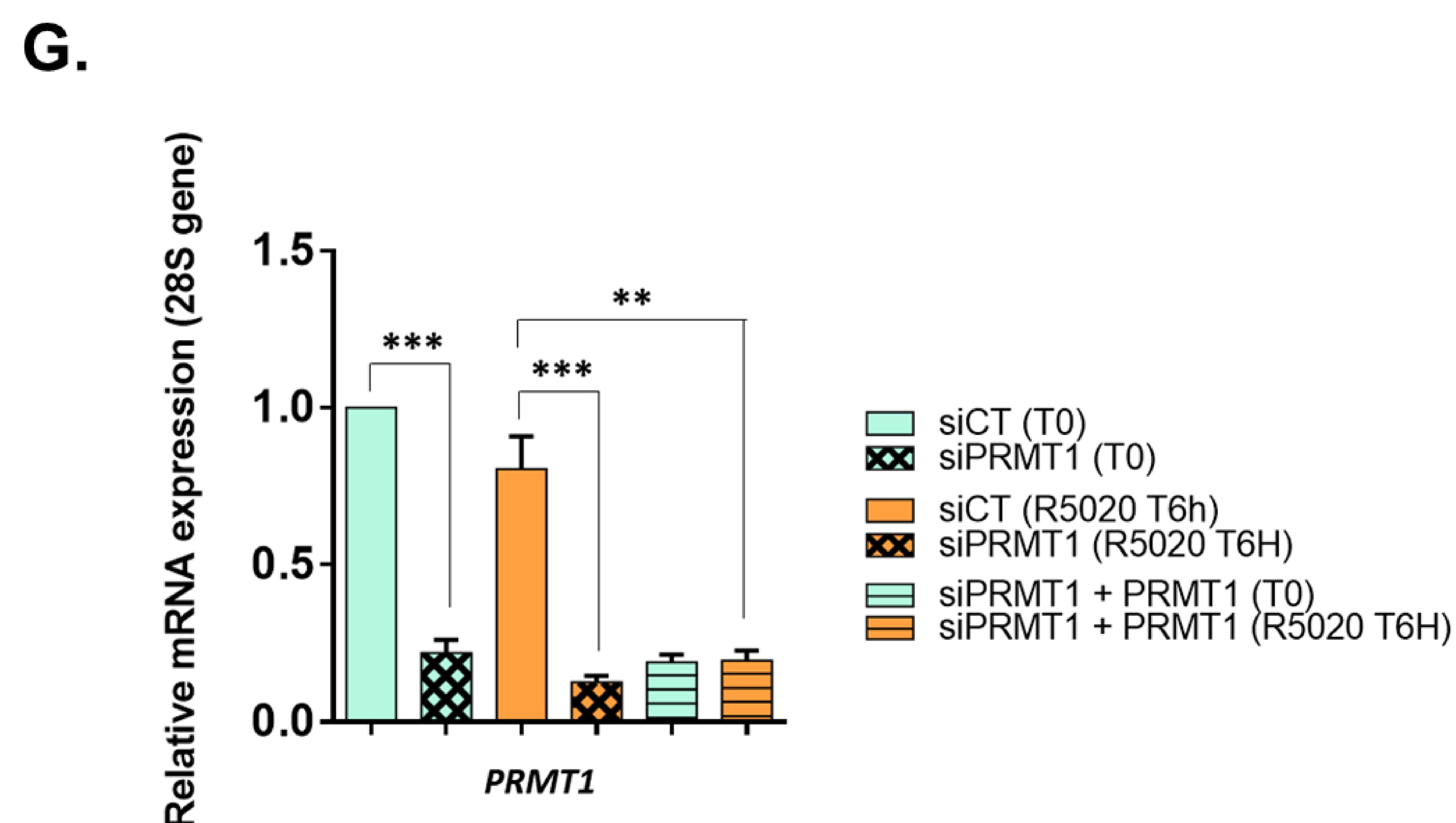
Figure S3, related to Fig. 4 | PRMT1 acts as a PR coregulator in breast cancer cells. A. RNAs were extracted for T47D cells, stimulated with R5020 for 3h or 6h (or with vehicle ethanol) and the expression of three endogenous PR-target genes was analyzed by RT-qPCR using specific primers. Results shown are mean ± SEM for three independent experiments. The *p*-value was calculated using a paired *t*-test: ** indicates $p \leq 0.01$ and ***$p \leq 0.001$. **B.** The expression of the indicated genes was analyzed by RT-qPCR using total RNA extracts from ZR-75 or **C.** T47D cells, previously transfected with siCT or siPRMT1 and stimulated with R5020 for 6h. The mean ± SEM of, at least, three independent experiments is shown. The *p*-value was calculated using a paired *t*-test: * indicates $p \leq 0.05$, ** $p \leq 0.01$ and *** $p \leq 0.001$.


A.
Relative mRNA expression (28S gene)
PRMT1
siCT (Eth)
siCT (R5020, 6h)
siPRMT1 (Eth)
siPRMT1 (R5020, 6h)
B.
R5020 (10nM)
Eth
R5020, 6h
siCT
siPRMT1
kDa
PR-B
PR-A
PRMT1
Tubulin α
C.
FKBP5
STAT5A
Eth
R5020, 6h


D.
Relative mRNA expression (28S gene)
CCND1
IGFBP5
IGF1R
siCT (Eth)
siCT (R5020)
siPRMT1 (Eth)
siPRMT1 (R5020)


E.
EGFR
MAPK
PgR
EGR1
NDRG1
SGK1
PDK1
P38-MAPK
Published activation pathways
R5020-regulated genes
Enzymes (Kinases)


F.
siCT
siPRMT1
R5020 10nM
kDa
P-PDK1 (S241)
P-ERK1 (T202)
P-ERK2 (Y204)
P-P38 (T180/Y182)
GAPDH


G.
Relative mRNA expression (28S gene)
PRMT1
siCT (T0)
siPRMT1 (T0)
siCT (R5020 T6h)
siPRMT1 (R5020 T6H)
siPRMT1 + PRMT1 (T0)
siPRMT1 + PRMT1 (R5020 T6H)

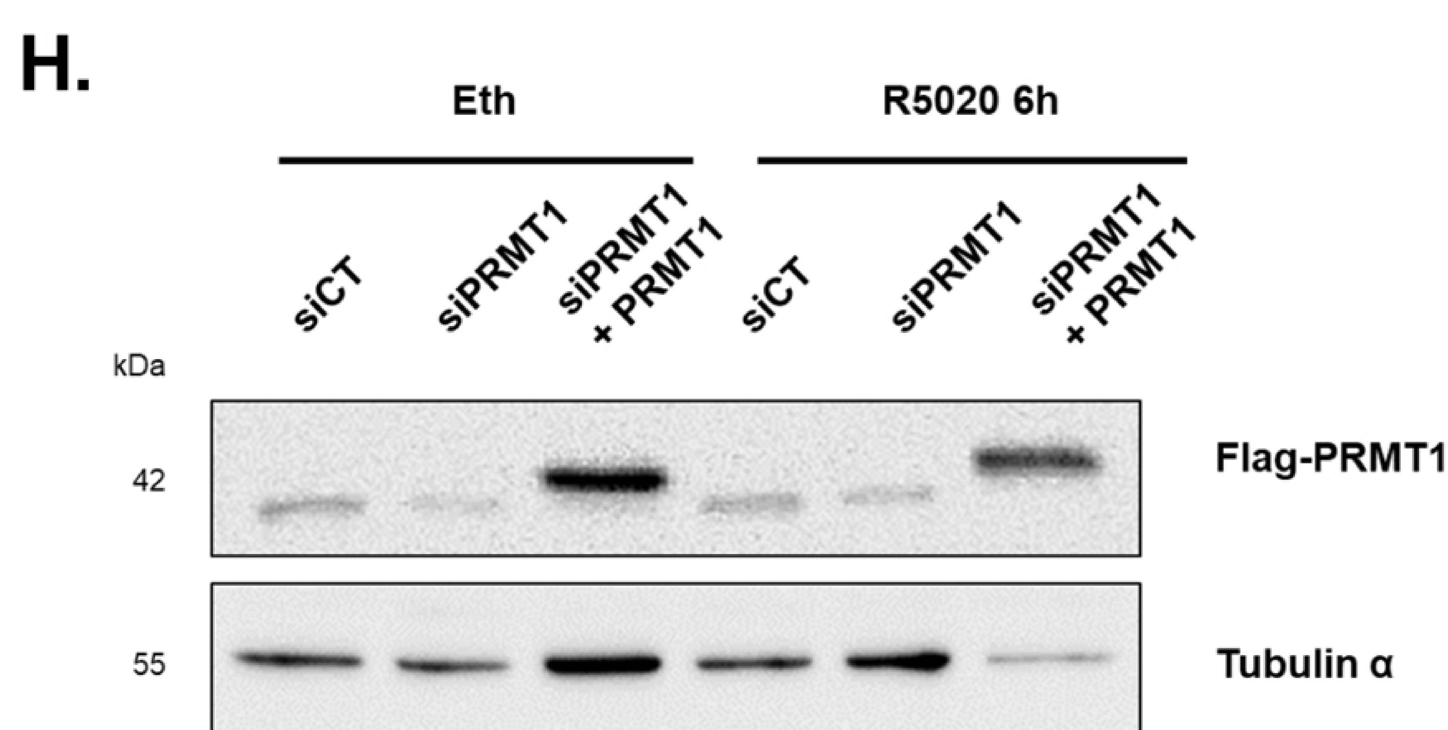
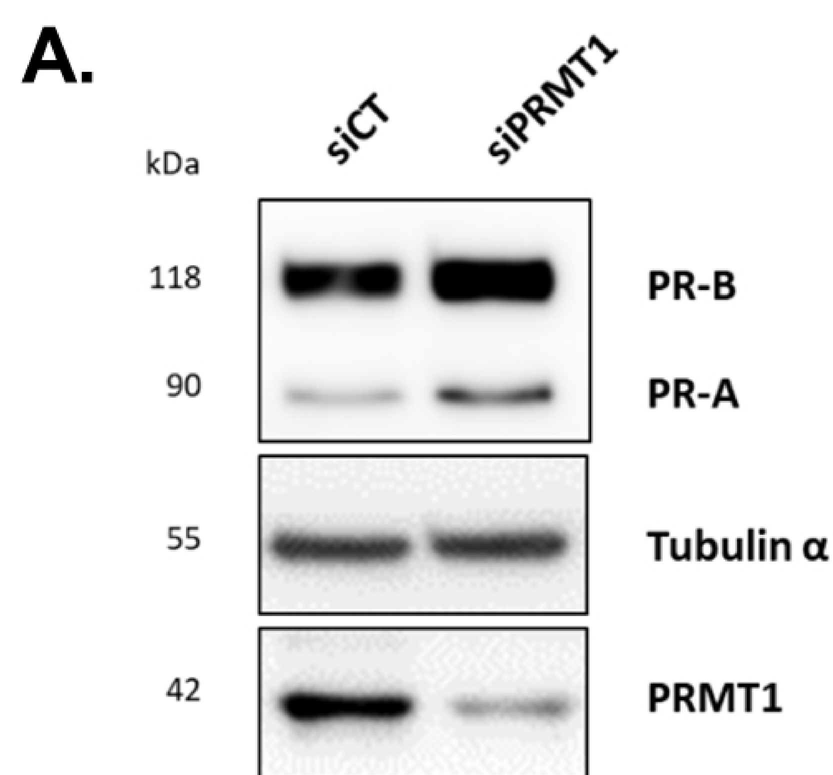
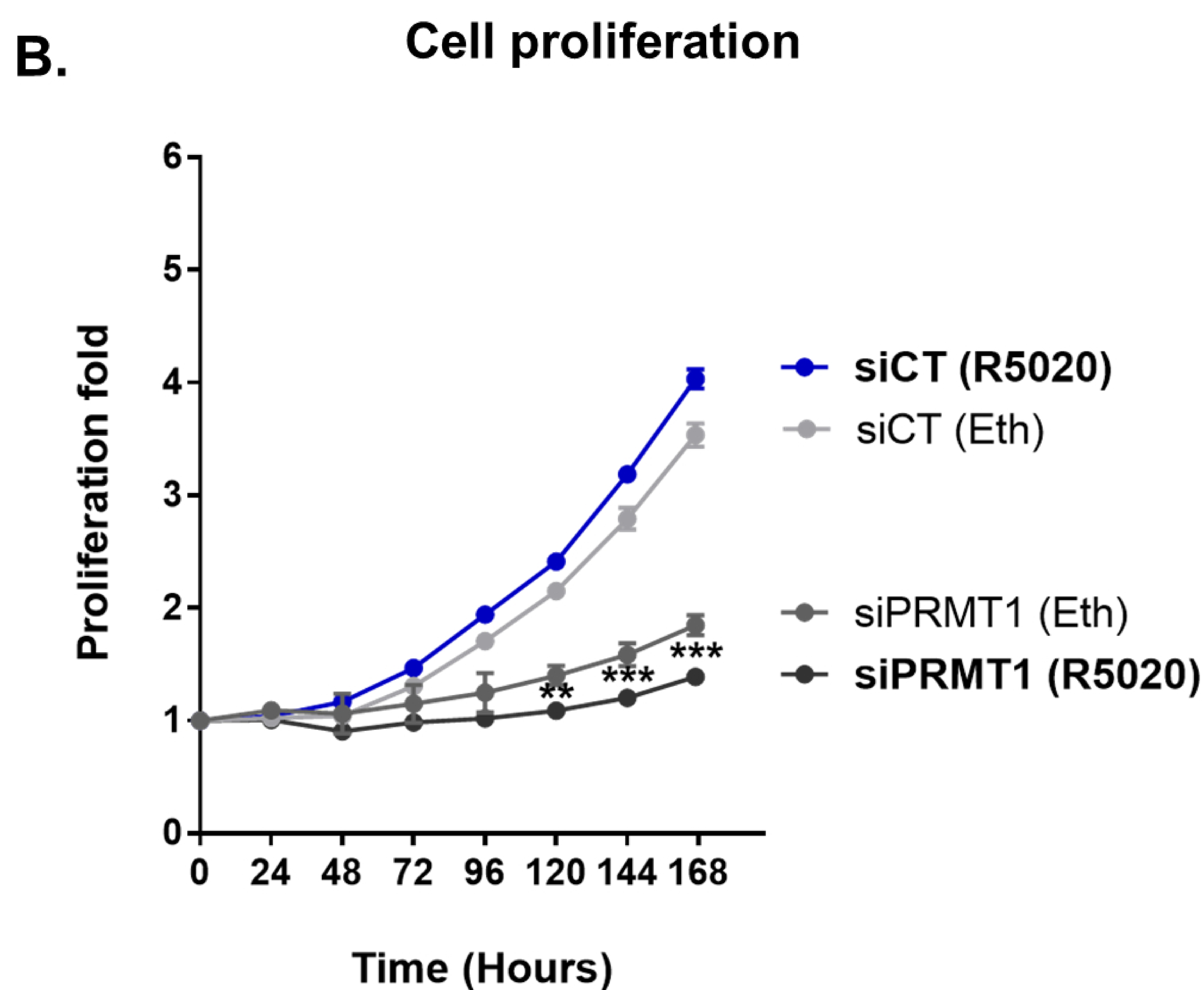
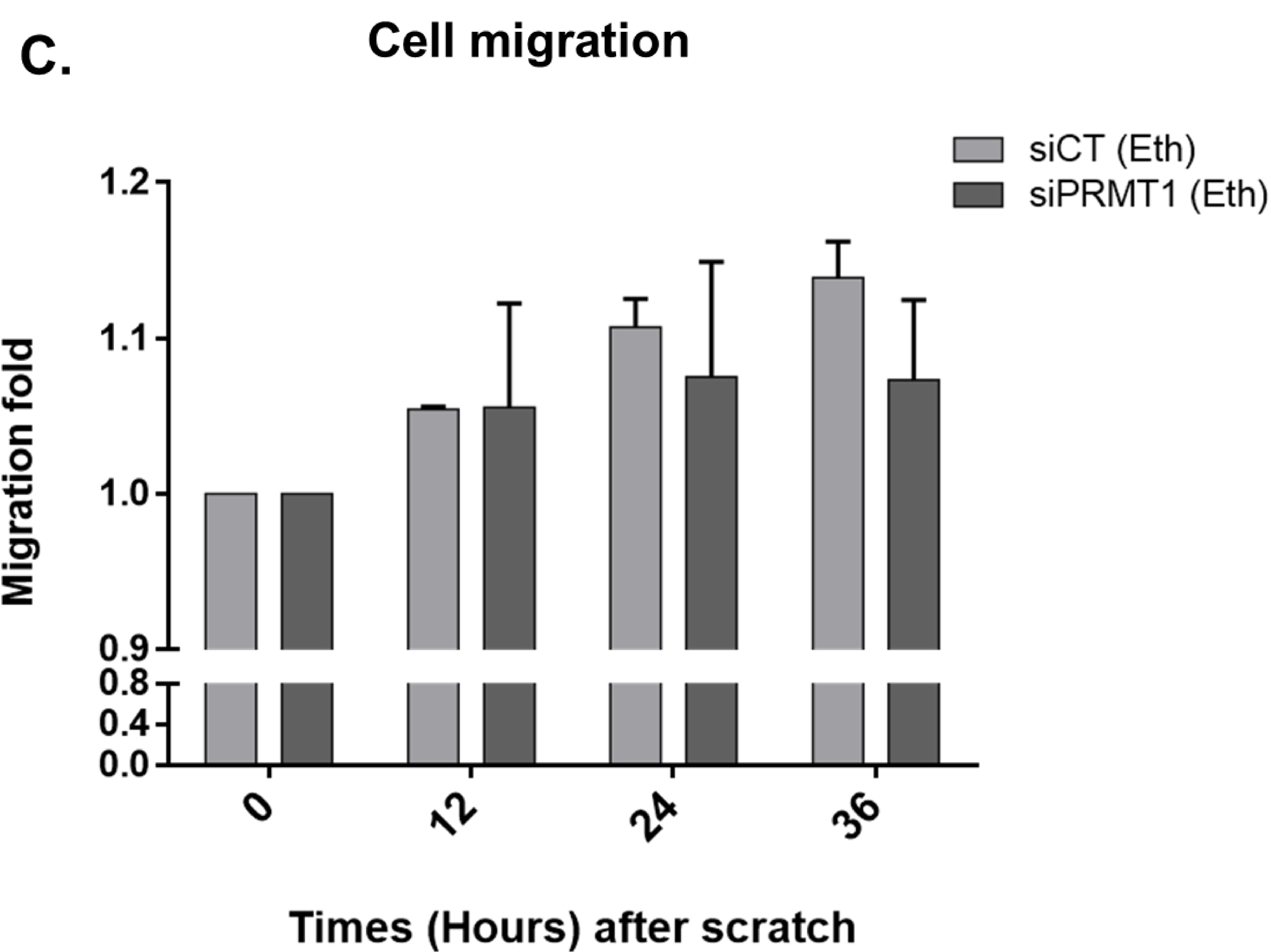
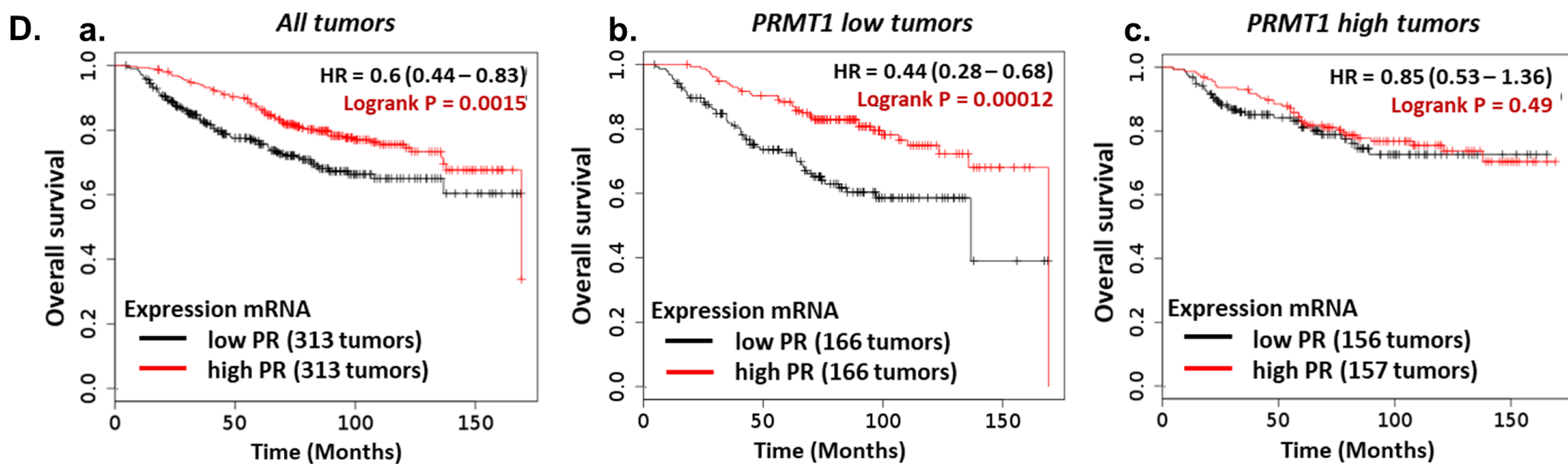

H.
Eth
R5020 6h
siCT
siPRMT1
siPRMT1 + PRMT1
kDa
Flag-PRMT1
Tubulin α

Figure S4, related to Fig. 5 | PRMT1 affects the expression of endogenous PR target genes in T47D cells. A-C. PRMT1 knockdown and hormonal treatment of T47D, stimulated for 6h of R5020 and used for RNA-seq analysis described in fig. 5. **A.** RT-qPCR analysis using *PRMT1* specific primers. **B**. Immunoblot using the indicated antibodies. **C.** RT-qPCR analysis using primers of *FKBP5* and *STAT5*, two well-characterized PR target genes. **D.** Genes randomly selected from the list of PRMT1-dependent genes identified by the RNA-seq analysis, were analyzed by RT-qPCR using total RNA extracts from T47D, transfected with either siCT or siPRMT1 and stimulated for 6h of R5020. The mean ± SEM of at least three independent experiments is shown. The *p*-value was calculated using a paired *t*-test: * indicates $p \leq 0.05$, $^{**}p \leq 0.01$ and $^{***}p \leq 0.001$. **E.** Scheme of the regulation of proteins involved in migration and proliferation pathways dependent on PR, according to the literature. **F.** Whole-cell extracts of T47D, depleted or not for PRMT1 using siRNA and treated with R5020 for the indicated times, were collected and subjected to immunoblot analysis using the listed antibodies. **G-H.** T47D cells were transfected with siRNAs (control or PRMT1) or co-transfected with siRNA plus a plasmid expressing the flag-tagged rat PRMT1 for 48h, and then treated with 10 nM of R5020 (6h). **G.** The expression of endogenous PRMT1 mRNA was analyzed by RT-qPCR using human *PRMT1* primers. Results shown are mean ± SEM for three independent experiments. The *p*-value was calculated using a paired *t*-test: ** indicates $p \leq 0.01$ and $^{***}p \leq 0.001$. **H.** The transfected rat PRMT1 was detected by immunoblot using anti-PRMT1 antibody.


A.
kDa
siCT
siPRMT1
118
90
55
42
PR-B
PR-A
Tubulin α
PRMT1


B.
Cell proliferation
Proliferation fold
Time (Hours)
siCT (R5020)
siCT (Eth)
siPRMT1 (Eth)
siPRMT1 (R5020)
**
***
***


C.
Cell migration
Migration fold
Times (Hours) after scratch
siCT (Eth)
siPRMT1 (Eth)


D.
a. All tumors
HR = 0.6 (0.44 – 0.83)
Logrank P = 0.0015
Overall survival
Expression mRNA
low PR (313 tumors)
high PR (313 tumors)
Time (Months)
b. PRMT1 low tumors
HR = 0.44 (0.28 – 0.68)
Logrank P = 0.00012
Expression mRNA
low PR (166 tumors)
high PR (166 tumors)
c. PRMT1 high tumors
HR = 0.85 (0.53 – 1.36)
Logrank P = 0.49
Expression mRNA
low PR (156 tumors)
high PR (157 tumors)

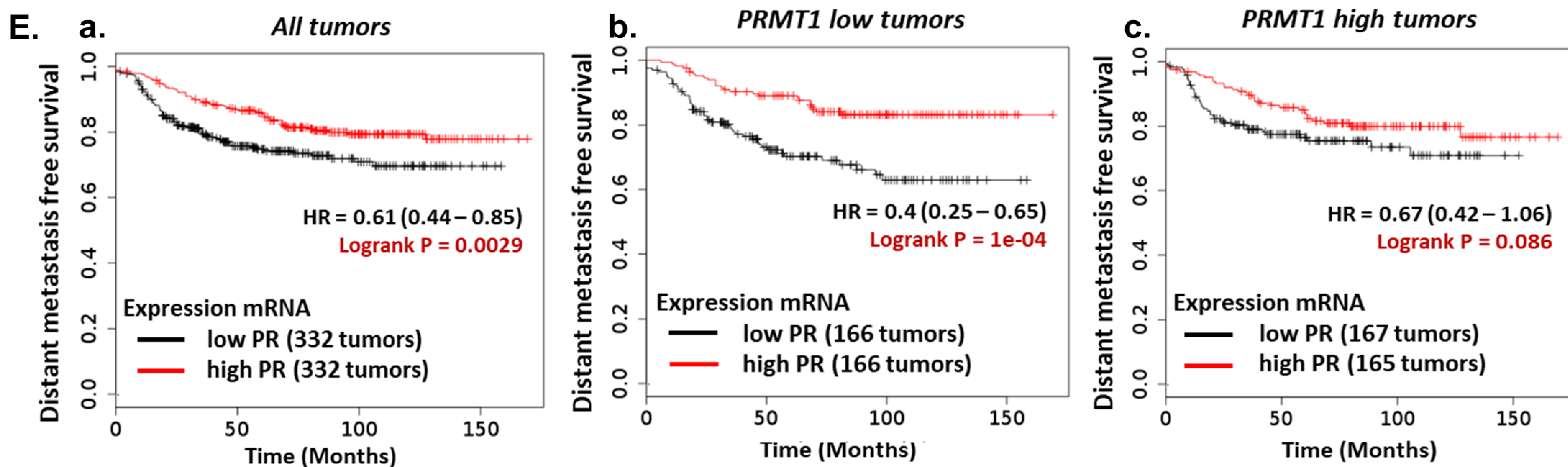
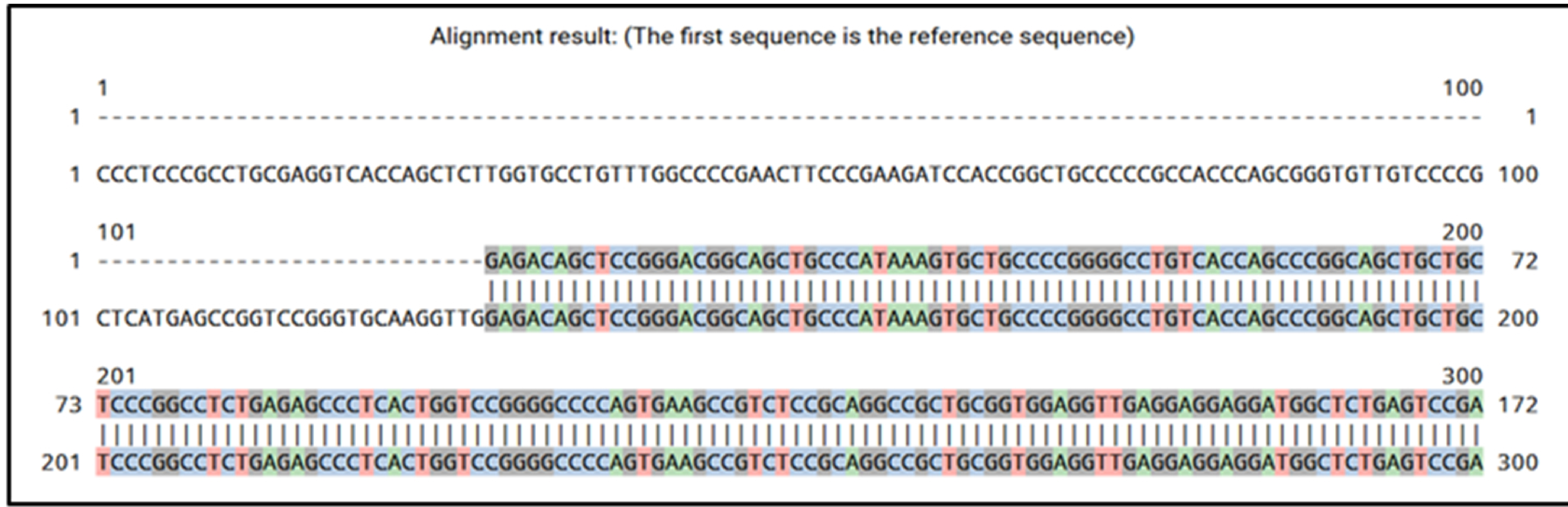
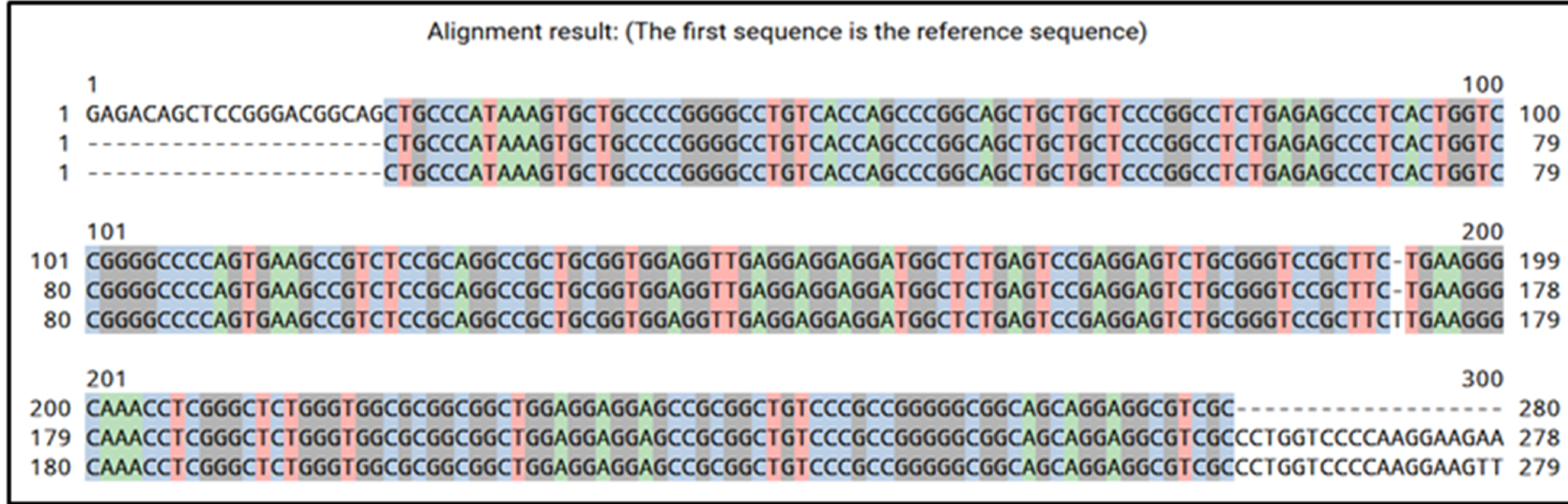

E.
a. All tumors
Distant metastasis free survival
HR = 0.61 (0.44 – 0.85)
Logrank P = 0.0029
Expression mRNA
low PR (332 tumors)
high PR (332 tumors)
Time (Months)
b. PRMT1 low tumors
HR = 0.4 (0.25 – 0.65)
Logrank P = 1e-04
Expression mRNA
low PR (166 tumors)
high PR (166 tumors)
c. PRMT1 high tumors
HR = 0.67 (0.42 – 1.06)
Logrank P = 0.086
Expression mRNA
low PR (167 tumors)
high PR (165 tumors)

Figure S5, related to Fig. 6 | Low PRMT1 reduces R5020-induced proliferation and migration of T47D cells and predicts improved survival of breast cancer patients. A. T47D cells were transfected with siCT or siPRMT1 and used for proliferation and migration assays. Whole cell extracts were assessed for PRMT1 inhibition by IB. **B-C.** T47D cells expressing the indicated siRNAs (PRMT1 or CT) were analyzed by Incucyte technology. **B.** Cells were stimulated with R5020 (10 nM) or vehicle ethanol every 48h for 7 days and their proliferation rate was assessed. Image acquisition was conducted as explained in fig. 6B. **C.** T47D cells were kept unstimulated (vehicle ethanol) and their migration was analyzed in a wound scratch assay with the Incucyte Live-Cell Imaging System and dedicated software (Essen Bioscience), as reported in the Transparent Methods section. Both **B.** and **C.** graphs show the mean ± SD of one experiment representative of three. The *p*-value was determined using the Student's t-test: ** indicates $p \leq 0.01$ and *** $p \leq 0.001$. **D-E.** Kaplan-Meier estimates **D.** overall survival and **E.** distant metastasis free survival in patients, in GEO, EGA, TCGA datasets with low (black) or high (red) PR expression as indicated using KM-plotter in a cohort of 1764 breast tumors **(a)**, or stratified in 2 groups following low PRMT1 **(b)** or high PRMT1 expression **(c)**.

A. T47D

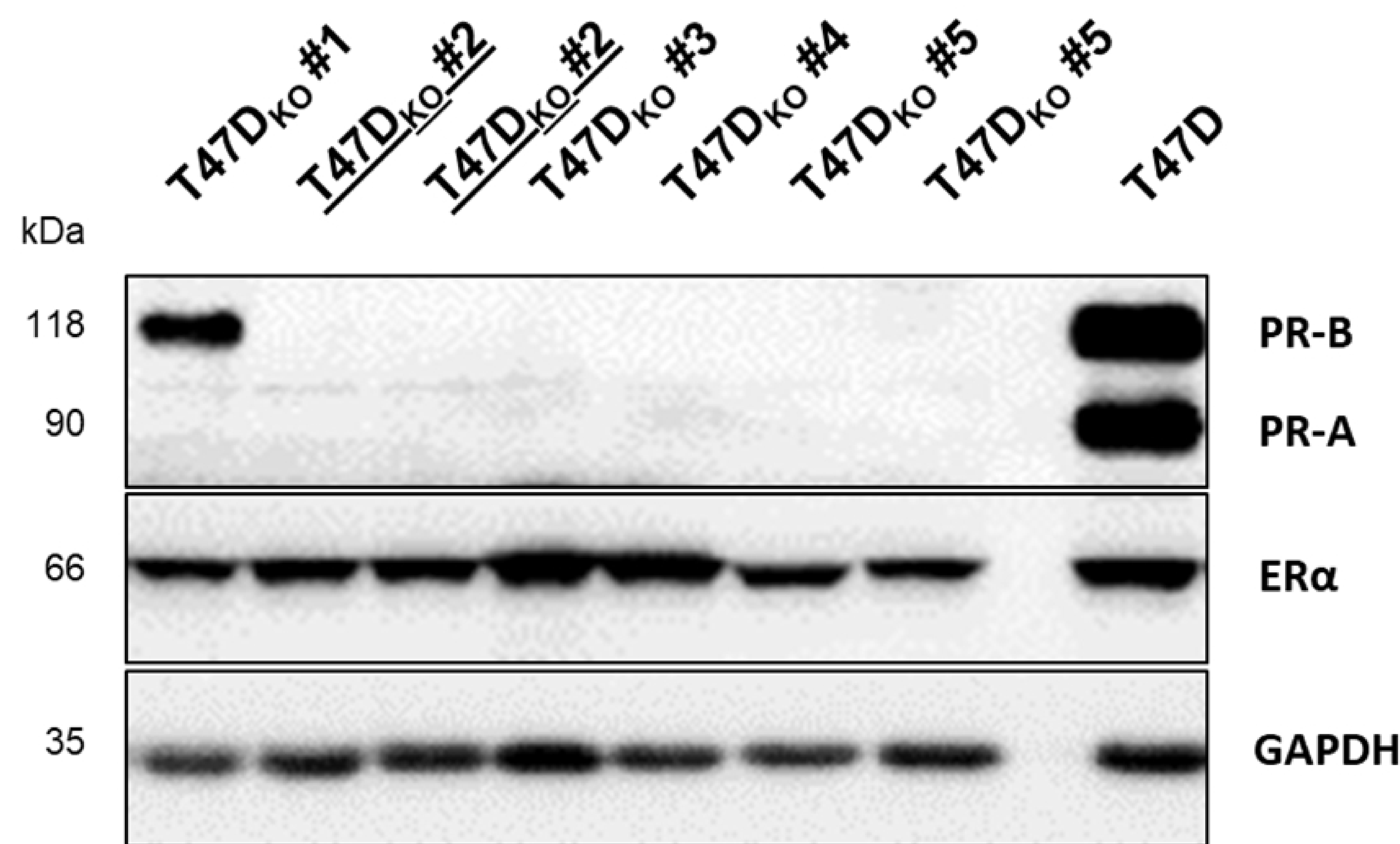


Clone #2



Clone #3

B.



C.

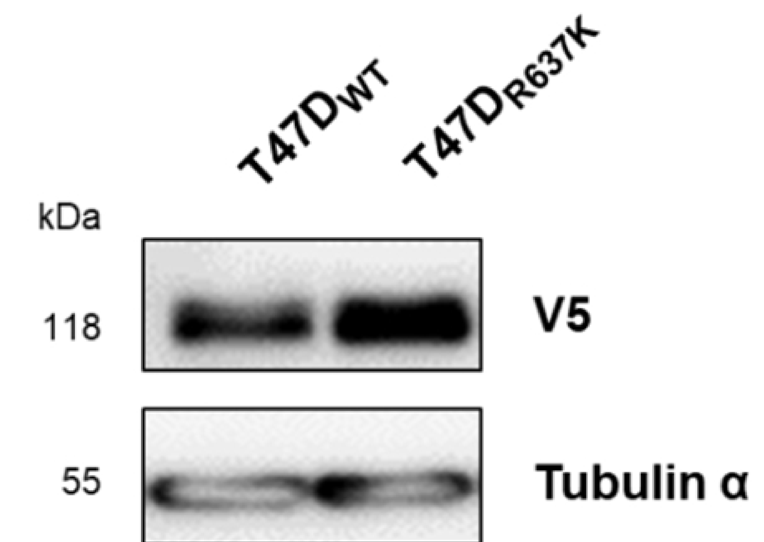


Figure S6, related to Fig. 7 | Inhibiting PR methylation decreases breast cancer cell proliferation and PR turnover. A. Chromatograms showing DNA sequences from mock and CRISPR-Cas9 cells. We performed genotyping PCRs. The amplified fragments were sequenced using an oligo targeting a sequence inside the fragment and analyzed using CRISP-ID, a web application. This tool allows the detection of the exact indel size and the location of a CRISPR-Cas9 targeted region, based on direct Sanger sequencing (as described in the Transparent Methods section). **B.** Whole-cell extracts of different clones of T47D PR_{KO} were collected and analyzed by immunoblot for their expression of PR, using PR-antibody, as well as ERα. The clone #2 (underlined) was chosen for stably re-expressing PR_{WT} or PR_{R637K}, as observed in experiences of fig. 7. T47D (PR-positive) cell line was used as control. **C.** Cell lysates of PR_{WT} and PR_{R637K}, used for cell proliferation test shown in fig. 7E and for the colony growth assay (fig. 7F), were collected and analyzed by immunoblot for their expression of PR-V5.

Transparent Methods

Cell culture and treatments

T47D (ATCC) were cultured in RPMI-1640 medium, supplemented with 10% fetal bovine serum (FBS), 2% penicillin-streptomycin (Life Technologies) and insulin (10 µg/ml). Cos7 and HeLa cells (ATCC) were maintained in DMEM, supplemented with 10% FBS and 2% penicillin-streptomycin (Life Technologies). All cell lines were grown in a humidified atmosphere with 5% CO_2 at 37°C, authenticated by Eurofins and tested for Mycoplasma infection by the MycoAlert Mycoplasma Detection Assay (Lonza, Rockland, ME USA).

Prior to experiments, T47D cells were grown in phenol red-free medium supplemented with 10% charcoal-stripped serum (Biowest). 48h later, medium was replaced by fresh serum-free medium. After 48h in serum-free conditions, cells were treated with 10 nM of R5020 (Perkin Elmer) or an equivalent amount of ethanol vehicle for the indicated time. When indicated, inhibitors (or DMSO vehicle) were added to cells: MS 023 Type I PRMT inhibitor (Tocris) for 48h, at the indicated concentration, cyclohemixide (Sigma, 50 µM) or MG132 proteasome inhibitor (Sigma, 10 µM) for 16h or 8h respectively, before R5020 treatment.

Plasmids and constructions

The GST-PR vectors (GST-PR-1, -PR-2, -PR-3, -PR-4, -PR-5 and -PR-3-R637A mutant) were constructed by inserting the cDNA fragments illustrated in Figure S2C, obtained by PCR amplification, into the pET41a vector (Novagen). All of the PR mutants were generated using a QuikChange XL Site-Directed Mutagenesis kit (Stratagene) according to the manufacturer's instructions and the sequences were verified by DNA sequencing. The mammalian expression vector pSG5-PR(B) was a gift from Pr. P. Chambon (Kastner et al., 1990). pSG5V5-PR(B) was obtained by inserting the V5-TAG coding sequence in frame with the coding sequence of PR(B) by PCR.

pSG5V5-PR(B) plasmid was used to generate PR-R637A. To rescue the phenotype of PR_{KO} clones, a guide-resistant mutant of the PR(B) isoform was created (pSG5V5gr) by substituting four nucleotides in the PR guide 1 (G633A, G6364, T639C and G641A) and four nucleotides in the PR guide 2 (C715T, T717A, C718T and G720A) targeting regions. This guide-resistant PR(B) was cloned into the stable mammalian expression vector pPRUpu. pSG5V5gr PR(B) and pPRUpuV5gr-PR(B) were used to obtain the PR-R637K. Sequences of the primers used for the constructions are listed in Table S2. The human HA-PRMT1 and HA-PRMT1(E153Q) in pSG5 vector were a gift of C. Teyssier (Teyssier et al., 2005) and the rat flag-PRMT1 in pSG5 vector was previously described (Robin-Lespinasse et al., 2007).

Generation of CRISPR cell lines: PR_{KO}

To knockout PR genes in T47D cells, we used the pLCV2 plasmid (a gift from F. Zhang, Addgene plasmid #52961). Oligonucleotides pairs were hybridized and cloned into the LentiCRISPR V2 vector linearized with BsmB1 to generate T47D clones KO: PR Guide#1 – Fwd. CACCGcccagtgaagccgtctccgc / Rev. AAACgcggagacggcttcactgggC; PR Guide #2 – Fwd. CACCGtctgcgggtccgcttctgaa / Rev. AAACttcagaagcggacccgcagaC, oligonucleotides targeting the regions 624-637 and 693-713 of the PR(B) coding sequence. T47D cells were transfected with the corresponding LentiCRISPR V2 plasmids and selected with blasticidin (5 µg/ml, Invitrogen, Grand Island, NY, USA) for 1 week. Cells were then cloned in 96-well plates by limiting dilution. Isolated clones were characterized by immunoblotting. For five different clones, we confirmed the knockout at DNA and protein levels. We performed genotyping PCRs using a forward primer upstream (5'-GGGGAGTCCAGTCGTCAT-3') and a reverse primer downstream (5'-ACTTTCGTCTTCCAGCAGC-3') of the sgRNA cleavage site. The amplified fragments were then sequenced using an oligo targeting a sequence inside the fragment (5'-CCAGAAAAGGACAGCGGAC-3') and analyzed using CRISP-ID, a web application that allows the detection of the exact indel size and location of a CRISPR-Cas9 targeted region, based on direct Sanger sequencing (Dehairs et al., 2016).

Generation of two stable cell lines: T47D$_{WT}$ and T47D$_{R637K}$

For the production of the rescue cell lines, CRISPR PR$_{KO}$ cells were transfected with pPRUpuPR(B)-WT and -R637K plasmids using Jetprime (Polyplus-transfection). Screening of stable transfected cells was performed using puromycin dihydrochloride, 1 µg/ml. Stable cell populations, called T47D$_{WT}$ and T47D$_{R637K}$, were maintained in complete RPMI-1640 medium containing 0.5 µg/ml of puromycin.

Generation of methylated-R637-PR antibody (anti-met-R637-PR)

Rabbits were immunized with a peptide corresponding to PR amino acids 628-640 (NH2-CQAGMVLGG([Me2as]R)KFK-CONH2), in which R637 was asymmetrically dimethylated by Covalab (Villeurbanne, France). To purify the met-R637-PR antibody, the dimethyl peptide used for the immunization and the corresponding control peptide (non-methyl) were coupled separately to cyanogen bromide activated agarose beads. The antisera were first bound on the non-methylated peptide column. The unbound antiserum was then applied to the methylated peptide column and eluted. The title and the specificity of the purified antibody were then tested by enzyme-linked immunosorbent assay (ELISA).

Glutathione transferase (GST) pull-down assay

The GST-PRMT1 and GST-PR fusion proteins were expressed in BL21 competent cells and purified using glutathione-sepharose 4B resin (GE Healthcare Life Sciences). SDS-PAGE and Coomassie staining were used to confirm the integrity of the fusion proteins. PR-B or ERα expression plasmids were transcribed and translated *in vitro* using T7-coupled reticulocyte lysate in the presence of [^{35}S] methionine. For *in vitro* protein-protein interaction assays, GST fusion proteins were incubated for 2h at room temperature with 200 µl of binding buffer (Tris 20 mM pH 7.4, NaCl 0.1 M, EDTA 1 mM, glycerol 10%, Igepal 0.25% with 1 mM dithiothreitol and 1% milk) and packed in minicolumns. After washing, the retained proteins were eluted and analyzed on a sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis (PAGE) and visualized by autoradiography.

In vitro methylation assays

GST-PRMT1 (Upstate Biotechnology) was incubated with different GST-tagged PR fragments, or with a GST-tagged hinge ERα as a positive control (Le Romancer et al., 2008), in the presence of S-adenosyl-l [methyl-^{3}H] methionine ([^{3}H] SAM 85 Ci/mmol from a 10.4 mM stock solution in dilute HCl/ethanol 9/1 (pH 2.0–2.5); Perkin Elmer) for 90 min at 37°C. Methylation reactions were quenched by the addition of 2x Laemmli sample buffer, heated at 95°C for 5 min and separated on SDS-PAGE. Following electrophoresis, gels were soaked in Amplify fluorographic reagent (Sigma) according to the manufacturer's instructions and visualized by autoradiography. The cold methylation assays were performed in the same experimental conditions, using 0.5 mM of nonradiolabeled AdoMet (SAM). The reaction mixtures were subjected to SDS-PAGE and then analyzed by western blot using the anti-met-R637-PR (homemade) antibody.

Dot immunoblot assay

A peptide corresponding to PR amino acids 628-640 (NH2-CQAGMVLGG([Me2as]R)KFK-CONH2), in which R637 was asymmetrically dimethylated, and the corresponding control peptide (non-methyl), were produced by Covalab (Villeurbanne, France). For dot blot assays, peptides were spotted onto nitrocellulose membrane allowing solution to penetrate (usually 3–4 mm diameter) by applying it slowly as a volume of 1 µL. The membrane was dried and analyzed by immunoblot using the anti-met-R637-PR (homemade) antibody.

siRNA and plasmid transfection

siRNA transfections were performed using Lipofectamine 2000 (Invitrogen, Thermofisher) according to the manufacturer's protocol. After 72h, the down-regulation was analyzed by immunoblot or by RT-qPCR. If requested, after 48h the medium was replaced by fresh medium without serum and cells were treated with R5020 (10 nM) or ethanol for different times.

Plasmid transfections were done using the JetPRIME reagent (Ozyme) according to the manufacturer's protocol. Cells were analyzed after the indicated times (48h or 72h). SiRNAs pool against PR, PRMT1, PRMT4 and PRMT6 were purchased from Thermofischer Scientific (catalog #AM16708) and the siRNA negative control from Eurogentec (catalog # SR-CL000-00). For the rescue experiments, the plasmid expressing rat-PRMT1 was co-transfected with siRNAs pool against PRMT1 using the JetPRIME reagent (Ozyme) according to the manufacturer's protocol.

Luciferase reporter assay

HeLa cells were plated in 96-well plates the day before transfection. Cells were transfected using Lipofectamine 2000 (Invitrogen) with the indicated plasmids according to the manufacturer's protocol. After transfection, the cells were grown for 48h in the presence or absence of 10 nM of R5020. Cell lysis and luciferase assays were performed with Promega luciferase assay kit. The results were normalized as indicated and presented as the mean ± SEM of three independent experiments.

Immunoprecipitation (IP), immunoblot (IB) and antibodies

After treatment, cells were lysed with RIPA buffer (50 mM Tris HCl pH 8, 150 mM NaCl, 1 mM ethylenediamine tetra-acetic acid (EDTA), 1% NP-40 and 0.25% deoxycholate) supplemented with protease inhibitors (Roche Molecular Biochemicals) and phosphatase inhibitors (1 mM sodium fluoride, 1 mM Na3VO4 and 1 mM β-glycerophosphate). For immunoprecipitation (IP), 1mg of protein extracts were incubated with primary antibodies at 4°C overnight. Protein A-Agorase beads were added in the medium for 2h at 4°C on a rotating wheel. After washing, the immunoprecipitates and their corresponding inputs (30µg) were denatured by boiling in Laemmli sample buffer and separated on SDS-PAGE. For immunoblot (IB), the gels were electroblotted onto a PVDF membrane and incubated with primary antibodies overnight, at 4°C. The following day, membranes were incubated with horseradish peroxidase (HRP)-conjugated anti-rabbit or anti-mouse immunoglobulins (Jackson Immunoresearch) and proteins were visualized by chemiluminescence (Clarity Western ECL Substrate,

BioRad) following the manufacturer's instructions. Quantification of the immunoblot band intensity was performed with Image J software. The primary antibodies used in the study, and their dilutions, are listed in Table S3.

Cycloheximide chase assay

3x10^5 cells were grown in complete RPMI medium for 24h and then in phenol red-free medium supplemented with 10% charcoal-stripped serum for 48h. 16h before starting the R5020 stimulation, 50 µg/mL cycloheximide (Sigma) or vehicle DMSO were added. After each R5020-time point, cells were lysed with protein lysis buffer with freshly-added protease inhibitors and analyzed by IB.

Immunofluorescence (IF)

T47D cells (3x10^5) and $T47D_{WT}$ or $T47D_{R637K}$ stable cells (2x10^5) were grown on coverslips in 12-well plates. After chemical treatment(s), cells were fixed in methanol for 2 min and washed twice in PBS. Non-specific binding was blocked using a 1% gelatin solution for 30 min at room temperature. Cells were incubated with the different primary antibodies (listed in Table S3) for 1h at 37°C and then with the secondary antibodies [Alexa Fluor 488 anti-mouse (Jackson ImmunoResearch, Cambridge, UK) (1/2000^e) or Alexa Fluor 568 anti-rabbit (Invitrogen, Carlsbard, USA) (1/1000^e)] in Dako diluent for 1h. To finish, coverslips were mounted on glass slides in mounting solution (Dako, Carpinteria, CA, USA). The fluorescent slides were viewed with the Nikon Eclipse Ni microscope.

Proximity ligation assays (PLA), image acquisition and analysis

PLA assays were performed to visualize protein/protein interactions *in situ*, using the Duolink kit (Sigma) according to the manufacturer’s instructions. Cells (3x10^5) were grown on coverslips in 12-well plates and treated (as explained in figures and legends), before fixation with methanol for 2 min. After saturation in the blocking solution, seeded cells were incubated with different pairs of primary antibodies at 37°C for 1h. The PLA probes consisting of secondary antibodies conjugated with

complementary oligonucleotides were then incubated for the same conditions. The step of nucleotides ligation (30 min at 37°C) is followed by the amplification phase, for 100 min at 37°C in a dark and humidified chamber. At the end, coverslips were mounted on glass slides in mounting solution (Dako, Carpinteria, CA, USA) and were analyzed under fluorescence microscopy on a Nikon Eclipse Ni microscope. Images were acquired under identical conditions at 60X magnification. Image acquisition was performed by imaging DAPI staining at a fixed Z-Position, while a Z-stack of ± 5 µm at 1 µm intervals was carried out. The final image was stacked to a single level before further quantification. On each sample, at least one hundred cells were counted. Analysis and quantifications of these samples were performed using the Image J software (Version 1.52, NIH, Bethesda, MD, USA). The primary antibodies used in the study, and their dilutions, are listed in Table S3.

RNA extraction and real-time qPCR analysis

Total RNA (1 µg) was extracted and purified using TRI-Reagent (Sigma-Aldrich, USA), prior to being reverse-transcribed using 100 ng of random primers following the Superscript II (ThermoFisher, USA) protocol. Real time PCR was performed with SYBR Green qPCR master mix (BioRad) in a Step One plus real-time PCR detection system (Applied Biosystems). Mean values of measurements were calculated according to the -ΔΔCt quantification method and were normalized against the expression of 28S ribosomal mRNA as reference. Results shown are mean ± SEM for, at least, three independent experiments. The *p*-value was calculated using a paired *t*-test comparing results for R5020-treated cells expressing siRNA against PRMT1 to the R5020-treated siCT sample: * indicates a $p \leq 0.05$, ** for $p \leq 0.01$ and *** for $p \leq 0.001$. Sequences of the oligonucleotides are listed in Table S4.

Chromatin ImmunoPrecipitation (ChIP)

Chromatin was prepared from 5x106 of T47D cells (untreated or treated with 10 nM of R5020 for 1h). Cells were crosslinked with 1% formaldehyde (Sigma-Aldrich, USA) for 10 min at room temperature and then treated with 0.125 M glycine for 5 min under a gentle shaking. Nuclei were lysed in 300 µL of

ice-cold RIPA buffer (50 mM Tris HCl pH 8, 150 mM NaCl, 1% NP-40, 0.5% NaDoc and 0.1% SDS) prior to Chromatin-DNA shearing with a Diogene Bioruptor. ChIP was performed with the primary antibodies listed in Table S3. The antibody–chromatin complexes were precipitated with salmon sperm DNA/protein A agarose beads for 3h by rotation. Samples were extracted and heated at 65°C for 5h to reverse cross-links. After DNA purification, 2 ng of input DNA were used for qPCR analysis to quantify co-precipitated chromatin-DNA. Relative enrichment of a given promoter region obtained with a specific antibody was compared with Input DNA, normalized to a reference locus (human chromosome 1 in which no histone modification was reported). Sequences of the primers used to amplify ChIP-enriched DNA are listed in Table S4.

RNA-sequencing and RNA-seq analysis

RNA-Sequencing experiment was performed using T47D cells. Cells were transfected with siCT or siPRMT1 (50 nM) for 72h and treated with R5020 (10 nM) for 6h before RNA extraction. Sequencing was done by the IGFL (Institute of Functional Genomic of Lyon) Sequencing Platform, to compare gene expression levels of R5020-induced genes between siCT and siPRMT1 conditions. cDNA libraries were prepared using the SENSE mRNA-Seq Library Prep Kit V2 (Lexogen, Vienna Austria). Quality of the cDNA was assessed and RT-qPCR was performed for selected PR target genes as quality control. All libraries were sequenced on an Illumina Nextseq500 and mapped on the hg19 version of the human genome using Bowtie2 (Galaxy Version 2.3.2.2). Count tables were prepared using htseq-count (Galaxy Version 0.9.1galaxy3). Differential gene expression analysis was performed with DEseq2 (Galaxy Version 2.1.8.3) using different thresholds. For R5020-induced genes in siCT-cells: FDR < 0.05; p-adjusted value < 0.01; fold-change > 2; expression > 10 reads per million. For R5020-induced genes in siPRMT1-cells: FDR < 0.05; p-adjusted value < 0.01; fold-change > 1.5; expression > 10 reads per million. Experiments were performed three independent times for siRNA transfection and for RNA extraction. The list of genes obtained by RNA-Seq analysis is provided in Table S1.

Analysis of cell migration

6×10^5 T47D cells were plated on 96-well ImageLock plates (Essen BioScience) for 24h at 37°C and then scratched (800 µm width) with the Wound Maker (Essen BioScience). Addition of R5020 or vehicle ethanol were added in the medium just after scratching and wound closure was followed and evaluated with the Incucyte Live-Cell Imaging System and dedicated software (Essen Bioscience). Cell migration was evaluated by monitoring the evolution of the size of wound closure (µm) for 24h maximum, in order to assess the contribution of cell proliferation to gap filling. Since wound width decreases as cell migration progresses over time, we represented the results as graphs indicating the rate of migration, corresponding to the change in wound area over time, extrapolated from three independent experiments, each one performed in triplicate.

Analysis of cell proliferation

4×10^3 T47D and 2×10^3 $T47D_{WT}$ or $T47D_{R637K}$ stable cells were seeded onto a 96-well plate 5h before incubation with the different hormones (E2, R5020 or ethanol). Images were acquired using an IncuCyte ZOOM over 7 days and cell proliferation was measured as the percentage of cell density observed over this period. Results are represented as graphs indicating the rate of proliferation over time, extrapolated from at least three independent experiments, each one performed in triplicate.

Colony formation assay

Cells were seeded into 6-well plates (600 cells/well) and left for 8–12 days until formation of visible colonies. Colonies were washed with PBS and fixed with 10% acetic acid/10% methanol for 20 minutes, then stained with 0.4% crystal violet in 20% ethanol for 20 minutes. After staining, the plates were washed and air-dried, and colony numbers were counted.

Supplemental Tables

Table S2, Related to Methods | Plasmids sequences

Name	Forward sequence (5’ – 3’)	Reverse sequence (5’ – 3’)
PR-1	GATATCTGACTGAGCTGAAGGCAAAGG	CTCGAGTTACTGCTCCACCAGGGCGAC
PR-2	GATATCTGGACGCGCCGATGGCGCCC	CTCGAGTTATGGGCTCTGGCTGGCTTC
PR-3	GATATCTGCAATACAGCTTCGAGTCATTACC	CTCGAGTTACTTTTTAAATTTTCGACC
PR-4	GATATCTGTTCAATAAAGTCAGAGTTG	CTCGAGTTATGGTGGAATCAACTG
PR-5	GATATCTGCTGATCAACCTGTTAATGAG	CTCGAGTTACTTTTTATGAAAGAGAAGG
PR-3/ PR-R637A	GCTGGCATGGTCCTTGGAGGTGCAAAATTTAAAAAGT AACTCGAG	CTCGAGTTACTTTTTAAATTTTGCACCTCCAAGGACCAT GCCAGC
PR-R637K	CAGGCTGGCATGGTCCTTGGAGGTAAAAAATTTAAA AAGTTCAATAAAGTC	GACTTTATTGAACTTTTTAAATTTTTTACCTCCAAGGAC CATGCCAGCCTG

Table S3, Related to Methods | List of antibodies and respective dilutions

PROTEIN NAME	Company	Reference	IB	IP	IF	PLA	ChIP	Species
EGFR	Ozyme (CST)	#4267	1/500e					Rabbit
EGR1	SCBT	Sc-515830	1/500e					Mouse
ERK1/2	CST	#4695	1/2000e					R
GAPDH	SCBT	Sc-4724	1/1000e					M
H3K4me3	Diagenode	C15410003					4µg	R
H4	CST	#13919	1/1000e					R
H4R3me2as	ActiveMotif	#39705	1/500e		1/1000e			R
IgG	CST	#2729		2 µg			4µg	R
Met-R637-PR	Home made		1/100e	15µL	1/300e			R
Pan-methyl-R	CST	#13522	1/500e	4µL		1/1000e		R
p-ERK1/2	CST	#5726	1/2000e					R
p-P38	CST	#9211	1/500e					R
p-PDK1	CST	#3438	1/1000e					R
p-PR	CST	#13736	1/500e					R
PR	SCBT	Sc-7208	1/2000e	2µg			4µg	R
PR	ThermoFischer	MA1-12626	1/1000e		1/500e	1/500e		M
PRMT1	Bethyl Lab.	#A300-722A		2µg			4µg	R
PRMT1	Millipore	#07-404	1/2000e			1/1000e		R
PRMT1	Sigma	P1620	1/500e			1/500e		M
SGK1	SCBT	Sc-28338	1/300e					M
Tubulin α	Sigma	T6074	1/10000e					M
V5-tag	Life Technol.	R920-25	1/1000e					M

Table S4, Related to Methods | Primers sequences

Experiment	Target gene	Forward sequence (5' – 3')	Reverse sequence (5' – 3')
RT-qPCR	***CCND1***	AAGCTCAAGTGGAACCT	AGGAAGTTGTTGGGGC
	CD44	TTTGCATTGCAGTCAACAGTC	GTTACACCCCAATCTTCATGTCCAC
	EGFR	GACAGGCCACCTCGTCG	CCGGCTCTCCCGATCAATAC
	EGR1	GGCGAGCAGCCCTACGAGC	GTATAGGTGATGGGGGGCAGTC
	IGFBP5	GGTTTGCCTCAACGAAAAGA	CGGTCCTTCTTCACTGCTTC
	IGF1R	TGTCCAGGCCAAAACAGGA	CGGGTTCACAGAGGCATACA
	FKBP5	GGATATACGCCAACATGTTCAA	CCATTGCTTTATTGGCCTCT
	NRDG1	GGCAACCTGCACCTGTTCATCAAT	TGAGGAGAGTGGTCTTTGTTGGGT
	PGR	TGCCTGAAGTTTCGGCCAT	CGCCAACAGAGTGTCCAAGA
	PRMT1	CGCCTCTTGAAGAAGTGTCCT	GATGCCAAAGTGTGCGTAGG
	STAT5A	AAGCCCCACTGGAATGATGG	GGAGTCAAACTTCCAGGCGA
	SGK1	CATAGGAGTTATTGGCAAT	CTTCCATCTCACTAACCA
	28S	CGATCCATCATCCGCAATG	AGCCAAGCTCAGCGCAAC
ChIP-qPCR	***EGFR* +1960**	GAATCTCTGGACTCTGTTCTCAGGTA	CTAGGACTATGTCATTAGCAGATCAG
	***FKBP5* (PRE)**	TAATAGAGGGGCGAGAAGGCAGA	GGTAAGTGGGTGTGCTCGCTCA
	***STAT5A* +6896**	GGACTACTGTGAATTGGCTCGT	GCTTTCTGTTTCTGTTCCTTGA
	hChr1 (NG)	CGGGGGTCTTTTTGGACCTT	GAAACACGGCTGCCAGAAAC

References

Dehairs, J., Talebi, A., Cherifi, Y., and Swinnen, J.V. (2016). CRISP-ID: decoding CRISPR mediated indels by Sanger sequencing. Sci Rep *6*, 28973.

Kastner, P., Krust, A., Turcotte, B., Stropp, U., Tora, L., Gronemeyer, H., and Chambon, P. (1990). Two distinct estrogen-regulated promoters generate transcripts encoding the two functionally different human progesterone receptor forms A and B. EMBO J. *9*, 1603–1614.

Le Romancer, M., Treilleux, I., Leconte, N., Robin-Lespinasse, Y., Sentis, S., Bouchekioua-Bouzaghou, K., Goddard, S., Gobert-Gosse, S., and Corbo, L. (2008). Regulation of estrogen rapid signaling through arginine methylation by PRMT1. Mol. Cell *31*, 212–221.

Robin-Lespinasse, Y., Sentis, S., Kolytcheff, C., Rostan, M.-C., Corbo, L., and Le Romancer, M. (2007). hCAF1, a new regulator of PRMT1-dependent arginine methylation. J. Cell. Sci. *120*, 638–647.

Teyssier, C., Ma, H., Emter, R., Kralli, A., and Stallcup, M.R. (2005). Activation of nuclear receptor coactivator PGC-1alpha by arginine methylation. Genes Dev. *19*, 1466–1473.