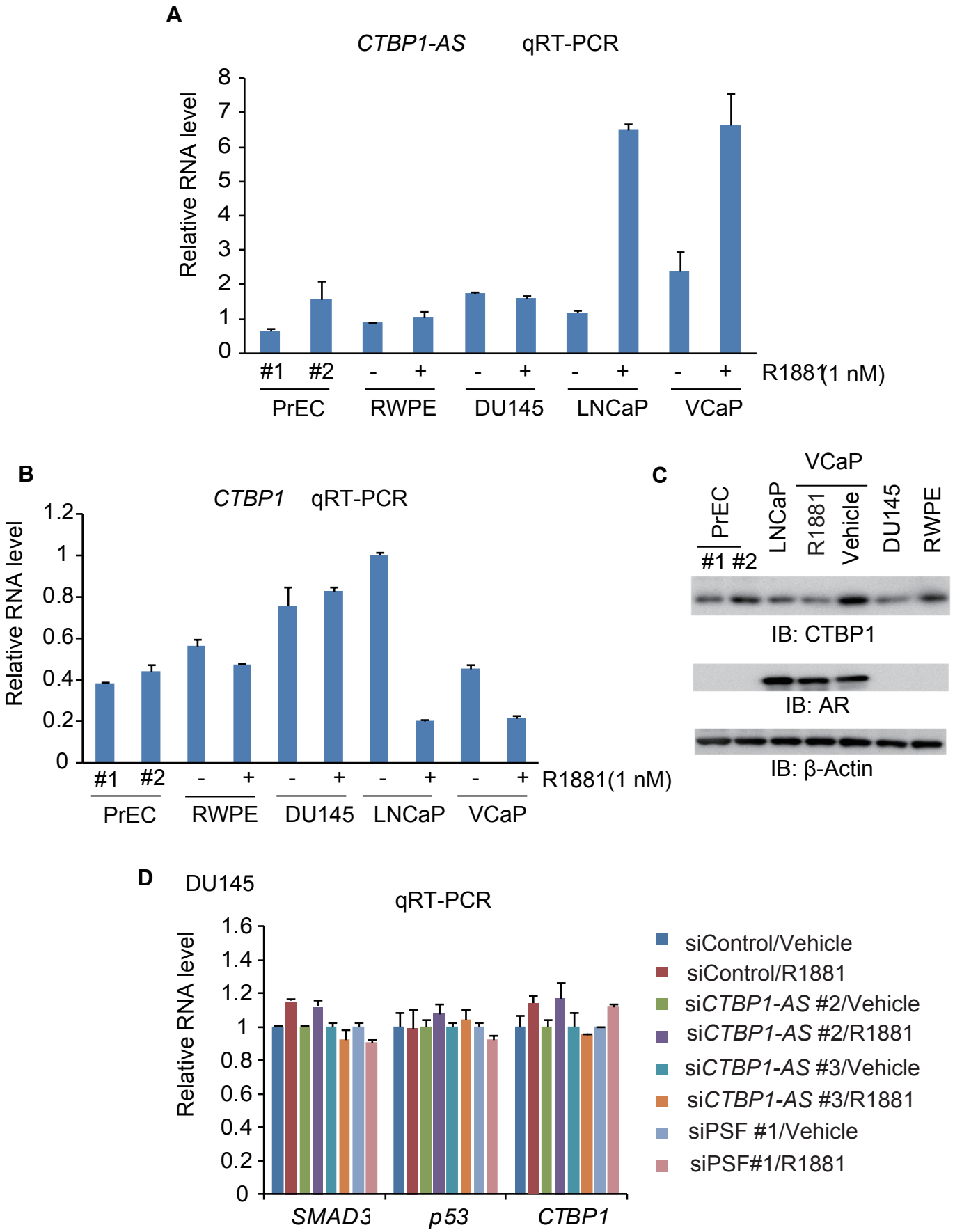
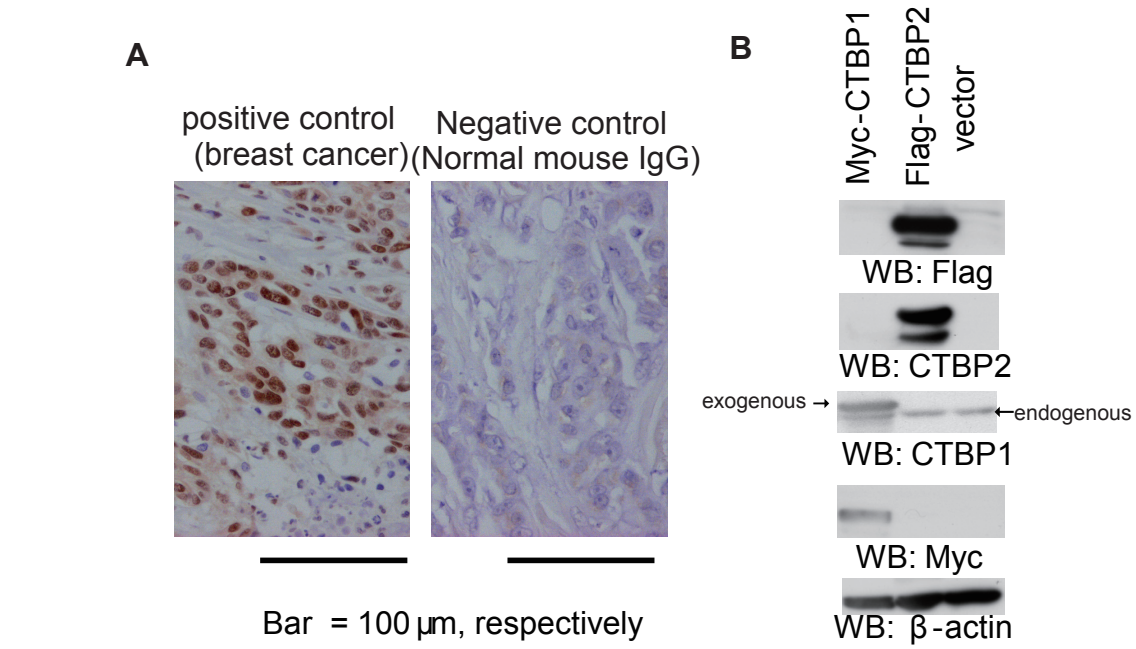
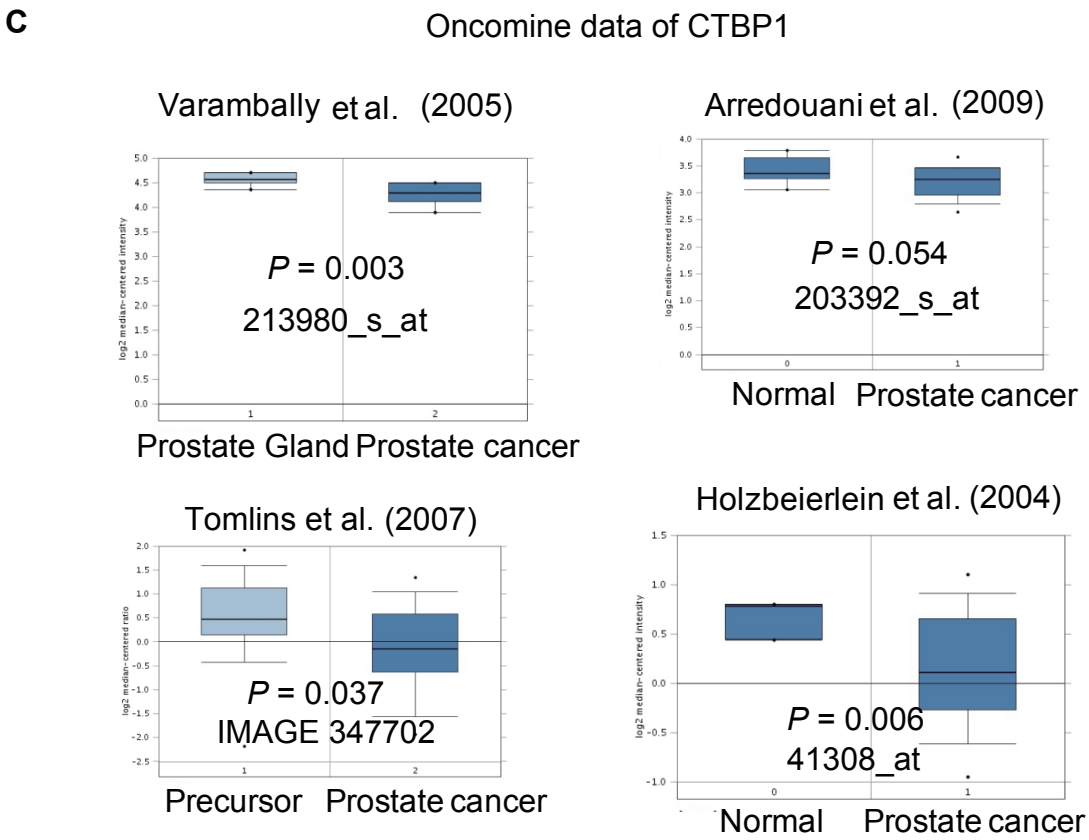
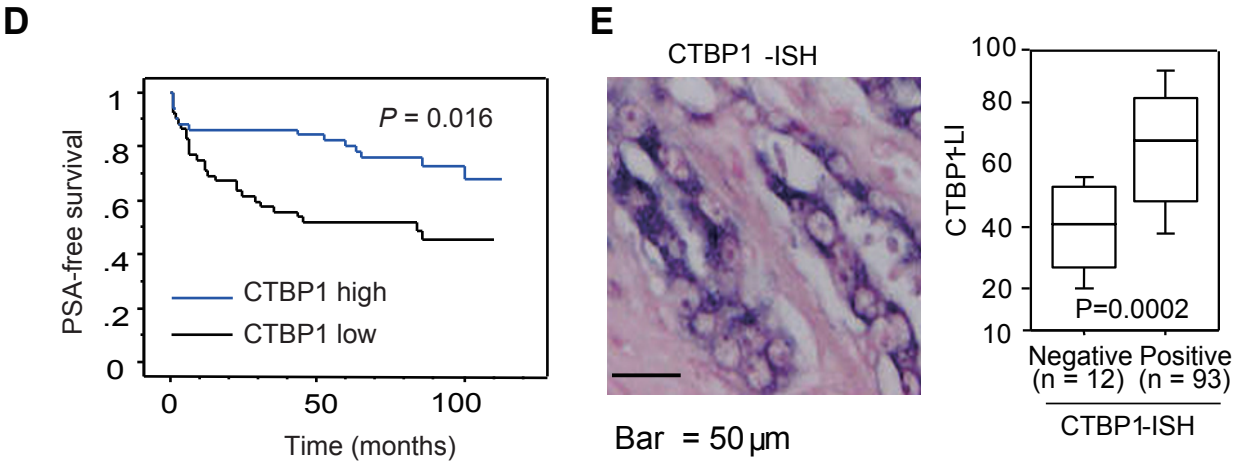


Supplementary Fig. 1







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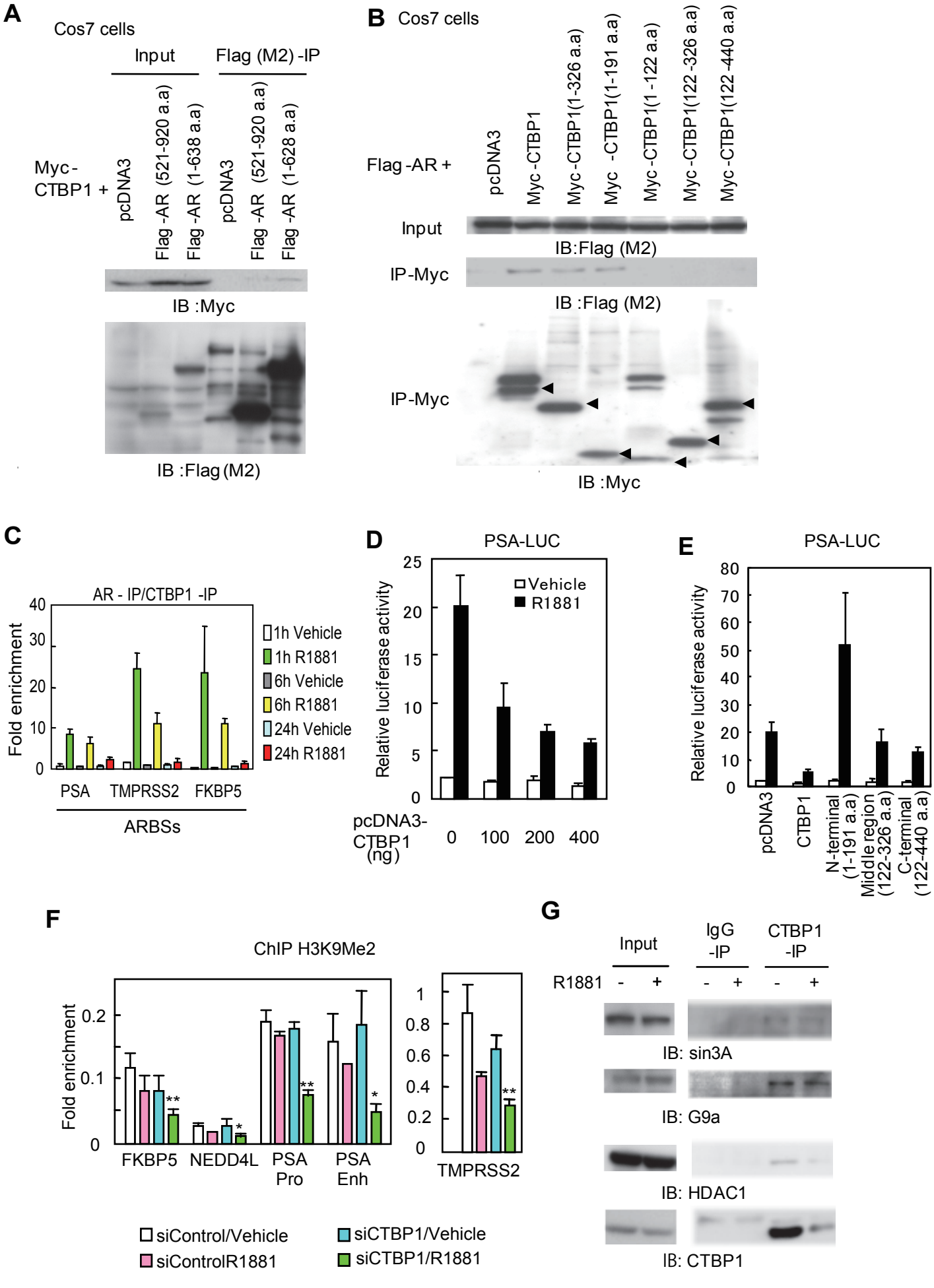

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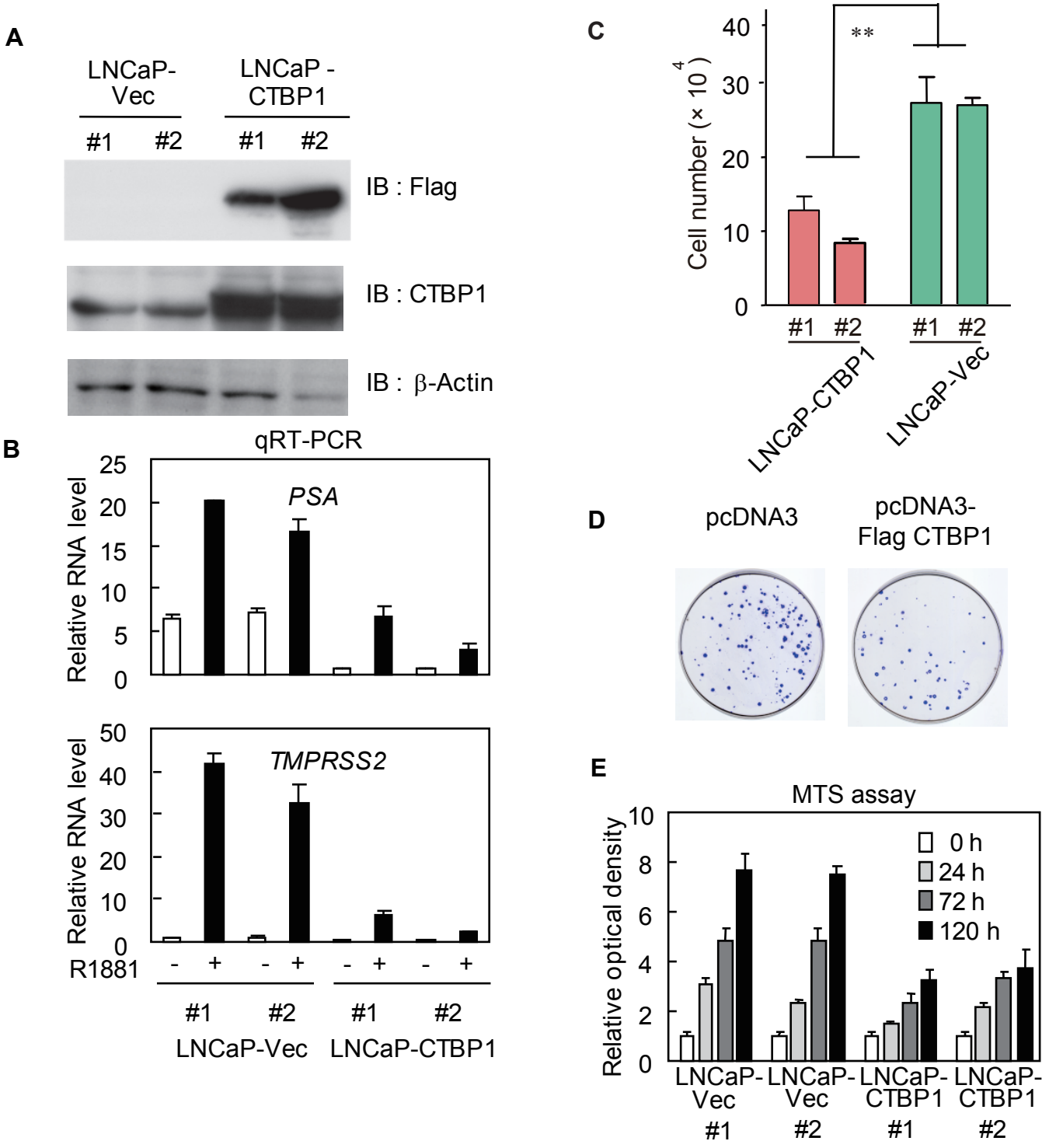

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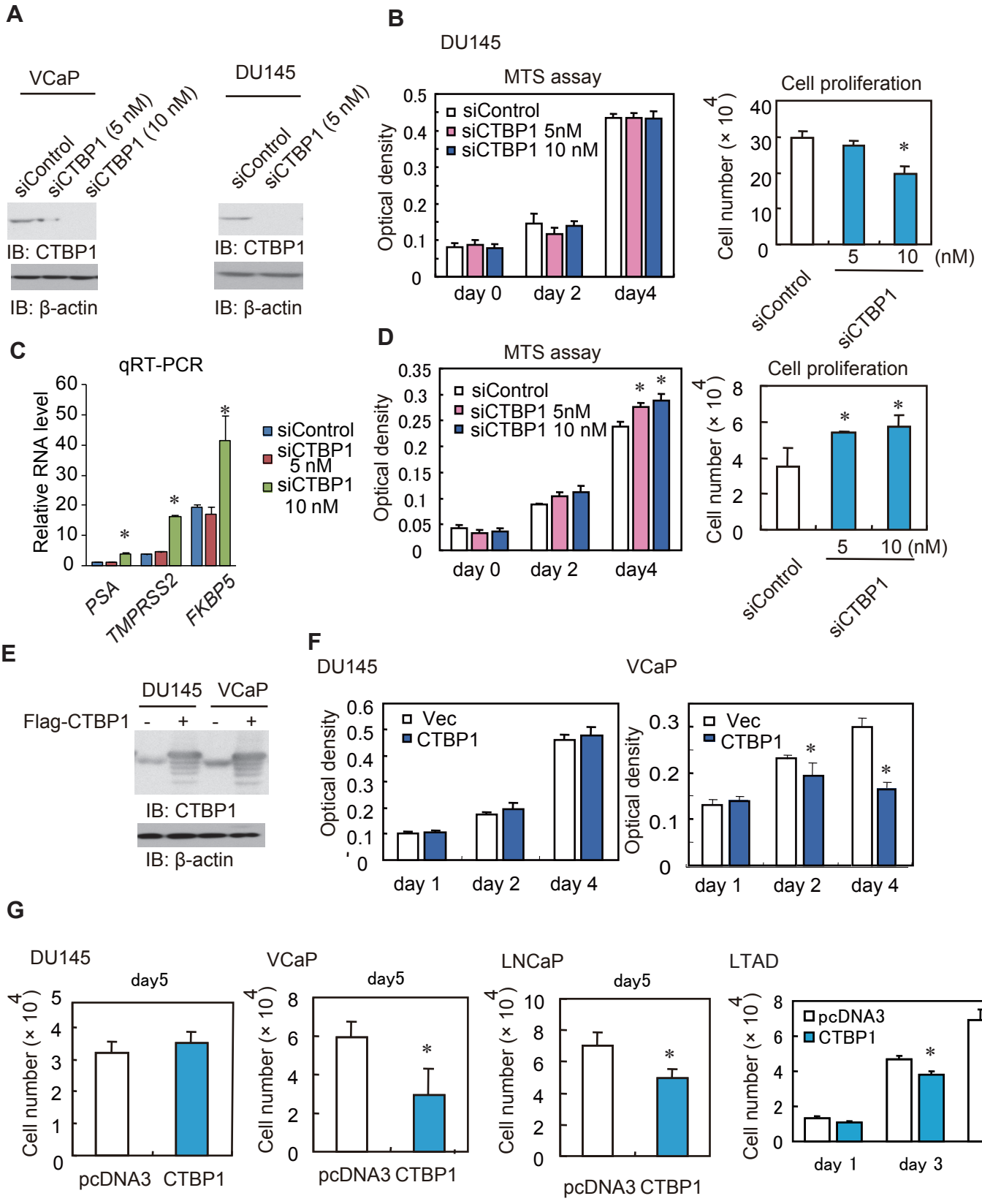

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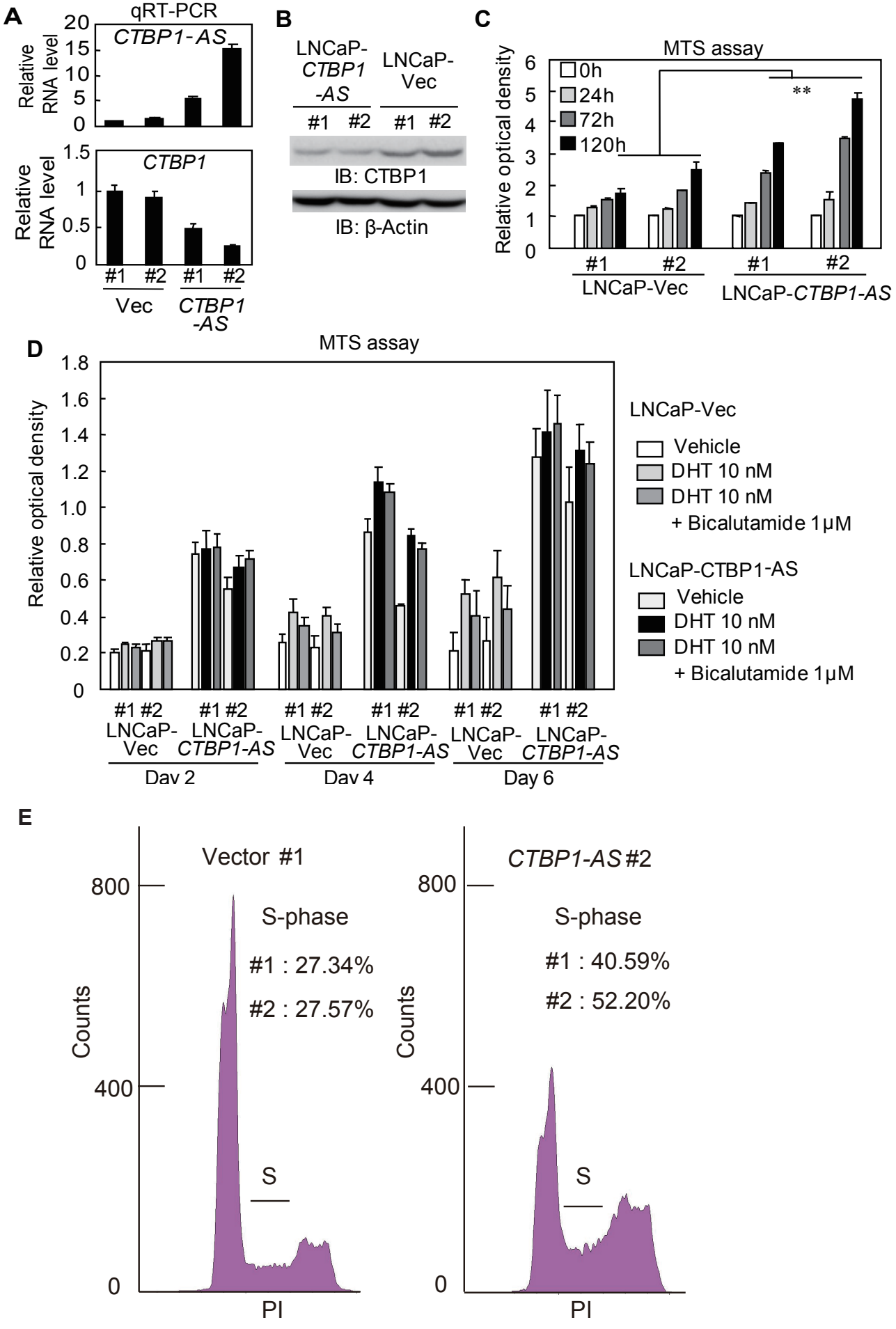

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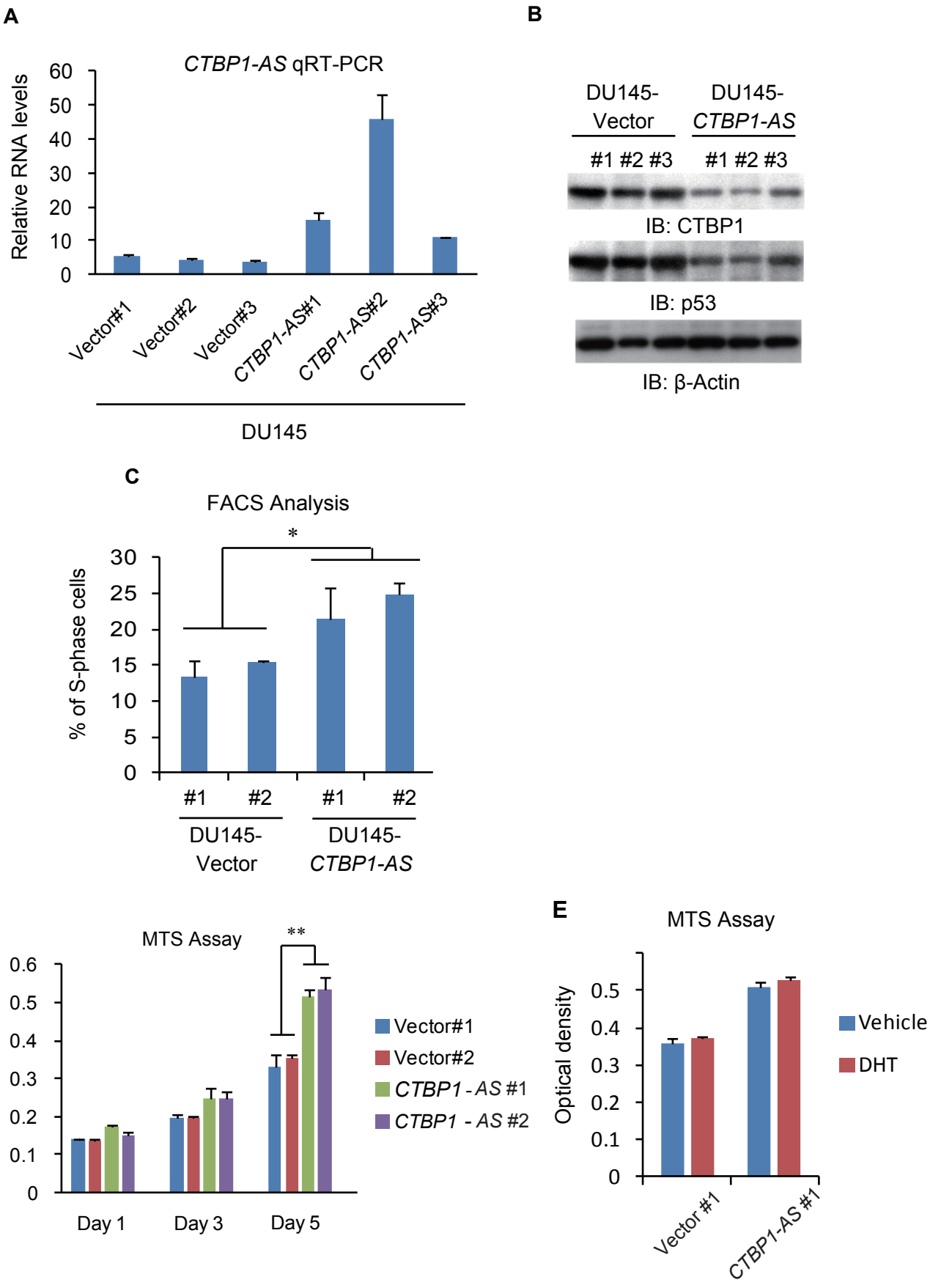

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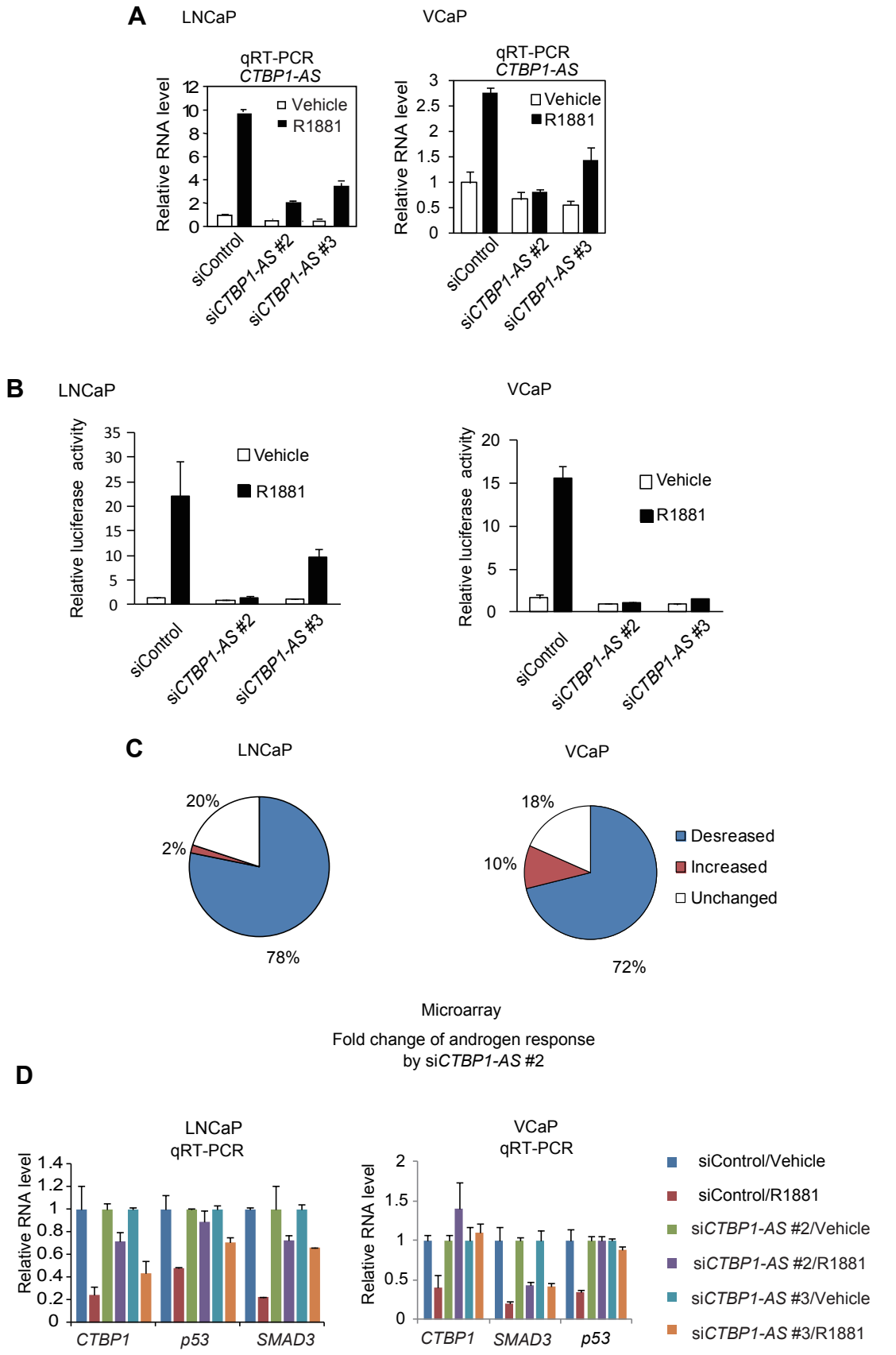

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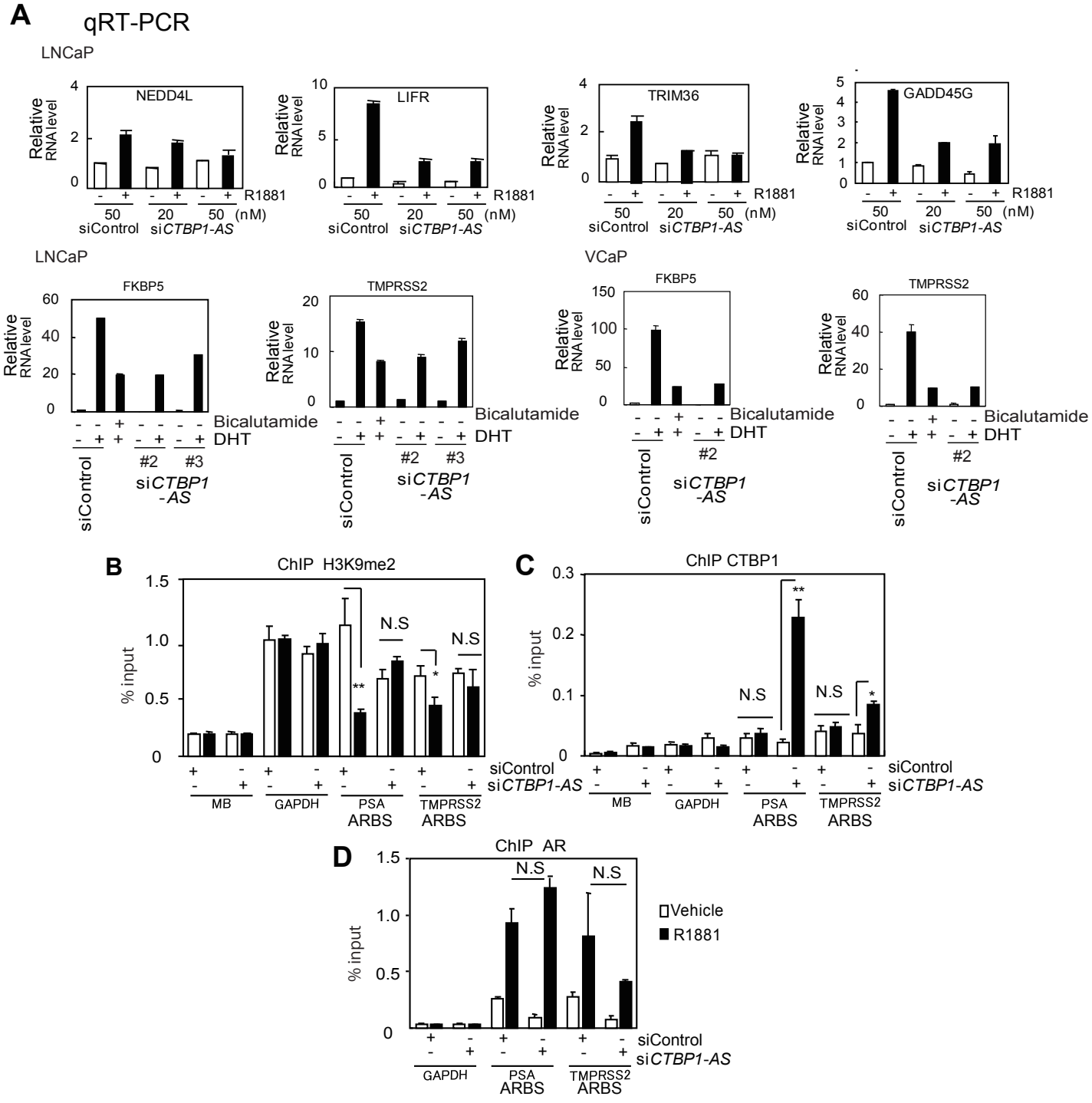

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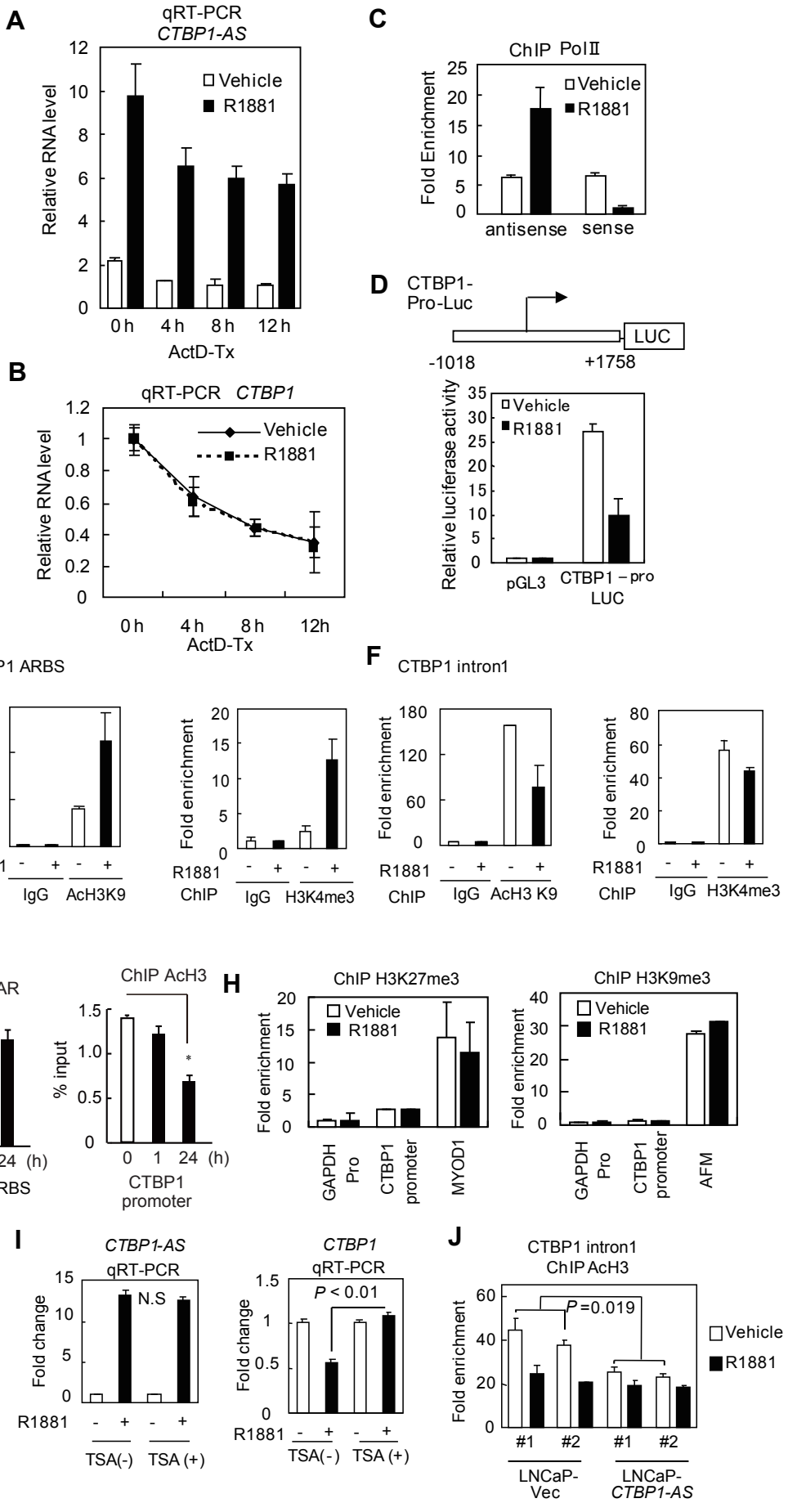

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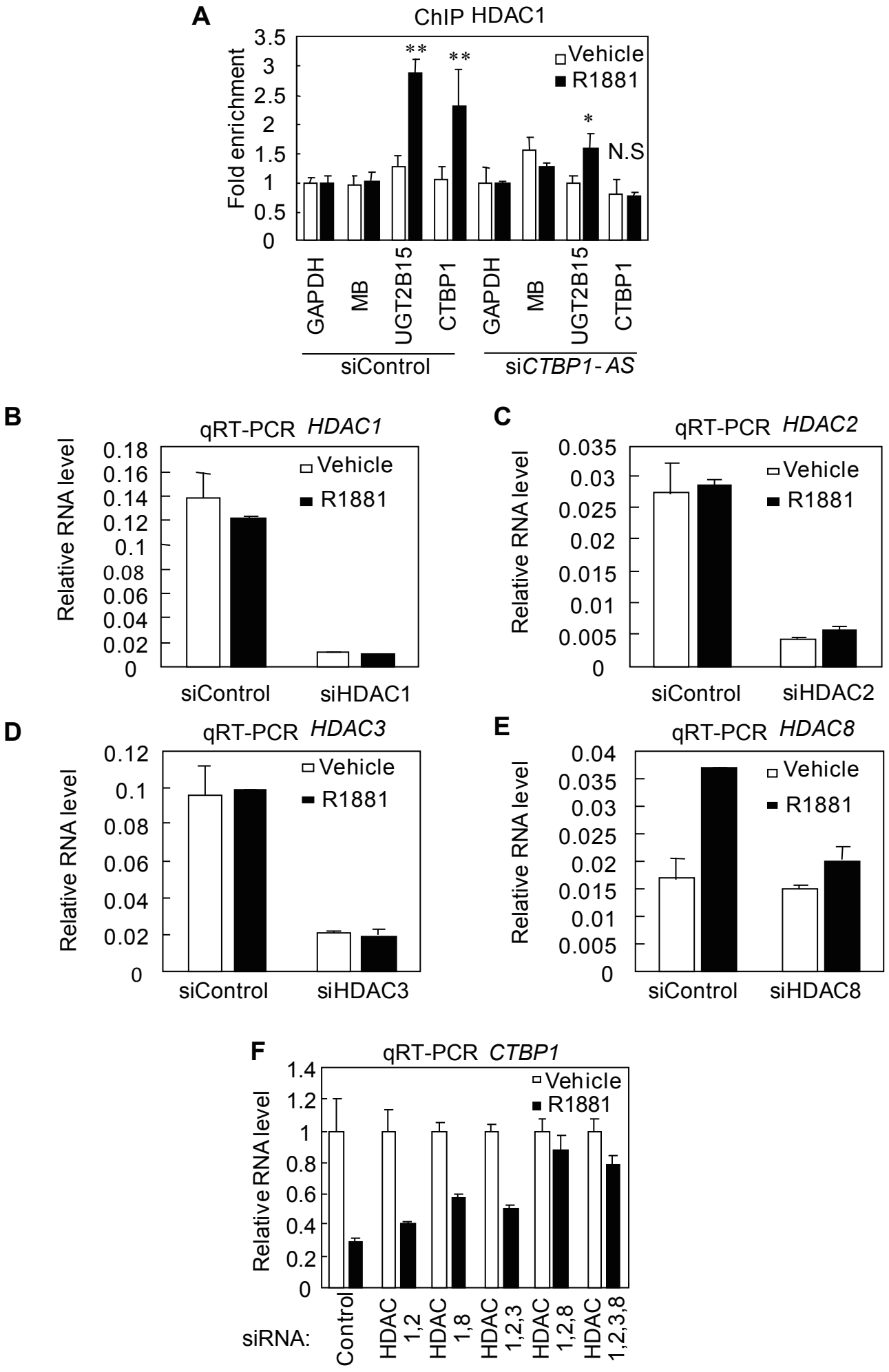

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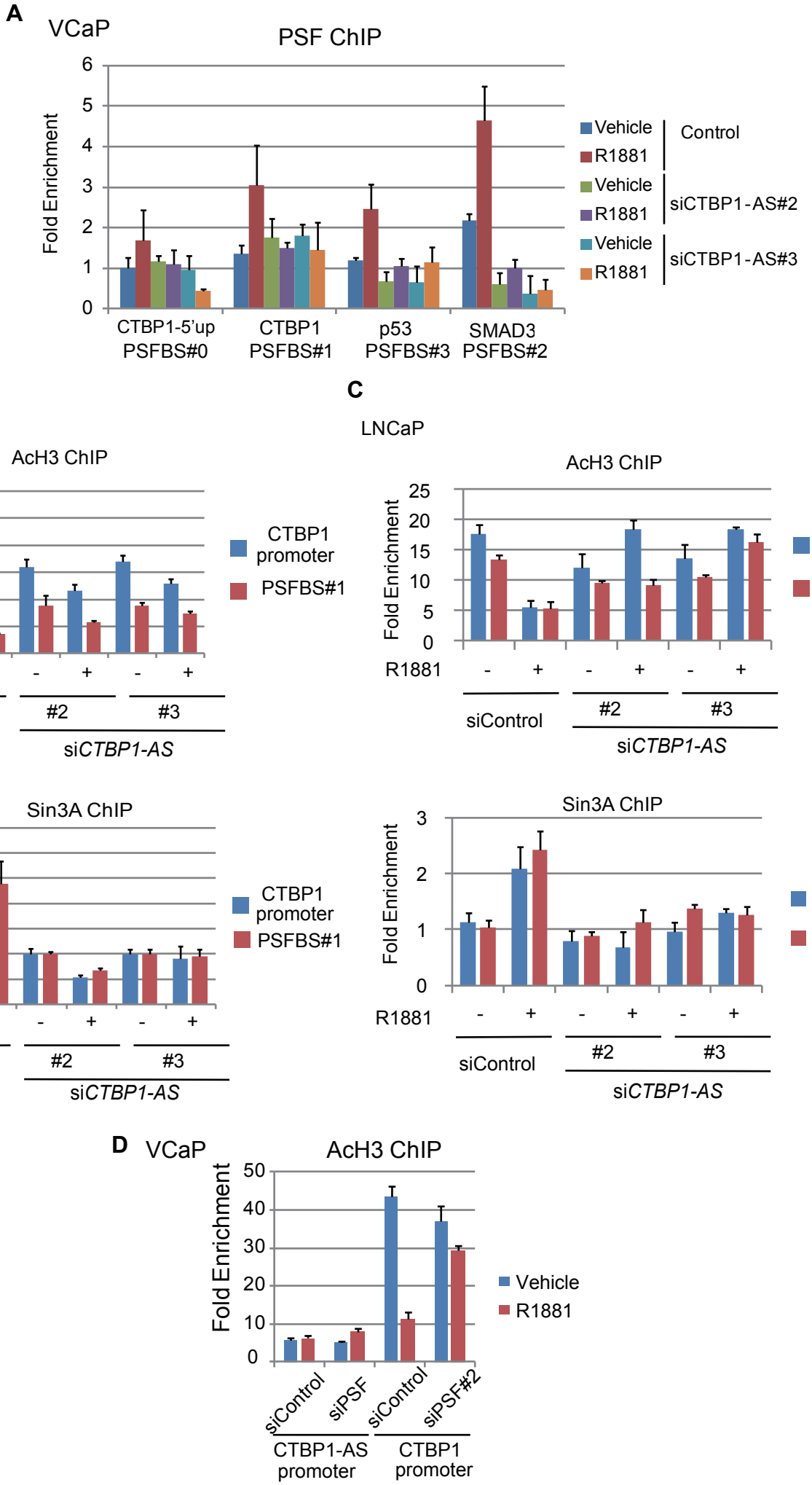

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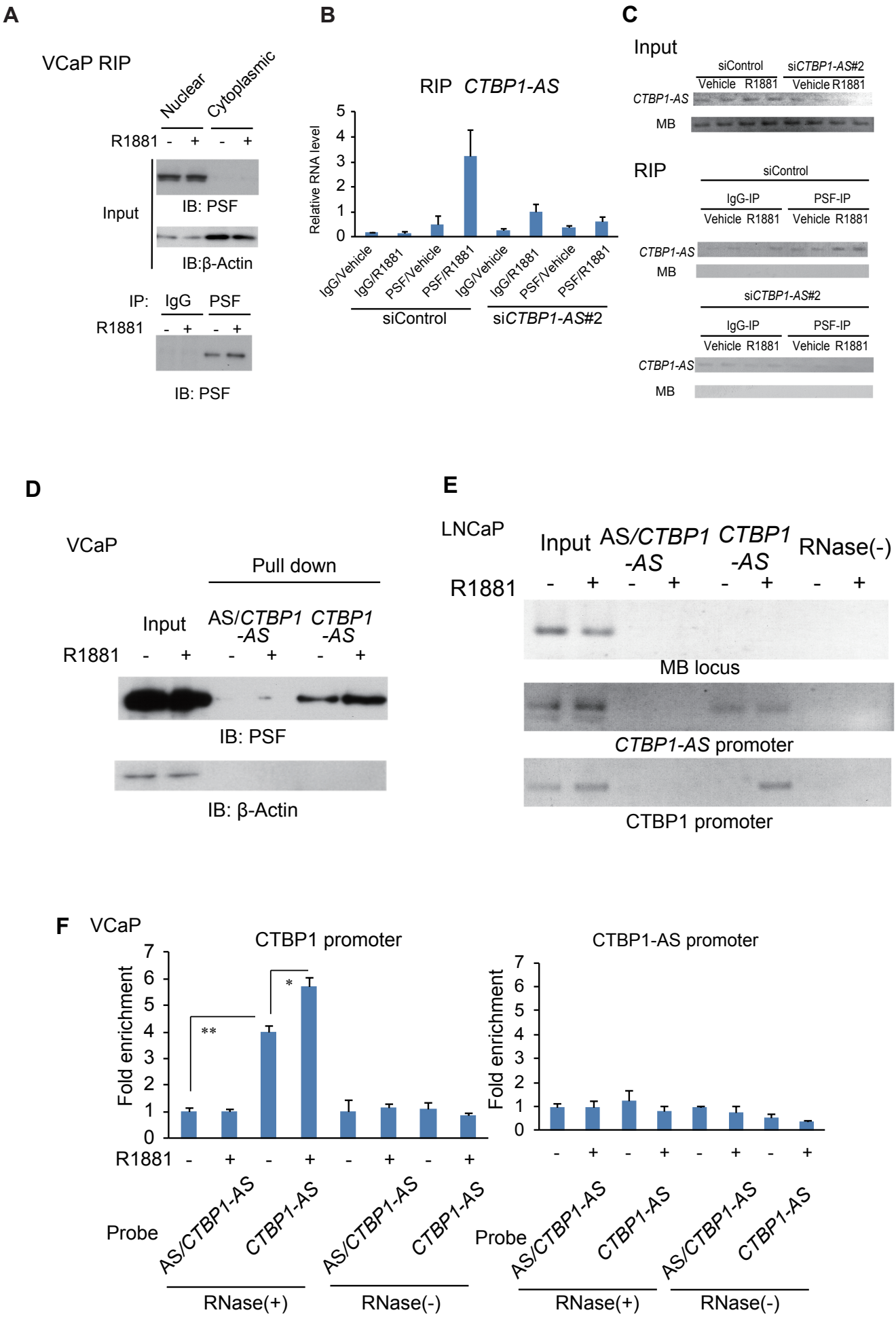

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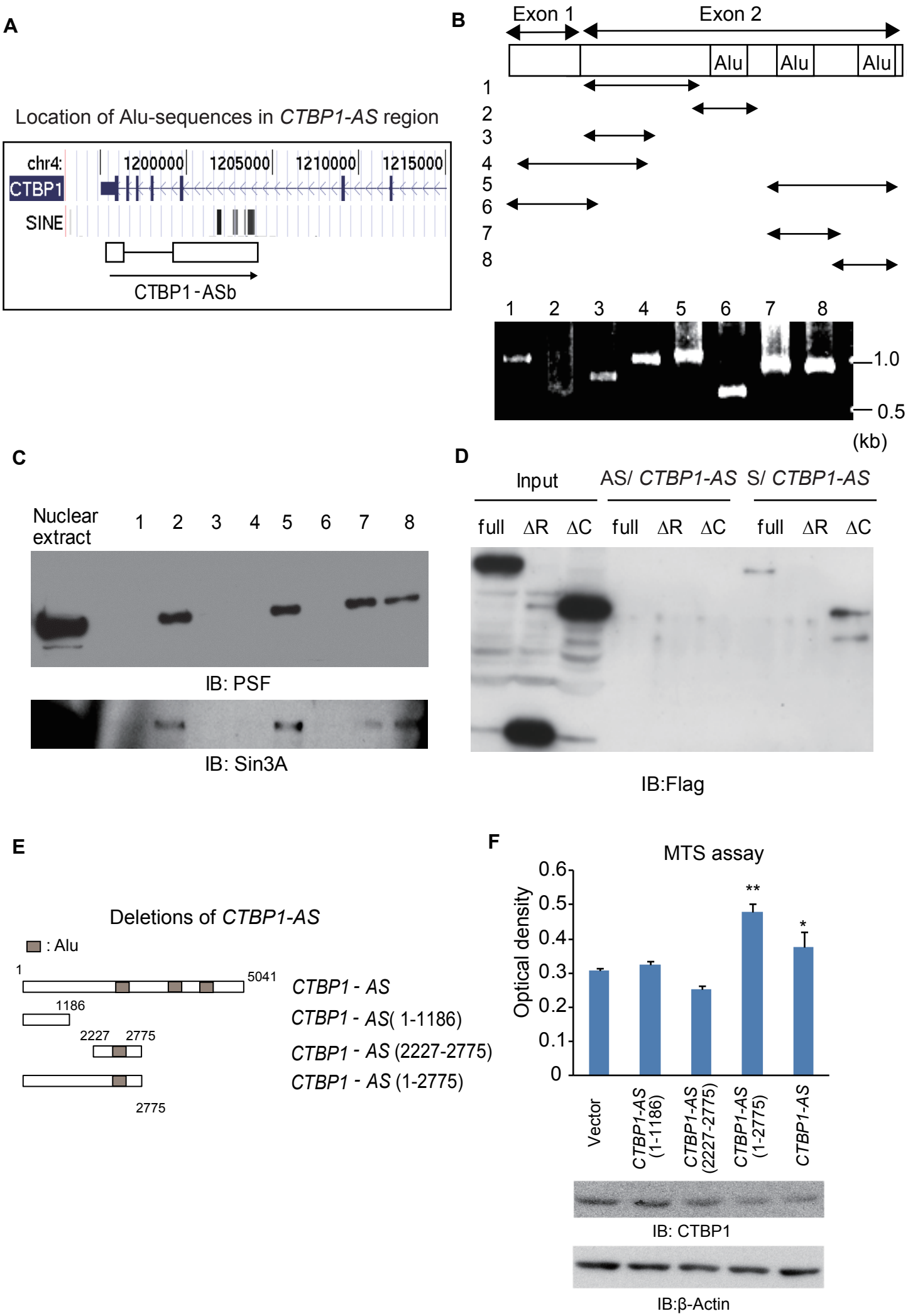

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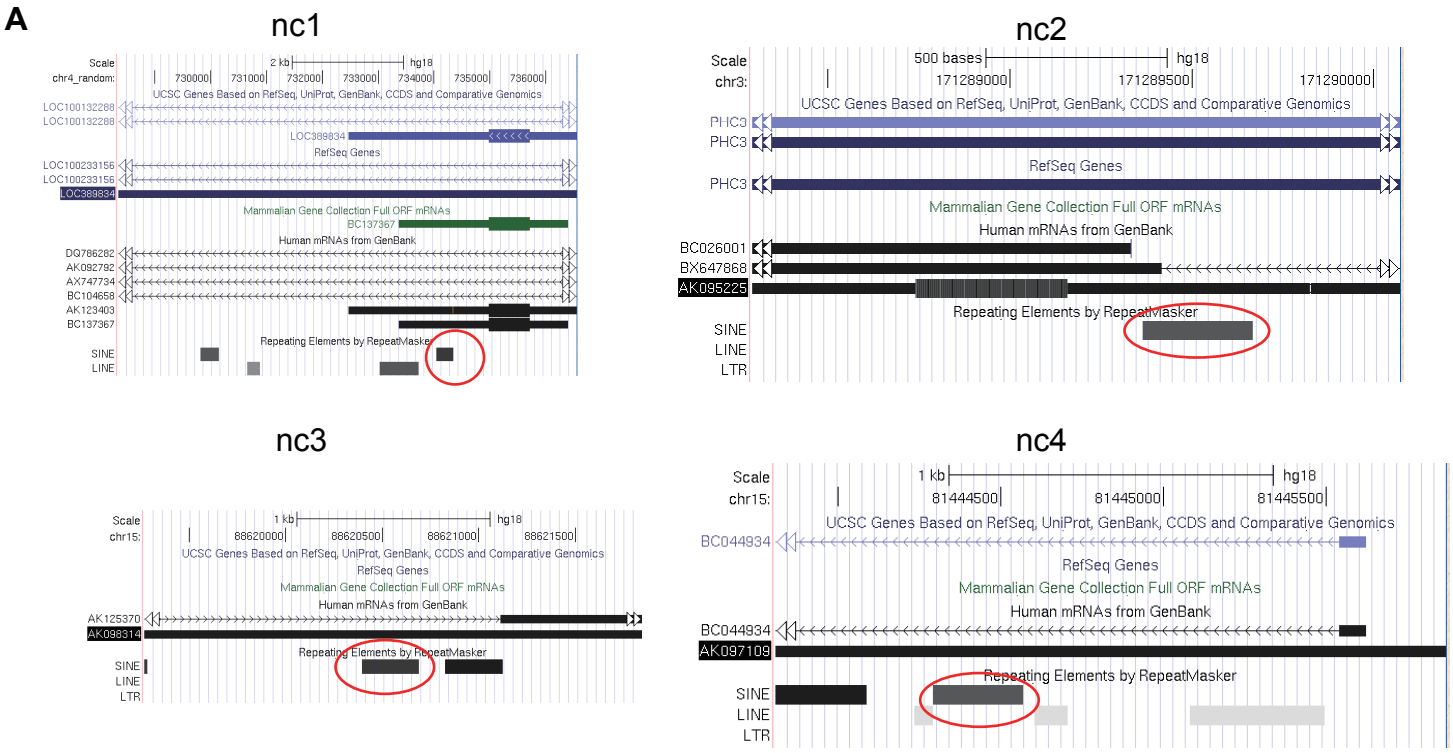

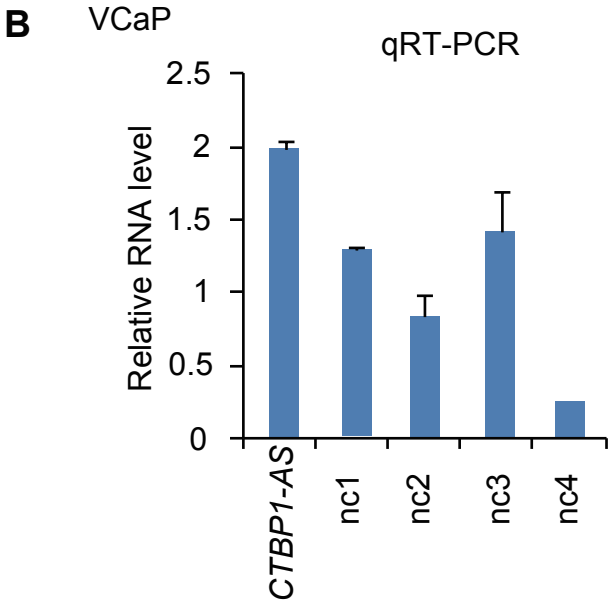

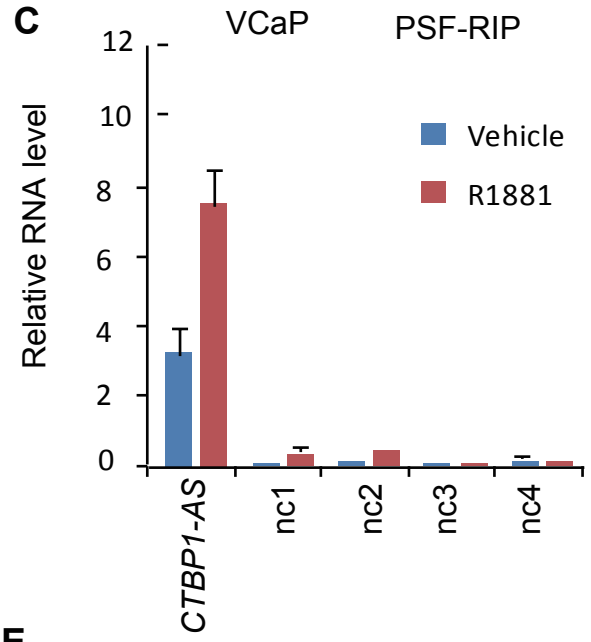

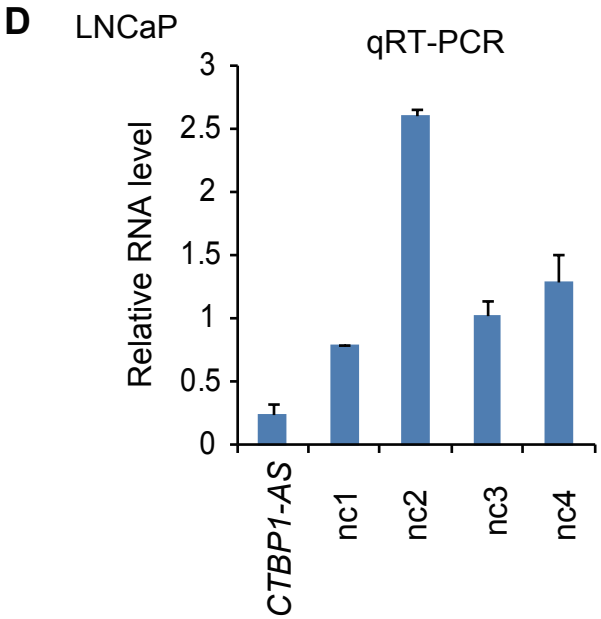

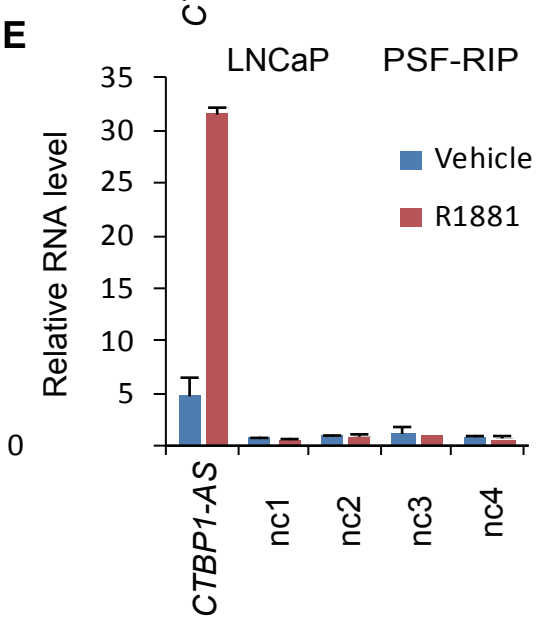

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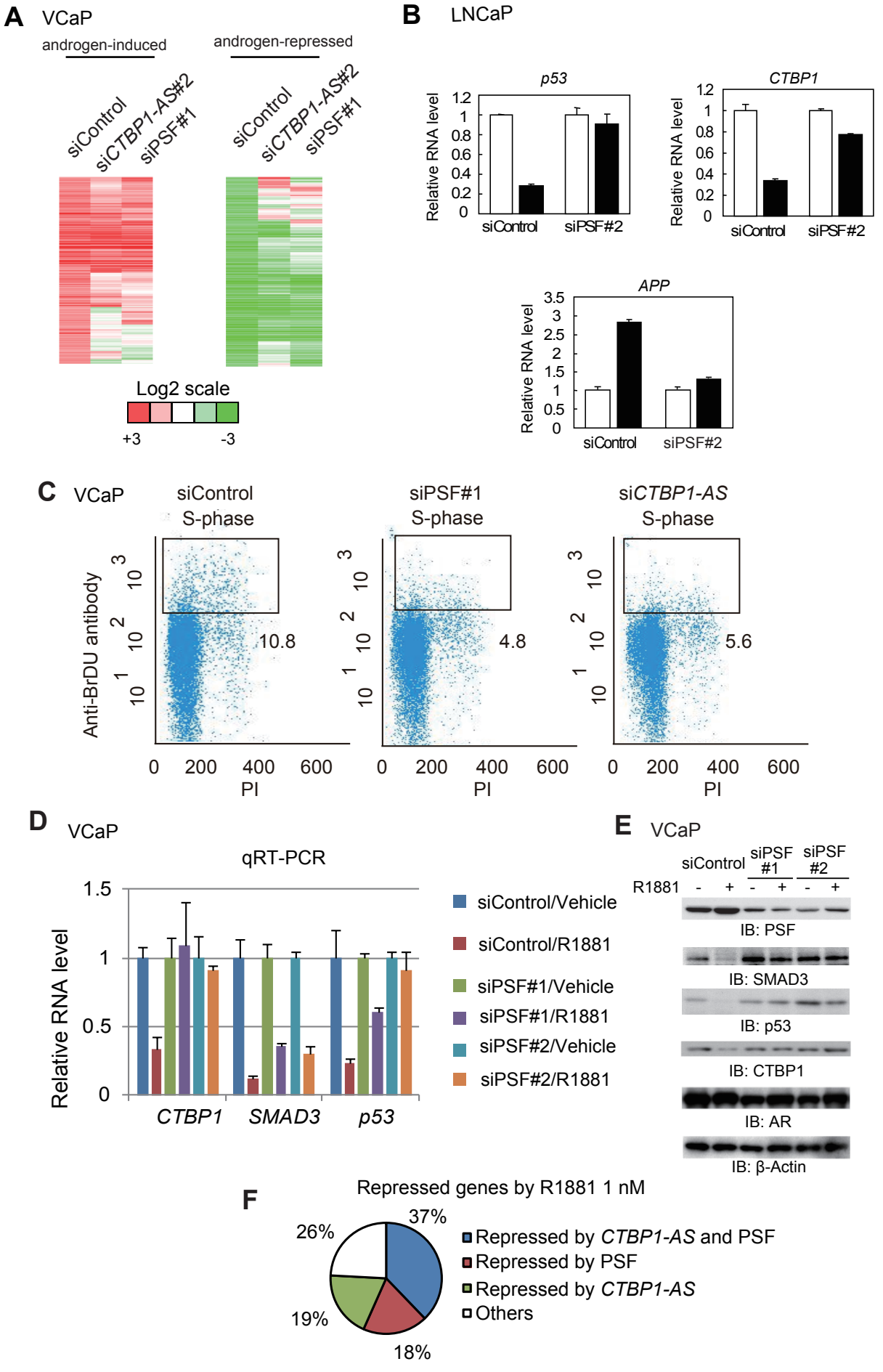

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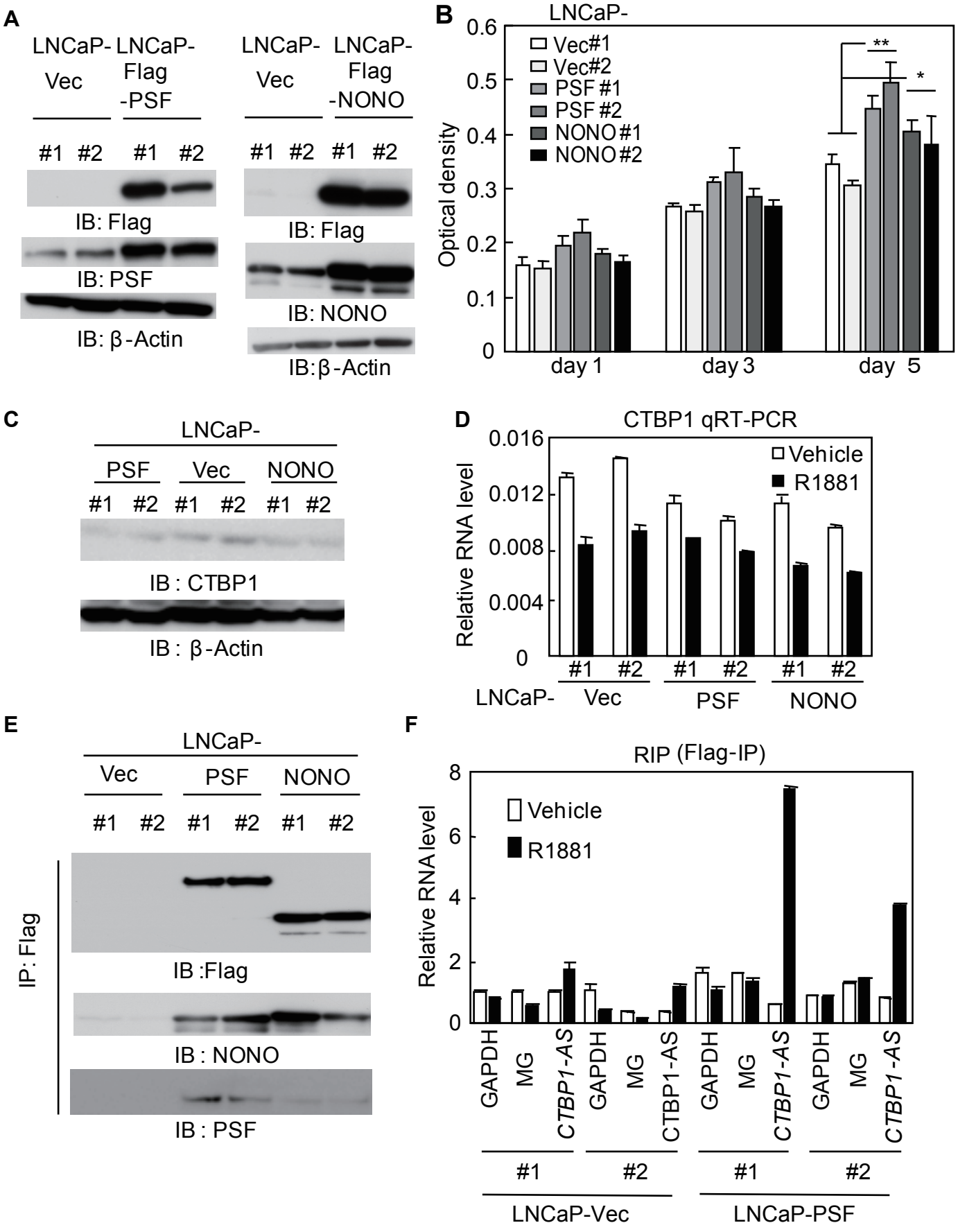

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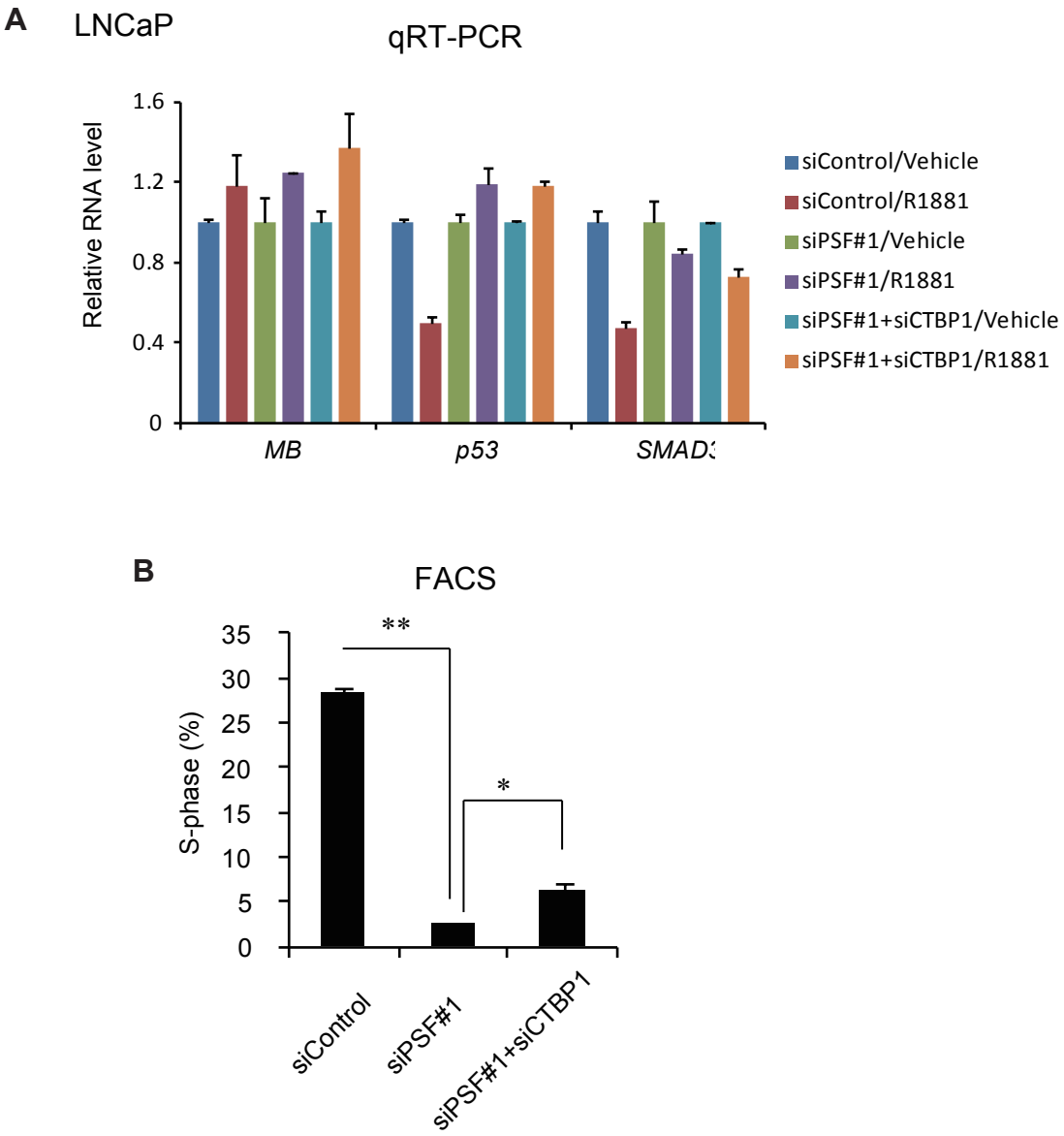

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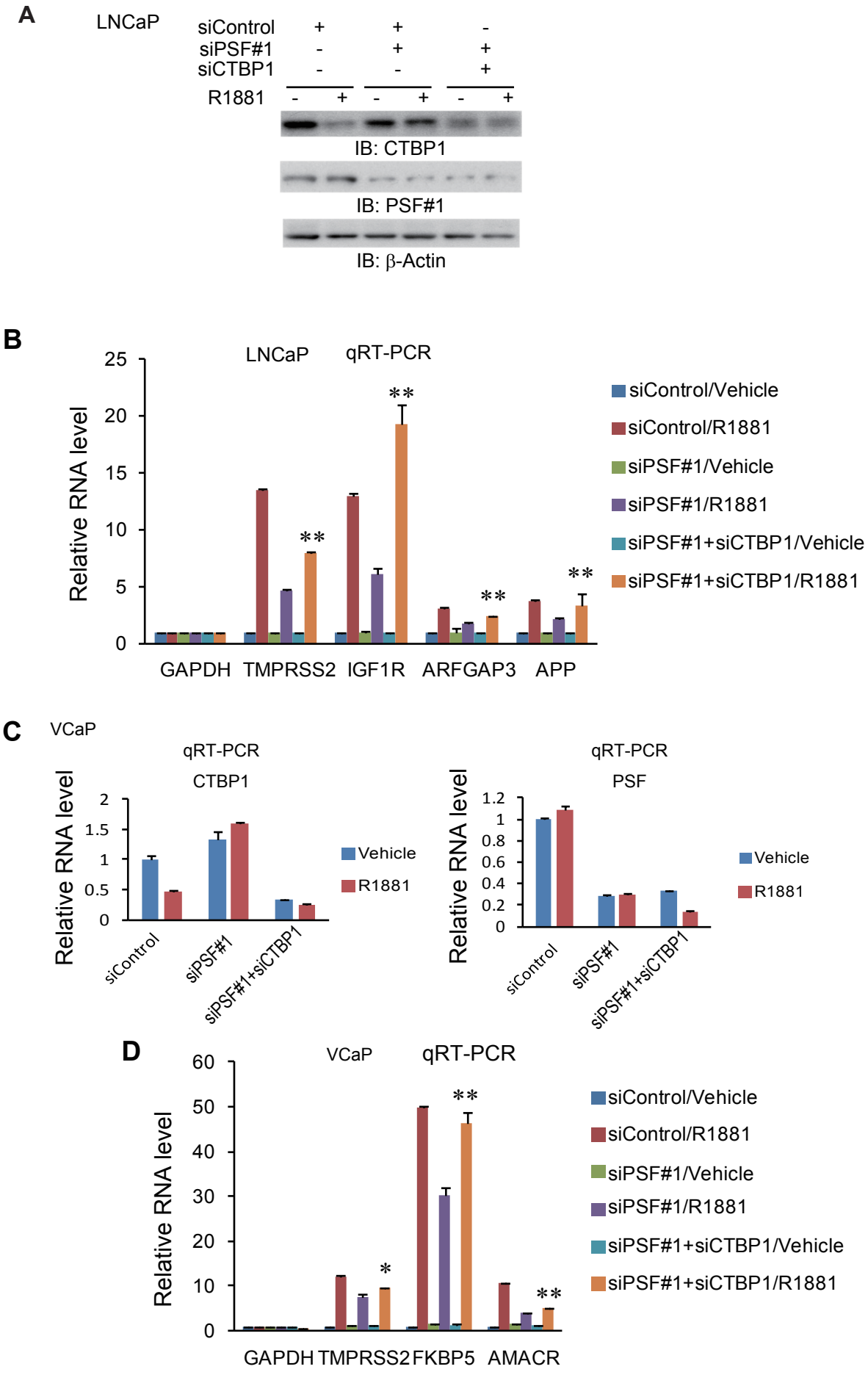

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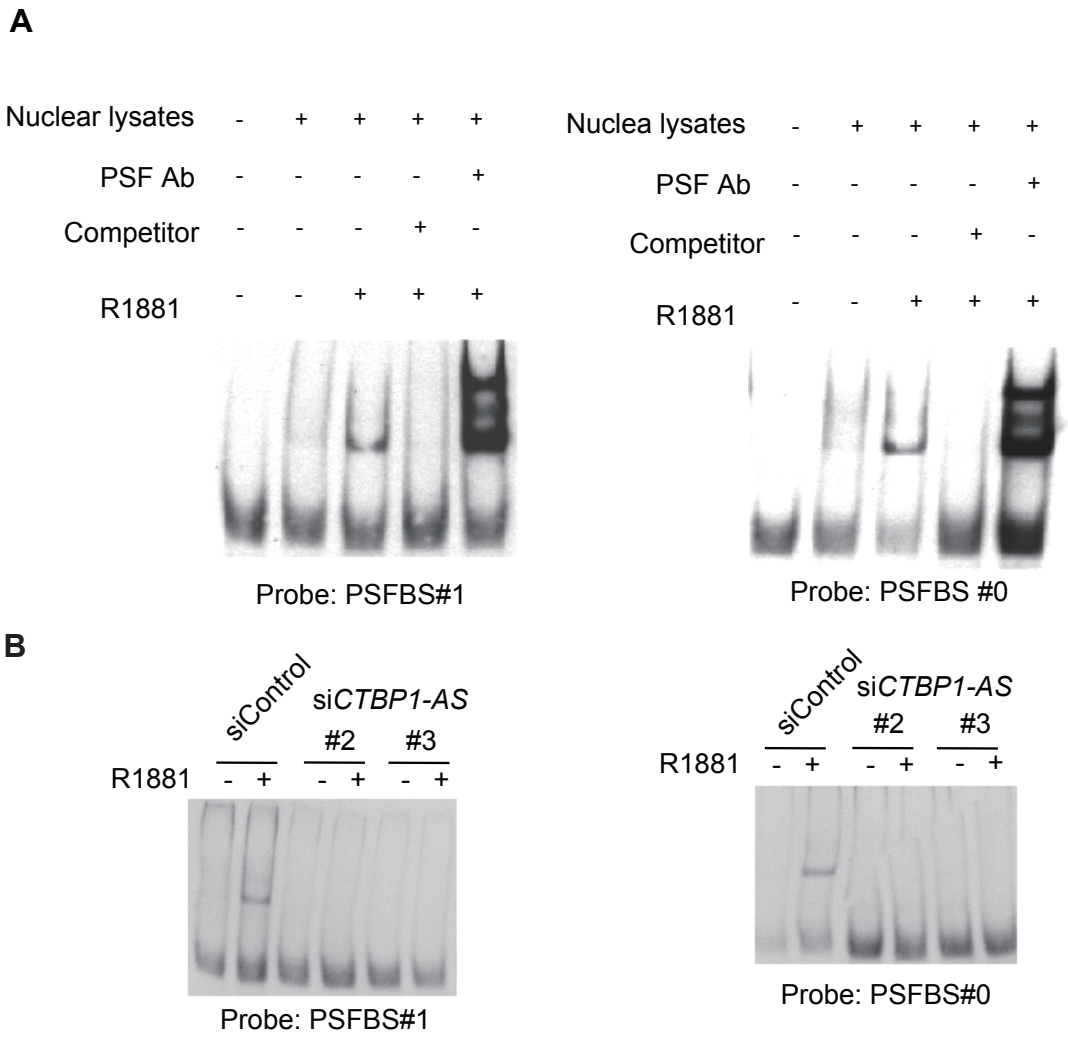

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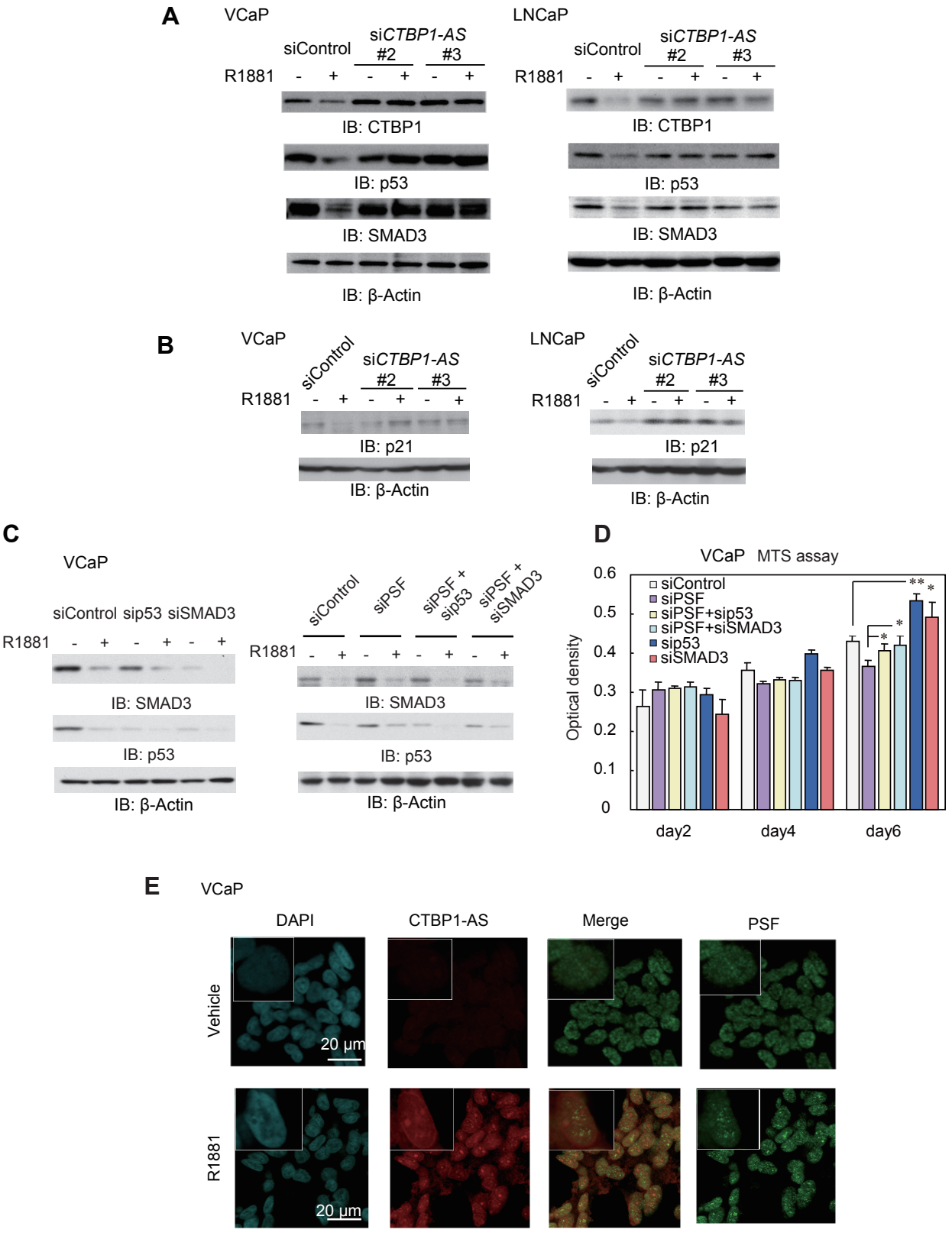

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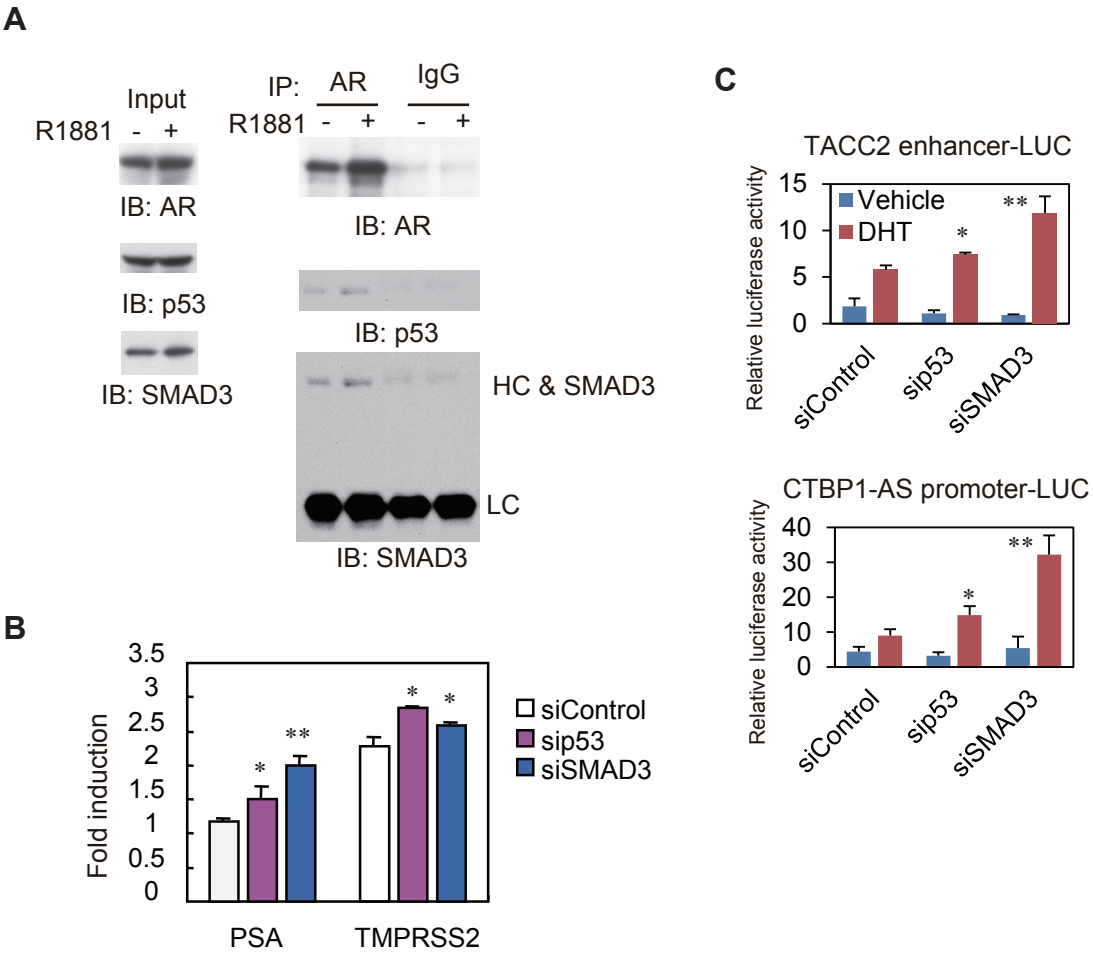

Supplementary Fig. 22

Supplementary Figure Legends

Supplementary Figure 1. Expression of CTBP1 and *CTBP1-AS* in prostate epithelial cells and prostate cancer cells.

(A, B) Expression of *CTBP1-AS* (A) and *CTBP1* (B) in prostate epithelial cells (PrECs), RWPE, DU145, LNCaP, and VCaP cells. RWPE, DU145, LNCaP and VCaP cells were treated with 1 nM R1881 or vehicle. PrECs derived from two individuals (#1 and #2) were used. *CTBP1-AS* (A) and *CTBP1* (B) expression levels were measured by qRT-PCR. Bar: s.d. (C) Expression levels of CTBP1 in prostate epithelial cells and prostate cancer cells. Western blot analysis for CTBP1 and AR was performed. β-actin was used as loading control. (D) qRT-PCR analysis of androgen-repressed genes in DU145 cells. DU145 cells were transfected with siControl, siPSF#2, si*CTBP1-AS*#2 or si*CTBP1-AS*#3 (10 nM). Two days later, cells were treated with 1 nM R1881 or vehicle for 24 h. Bar: s.d.

Supplementary Figure 2. Analysis of CTBP1 expression in prostate cancer samples.

(A) Specificity of CTBP1 antibody in CTBP1 immunohistochemistry. Stainings of positive control (breast cancer specimen) and negative control (normal mouse IgG was used as primary control) were shown. (B) Western blot analysis of CTBP isoforms by CTBP1/2 specific antibody. CTBP2 antibody was purchased from Abcam. CTBP1 antibody did not detect CTBP2 overexpression but CTBP1. (C) Microarray analysis of CTBP1 in the Oncomine database (Holzbeierlein et al., 2004, Arredouani et al., 2009, Tomlins et al., 2007, Varambally et al., 2005). Four examples of CTBP1 decrease in cancer compared with normal or prostate cancer precursor tissues are indicated, although an oligonucleotide-based microarray study (Welsh et al., 2001) has detected CTBP1 increase in cancer. (D) CTBP1 high expression is associated with prolonged PSA-free survival. Kaplan-Meier analysis using the log-rank test was performed. (E) *in situ* hybridization (ISH)

analysis of CTBP1. CTBP1 mRNA was detected in benign epithelium and cancerous tissues. A positive correlation was observed between CTBP1 transcript levels evaluated by ISH and protein expression levels evaluated by immunohistochemistry.

Supplementary Figure 3. Transcriptional repression of AR by CTBP1

(A) The N-terminal region of AR interacts with CTBP1 in a ligand-dependent manner. FLAG-tagged AR deletion mutants 521-638 a.a and 1-638 a.a were constructed. Cos7 cells were co-transfected with combinations of each mutant with Myc-CTBP1. (B) The N-terminal region of CTBP1 interacts with AR in a ligand-dependent manner. Six Myc-tagged CTBP1 deletion mutants were constructed as indicated. Cos7 cells were co-transfected with combinations of each mutant with FLAG-AR. (C) Re-ChIP analysis of CTBP1 and AR colocalization at ARBSs. (D, E) Addition of CTBP1 represses AR-mediated transcriptional activity. LNCaP cells were transfected with luciferase vector including the PSA promoter and enhancer regions. Twenty-four hours post transfection, LNCaP cells were treated with 10 nM R1881 or vehicle for 24 h. Cells were co-transfected with a CTBP1-expressing vector (100, 200, or 400 ng) or empty vector and luciferase vector (D). Cells were also co-transfected with CTBP1 deletion mutants and luciferase vector (E) (F) Analysis of histone H3K9me2 levels by ChIP assay. LNCaP cells were transfected with siControl or siCTBP1 (50 nM) and then treated with 10 nM R1881 or vehicle for 6 h. (G) Immunoprecipitation of CTBP1 in LNCaP cells treated with 10 nM R1881 or vehicle for 12 h. Western blot analysis was performed with antibodies against CTBP1, G9a, HDAC1, and Sin3A. $*$ $P < 0.05$, $**P < 0.01$ compared with siControl. Bar: s.d.

Supplementary Figure 4. Analysis of CTBP1 function by overexpression in LNCaP cells

(A) Overexpression of CTBP1 in LNCaP cells. LNCaP cell lines stably expressing CTBP1 were

established. LNCaP cells were transfected with FLAG-CTBP1 pcDNA3. Selection was performed using 500 μg/ml G418. Expression of CTBP1 was validated by western blot analysis. (B) Analysis of androgen-regulated genes in LNCaP cells stably expressing CTBP1. LNCaP cell lines stably expressing vector control or CTBP1 were treated with or without 10 nM R1881. Expression levels of *PSA* and *TMPRSS2* were analyzed by qRT-PCR. Bar: s.d. (C) CTBP1 overexpression repressed LNCaP cell proliferation. LNCaP cells expressing CTBP1 stably or vector controls were plated and cell numbers were counted after 5 days incubation. (D) Colony formation assay for CTBP1-expressing LNCaP cells. LNCaP cells were transfected with FLAG-CTBP1 pcDNA3 or empty vector. Representative dishes stained with crystal blue after selection for 2 weeks with 500 μg/ml G418 are shown. (E) MTS assay on LNCaP cells stably expressing CTBP1. Growth rates of cells stably expressing vector control or CTBP1 were measured by MTS assay. Data are expressed as mean ± s.d. ($n = 6$) $*P < 0.05$, $**P < 0.01$.

Supplementary Figure 5. Transcriptional repression of AR target gene induction by CTBP1 in both LNCaP and VCaP cells

(A) Western blot analysis of VCaP and DU145 cells transfected with siControl or siCTBP1. (B) Growth stimulatory effects by siCTBP1 were not observed in DU145 cells. MTS assay and cell counting were performed to analyze the cell proliferation. Bar: s.d. (C) VCaP cells transfected with siControl or siCTBP1 (5 or 10 nM) were treated with 1 nM R1881 for 4 h. Fold induction following androgen treatment is shown. RNA levels were measured by qRT-PCR. Bar: s.d. (D) CTBP1 knockdown accelerates cell proliferation. (left) MTS assay of VCaP cells transfected with siCTBP1 ($n = 4$). Bar: s.d. (right) The numbers of LNCaP cells transfected with siControl or siCTBP1 were counted 3 days after transfection. Bar: s.d. (E) Western blot analysis of VCaP and DU145 cells transfected with Flag-CTBP1 or empty vector. (F) CTBP1 overexpression repressed

the cell proliferation of VCaP cells. MTS assay of VCaP and DU145 cells transfectd with Flag-CTBP1 or empty vector (pcDNA3) were performed (n = 4) Bar: s.d. (G)Analysis of cell counting after CTBP1 overexpression at day 4 after transfection in DU145, VCaP and LNCaP cells and day 1, 3 and 5 in LTAD cells. CTBP1 overexpression reduced the cell numbers of AR-positive cells. Bar: s.d. $*P < 0.05$, $**P < 0.01$.

Supplementary Figure 6. Functional analysis of *CTBP1-AS* by overexpression in LNCaP cells.

(A) (Top) Establishment of LNCaP cell lines stably expressing *CTBP1-AS*. *CTBP1-AS* expression levels were measured by qRT-PCR. (Bottom) mRNA expression levels of CTBP1 in LNCaP cells stably expressing *CTBP1-AS*. Expression levels of CTBP1 were analyzed by qRT-PCR. (B) Protein expression levels of CTBP1 in LNCaP cells stably expressing *CTBP1-AS*. CTBP1 proteins were detected by western blotting. (C) MTS assay on LNCaP cells stably expressing *CTBP1-AS*. MTS assay was performed at 24, 72, or 120 h after plating cells. The fold change over 0 h is indicated ($n = 4$). (D) MTS assay on LNCaP cells stably expressing vector control or *CTBP1-AS*. Cells were plated in phenol red-free medium with charcoal-stripped serum, incubated for 48 h, and then treated with 10 nM DHT, 10 nM DHT + 10 μM bicalutamide, or vehicle. MTS assay was performed on day 2, 4, and 6 post treatment ($n = 4$). (E) Cell cycle analysis on LNCaP cells stably expressing *CTBP1-AS*. Cells were treated with 10 % FCS and FACS analysis was performed after 24 h incubation. $*P < 0.05$, $**P < 0.01$.

Supplementary Figure 7. Overexpression of *CTBP1-AS* accelerates proliferation of DU145 AR-negative prostate cancer cells

(A) Establishment of DU145 cells stably expressing *CTBP1-AS*. *CTBP1-AS* expression levels

were measured by qRT-PCR. (B) Protein expression levels of CTBP1, p53 in DU145 cells stably expressing *CTBP1-AS*. CTBP1 and p53 proteins were detected by western blotting. (C) FACS analysis of DU145 cells stably expressing *CTBP1-AS*. Prior to FACS analysis, cells were treated with 20% serum for 24 h after incubation in serum-free medium for 24 h (n = 2). Bar: s.d.∗ P < 0.05. (D) MTS assay of DU145 cells stably expressing *CTBP1-AS*. MTS assay was performed at day 1, 3, and 5 after plating cells (n = 4). Bar: s.d. ∗∗P < 0.01. (E) DU145 cell proliferation was not affected by androgen treatment. *CTBP1-AS*-overexpressing DU145 cells or vector control cells were treated with 10 nM DHT or vehicle for 5 days.

Supplementary Figure 8. Analysis of *CTBP1-AS* function with other independent siRNAs

(A) LNCaP and VCaP cells were transfected with siControl or 2 independent si*CTBP1-AS* RNAs (si*CTBP1-AS*#2 and si*CTBP1-AS*#3, 10 nM). After incubation for 72 h, cells were treated with 1 nM R1881 or vehicle for 24 h. Expression levels of *CTBP1-AS* were analyzed by qRT-PCR. (B) AR transcriptional activity was repressed by both si*CTBP1-AS* RNAs (si*CTBP1-AS*#2 and si*CTBP1-AS*#3, 10 nM). LNCaP and VcaP cells were transfected with siControl or 2 independent si*CTBP1-AS* RNAs (si*CTBP1-AS*#2 and si*CTBP1-AS*#3, 10 nM). After incubation for 72 h, the cells were transfected with luciferase vector containing the PSA-promoter region. Next day, cells were treated with 1 nM R1881 or vehicle for 24 h before measuring luciferase activity. Bar: s.d. (C) Androgen-mediated gene induction was repressed by transfection with another si*CTBP1-AS* RNA. LNCaP and VcaP cells were treated with siControl or si*CTBP1-AS*#2 (10 nM). After treatment with 1 nM R1881 or vehicle treatment for 24 h, microarray analysis was performed. We selected genes induced by androgen (> 1.4-fold). Change in the androgen response in LNCaP cells by si*CTBP1-AS* is summarized. (D) Androgen-mediated transcriptional repression was induced by *CTBP1-AS*. LNCaP cells treated with siControl or 2 independent si*CTBP1-AS* RNAs

(si*CTBP1-AS*#2 and si*CTBP1-AS*#3, 10 nM). After androgen treatment for 24 h. CTBP1, *p53* and *SMAD3* expression levels were measured by qRT-PCR.

Supplementary Figure 9. *CTBP1-AS* regulates androgen-regulated gene induction

(A) (Upper) qRT-PCR analysis of androgen regulated genes. Two days before androgen stimulation, siControl or si*CTBP1-AS* was transfected to LNCaP cells. LNCaP cells were treated with 10 nM R1881 or vehicle for 24 h. (Lower) qRT-PCR analysis of androgen regulated genes. Two days before androgen stimulation, siControl or si*CTBP1-AS* #2, #3 was transfected to LNCaP and VCaP cells. Cells were treated with 10 nM DHT, 10 nM DHT + 10 μM bicalutamide, or vehicle for 24 h. (B-D) CTBP1 recruitment to AR-binding sites persisted by *CTBP1-AS* knockdown. Two days before androgen stimulation, siControl or si*CTBP1-AS* was transfected to LNCaP cells. LNCaP cells were treated with 10 nM R1881 or vehicle for 24 h. ChIP analysis was perfomed using anti-H3K9me2 (B), CTBP1 (C) and AR (D) antibodies. Fold enrichment was measured by qPCR and normalized by IgG-control. Bar: s.d. $*P < 0.05$, $**P < 0.01$. N.S: not significant.

Supplementary Figure 10. CTBP1 repression is not mediated by CTBP1 transcript stability but epigenetic change by androgen treatment.

(A,B) LNCaP cells were treated with 10 nM R1881 or vehicle for 12 h, at which time the medium was replaced with a fresh medium containing actinomycin D (1 μg/ml) for the indicated times. Total RNA was extracted, and *CTBP1-AS* expression levels were measured by qRT-PCR analysis (A). *CTBP1* mRNA levels were measured by qRT-PCR analysis. The expression level at each time point is plotted relative to that of the 0-h sample (B). (C) ChIP analysis of PolII at both CTBP1 and *CTBP1-AS* promoters. LNCaP cells were treated with 10 nM R1881 or vehicle for 24 h. Cell

lysates were immunoprecipitated with rabbit IgG or anti-PolII antibody. (D) The *CTBP1* promoter region (-1 kb to +1.7 kb) was inserted into the pGL3 luciferase vector. Luciferase assays were performed in LNCaP cells. Bar: s.d. (E, F) ChIP analysis was performed with normal IgG, anti-histone AcH3K9, or anti-histone H3K4me3 antibody in LNCaP cells treated with 10 nM R1881 or vehicle for 24 h. Fold enrichments at the CTBP1 and *CTBP1-AS* promoters were measured by qPCR. Bar: s.d. (G) ChIP analysis of histone H3 acetylation at CTBP1 promoters and AR binsings at ARBS. Cells were treated with 1 nM R1881 for 1 or 24 h. Bar: s.d. (H) ChIP analysis of histones H3K27me3 and H3K9me3 in LNCaP cells. Fold enrichment was measured by qPCR and normalized to IgG control. Bar: s.d. (I) LNCaP cells were treated with 100 nM TSA for 2 h, and then with 10 nM R1881 or vehicle for 24 h. Fold changes of *CTBP1-AS* and CTBP1 were measured by qRT-PCR. Expression levels were normalized to GAPDH. N.S: not significant. Bar: s.d. (J) ChIP analysis of AcH3 in LNCaP cells stably expressing *CTBP1-AS* or vector control. Bar: s.d.

Supplementary Figure 11. Class I histone deacetylases (HDACs) are involved in CTBP1 repression

(A) HDAC1 is recruited to the CTBP1 promoter. LNCaP cells were treated with siControl or si*CTBP1-AS* (10 nM), incubated for 48 h, and then treated with 10 nM R1881 or vehicle for 24 h. ChIP analysis was performed using anti-HDAC1 antibody. Fold enrichments were measured by qPCR (n = 3) and normalized to IgG-IP control. $*P < 0.05$, $**P < 0.01$. Bar: s.d. N.S: not significant. (B–E) Specific knockdown of HDAC isoforms by siRNA. LNCaP cells were transfected with siControl, siHDAC1, siHDAC2, siHDAC3, or siHDAC8 (10 nM), 48 h before androgen stimulation. mRNA levels were measured by qRT-PCR and normalized to GAPDH. (F) Combinational knockdown of HDAC isoforms by siRNA. LNCaP cells were transfected with

siRNA mix (10 nM) as indicated, incubated for 48 h, and then treated with 10 nM R1881 or vehicle for 24 h. The CTBP1 mRNA level was measured by qRT-PCR (n = 3).

Supplementary Figure 12. Analysis of PSF binding and histone deacetylation in VCaP cells

(A) PSF ChIP analysis in VCaP cells. VCaP cells were treated with 1 nM R1881 or vehicle for 24 h. Prior to androgen treatment, cells were transfected with siControl, si*CTBP1-AS*#2, or si*CTBP1-AS*#3 (10 nM). Results are presented as fold enrichment over input. (B, C) Histone deacetylation by *CTBP1-AS* at the CTBP1 promoter and PSFBS#1 in VCaP (B) and LNCaP (C) cells. AcH3 and Sin3A recruitments were analyzed. (D) ChIP analysis using anti-AcH3 was performed on sense CTBP1 and antisense *CTBP1-AS* promoters after treatment with 1 nM R1881 or vehicle for 24 h in VCaP cells transfected with 10 nM siControl or siPSF. Bar: s.d.

Supplementary Figure 13. Analysis of *CTBP1-AS* and PSF interaction by RIP in VCaP cells

(A) Confirmation of immunoprecipitation of PSF by western blotting. Nuclear extracts of VCaP cells treated with 1 nM R1881 or vehicle were immunoprecipitated with anti-PSF antibody and normal IgG. Small amounts of beads were boiled in SDS lysis buffer and analyzed by western blotting. (B) RIP analysis of RNA immunoprecipitated by anti-PSF antibody. RNA extracted from the beads was reverse transcribed to cDNA, and levels of *CTBP1-AS* and MB RNAs were then analyzed by qRT-PCR (n = 3). MB: myoglobin. Bars: s.d. (C) Electrophoresis of gels for each samples (2 independent experiments) are also shown. Bar: s.d. (D) *CTBP1-AS* RNA pull down assays in VCaP cells. Probes for a *CTBP1-AS* fragment and an antisense fragment to *CTBP1-AS* (AS/*CTBP1-AS*) were prepared. (E) Conventional ChIP analysis of Figure 5K. ChIP DNA samples were analyzed by PCR. Electrophoresis gels are shown. (F) RNA pull-down after PSF ChIP analysis in VCaP cells. Probes for a *CTBP1-AS* fragment and an antisense fragment to *CTBP1-AS*

(AS/*CTBP1-AS*) were prepared. RNA-dependent pull-down was detected by elution with RNase. ∗$P < 0.05$, ∗∗$P < 0.01$. Bar: s.d. Fold enrichments over AS /*CTBP1-AS* probe pull-down samples were calculated by qPCR ($n = 3$).

Supplementary Figure 14. *CTBP1-AS* interacts with PSF at Alu sequences

(A) Image from UCSC genome browser showing SINE clusters in the *CTBP1-AS* sequence. (B) Construction of *CTBP1-AS* fragments for probe preparations. (C) RNA pull-down assay. Each probe was added to LNCaP nuclear extracts and incubated with rotation. Western blot analysis was performed using anti-PSF and anti-Sin3A antibodies. (D) *CTBP1-AS* interacts with the RNA-binding domain of PSF. Plasmids expressing PSF with deletions in the RNA-binding (ΔR) or C-terminal (ΔC) domain. RNA pull downs were performed by adding *CTBP1-AS* fragments to nuclear lysates from Cos7 cells transfected with mutant and full-length PSF. (E) Schematic view of deletions of *CTBP1-AS*. (F) (Upper panel) MTS assay ($n = 4$) of LNCaP cells transfected with deletions of *CTBP1-AS*. MTS assay was performed 4 days after plating. *$P < 0.05$, **$P < 0.01$ compared with vector control, Bar: s.d. (Middle panel) CTBP1 expression levels in LNCaP cells transfected with deletions. (Lower panel) Immunoblot of β-actin for loading control.

Supplementary Figure 15. RIP analysis of ncRNAs containing SINE sequences expressed in nuclei of prostate cancer cells

(A) UCSC genome browser view of the 4 selected ncRNAs. We used microarray data to select ncRNAs expressed at comparable levels to *CTBP1-AS* in prostate cancer cells. Some of these ncRNAs possess SINE sequences in the forward direction of the transcripts. (B, D) Expression levels of 4 ncRNAs in VCaP (B) and LNCaP (D) cells by qRT-PCR analysis ($n = 3$), Bar: s.d. (C, E) RIP analysis of 4 ncRNAs and *CTBP1-AS*. VCaP (C) and LNCaP (E) cells were treated with 1

nM R1881 or vehicle. Anti-PSF was added to the nuclear extracts and RIP assays were performed. RNA levels normalized to negative control (MB) and IgG control are shown. Bar: s.d.

Supplementary Figure 16. PSF is responsive for androgen-mediated gene repression and androgen-mediated cell proliferation in VCaP cells

(A) The androgen-mediated gene regulation is repressed by si*CTBP1-AS* and siPSF. VCaP cells were transfected with siControl, si*CTBP1-AS*#2, and siPSF#2 (10 nM). Cells were treated with 1 nM R1881 or vehicle for 24 h. Fold changes induced by androgen treatment are shown as a heatmap. (B) qRT-PCR analysis (n = 3) of CTBP1, p53 and APP in LNCaP cells transfected with siControl or siPSF#2 (10 nM). LNCaP cells were transfected with siPSF#2 (10 nM) 3 days prior to androgen treatment. LNCaP cells were treated with 1 nM R1881 for 24 h. (C) Cell cycle analysis by flow cytometry was performed to evaluate the roles of PSF and *CTBP1-AS* in cell cycle progression. BrdU-labeled VCaP cells were measured to assess the percentages of S-phase cells using bivariate FACS analysis. (D) Relative expression levels of p53 and SMAD3 in VCaP cells transfected with siControl siPSF#1, and siPSF#2 (10 nM) by qRT-PCR. Cells were treated with 1 nM R1881 or vehicle for 24 h. (E) Expression levels of PSF, AR, CTBP1, p53 and SMAD3 in VCaP cells transfected with siControl, siPSF#1 and siPSF#2 (10 nM) by western blot analysis. Cells were treated with 1 nM R1881 or vehicle for 24 h. (F) Summary of androgen-repressed genes. The percentages of androgen-repressed genes with reduced repression after treatment with siPSF#1 or si*CTBP1-AS*#2 (10 nM) are indicated.

Supplementary Figure 17. Overexpression of either PSF or NONO induces cell proliferation of prostate cancer cells

(A) Western blot analysis of LNCaP cells stably expressing FLAG-PSF or FLAG-NONO. Antibodies against FLAG, PSF, and NONO were used. β-Actin was used as a loading control. (B) MTS assay on cell lines stably expressing PSF or NONO (n = 4). Bar: s.d. (C, D) Western blot (C) and qRT-PCR (D) analyses were performed to measure CTBP1 expression in LNCaP cells stably expressing FLAG-PSF or FLAG-NONO. (E) Anti-FLAG immunoprecipitation followed by western blot analysis. Exogenous FLAG-PSF and FLAG-NONO were immunoprecipitated by anti-FLAG antibodies. (F) Interaction of exogenous PSF with *CTBP1-AS*. RIP was performed with anti-FLAG antibody to assess the interaction between exogenous PSF and *CTBP1-AS*.

Supplementary Figure 18. The role of CTBP1 in PSF-dependent cell cycle control

(A) qRT-PCR analysis (n = 3) of p53 and SMAD3 in LNCaP cells transfected with siControl (10 nM), siPSF#1 (10 nM) or siPSF#1 (10 nM) + siCTBP1 (10 nM). Cells were transfected 3 days prior to androgen treatment and then treated with 1 nM R1881or 24 h. (B) FACS analysis (n = 2) of LNCaP cells transfected with siControl (10 nM), siPSF#1 (10 nM) or siPSF#1 (10 nM) + siCTBP1 (10 nM) 4 days prior to FACS analysis. The percentage of cells in S-phase is shown. $*P < 0.05$, $**P < 0.01$. Bar: s.d.

Supplementary Figure 19. CTBP1 knockdown partially reversed the inhibition of androgen-mediated gene induction by PSF knockdown

(A) LNCaP cells were transfected with siControl (10 nM), siPSF#1 (10 nM) and siPSF#1 (10 nM) + siCTBP1 (10 nM). Three days after transfection, cells were treated with 1 nM R1881 or vehicle for 24 h. Expression levels of PSF and CTBP1 were analyzed by western blotting. (B) qRT-PCR analysis (n = 3) of androgen-upregulated genes inhibited by siPSF#1 (10 nM) in LNCaP cells. siCTBP1 (10 nM) addition partially but significantly reversed gene induction. $*P < 0.05$, $**P <$

0.01. Bar: s.d. (C) VCaP cells were transfected with siControl (10 nM), siPSF#1 (10 nM) and siPSF#1 (10 nM) + siCTBP1 (10 nM). Three days after transfection, cells were treated with 1 nM R1881 or vehicle for 24 h. Expression levels of PSF and CTBP1 were analyzed by qRT-PCR (n = 3). (D) qRT-PCR analysis (n = 3) of androgen-upregulated genes inhibited by siPSF#1 (10 nM) in VCaP cells. siCTBP1 (10 nM) addition partially but significantly reversed gene induction. $*P < 0.05$, $**P < 0.01$. Bar: s.d.

Supplementary Figure 20. PSF DNA-binding ability is regulated by *CTBP1-AS* induction

(A) LNCaP cells were treated with 1 nM R1881 or vehicle for 24 h. Nuclear extracts were used for EMSA. We used 2 PSF-binding sequences (approximately 40 bp) located at the peaks from ChIP-seq analysis (PSFBS#0 an, PSFBS#1). To detect supershift, 1 μg of PSF antibody was used. (B) *CTBP1-AS* knockdown inhibited PSF DNA binding ability *in vitro*.

Supplementary Figure 21. Cooperative *trans*-regulatory function of *CTBP1-AS* and PSF in VCaP cells

(A) LNCaP and VCaP cells transfected with siControl si*CTBP1-AS*#2, or si*CTBP1-AS*#3 (10 nM) were treated with 1 nM R1881 or vehicle for 24 h. Western blot analysis was performed to assess the expression levels of p53, SMAD3, and CTBP1. (B) LNCaP and VCaP cells transfected with siControl si*CTBP1-AS*#2, or si*CTBP1-AS*#3 (10 nM) were treated with 1 nM R1881 or vehicle for 48 h. Western blot analysis was performed to assess the expression levels of p21. (C) Knockdown of p53 and SMAD3 in VCaP cells. (D) SMAD3 and p53 knockdowns promote prostate cancer cell proliferation. MTS assay of VCaP cells transfected with siControl, sip53 and siSMAD3 (n = 4). Bar: s.d. $*P < 0.05$, $**P < 0.01$. (E) Detection of *CTBP1-AS* by RNA FISH and PSF immunofluorescence analysis in VCaP cells.

Supplementary Figure 22. SMAD3 and p53 interact with AR for modulating AR-mediated signaling

(A) Endogenous interaction of AR with p53 and SMAD3 in LNCaP cells. LNcaP cells were treated with 1 nM R1881 for 1 h. Cell lysates were immunoprecipitated with rabbit anti-AR antibody or normal rabbit IgG. We detected p53 by mouse p53 antibody and SMAD3 by rabbit SMAD3 antibody. For the detection of SMAD3, secondary antibody raised against light chain was used because SMAD3 bands overlapped with heavy chain. Although heavy chain bands were still detected, we determined SMAD3 bands in AR-IP lanes by comparing them with IgG control laness. (B) Endogenous SMAD3 and p53 repressed androgen-mediated gene induction. LNCaP cells were transfected with siControl, sip53 or siSMAD3. After 48 h incubation, cells were treated with vehicle or R1881 1 nM for 3 h. Fold induction over vehicle control was measured using qRT-PCR (n = 2). Bar: s.d. (C) Endogenous SMAD3 and p53 repressed AR-mediated enhancer activity (n = 3). LNCaP cells were transfected with siControl, sip53 or siSMAD3. After 48 h incubation, cells were transfected with TACC2-ARBS-LUC (Takayama et al., 2012) or CTBP1-ARBS-LUC. Cells were treated with R1881 1 nM for 24 h. $*P < 0.05$, $**P < 0.01$.

Supplementary Table I. ChIP-PCR primer sequences

	Fw (5'→3')	Rv (5'→3')
CTBP1-ARBS	GCACTGTGTGGCATAAAAAGAAAA	TGGAACGTGCCCCAGAA
CTBP1-intron1	GGACGCCTGTATGGAAGCA	TCCGCAGACGCCTTTTG
CTBP1 intron5	GCCTCATAACGCACTAGGATAACA	CACAGCGTTCTAAATTCTCCATGT
TMPRSS2-ARBS	CAAATGGCCACCTGGTGAA	GTTGGAGCTAGTGCTGCATGTC
NEDD4L-ARBS	CATTCTTCAACTCTTTACCCTACAAATC	AATTCTTTTCATAGGTGAGCAAACTG
AFM	GCAGAACCTAGTTCCTCCTTCAAC	AGTCATCCCTTCCTACAGACTGAGA
MYOD	CCGCCTGAGCAAAGTAAATGA	GGCAACCGCTGGTTTGG
PSFBS #0	GGCATGGAGACGGAATGATG	CGCGTCCTTACGAACCA
PSFBS #1	CAGGAAGTTGAGAGGGCAAG	CCCCAGCTCTTTGGACATTTG
PSFBS #2	CAGTCTCCTGCCTCCTTTTG	CCCTAACCCCTCCAGCTAAG
PSFBS#3	AATGTTTCCCTCCCAAGGAC	CTCAGGCCTGTTTCTCCAAG
PHGDH	CAAGCAATTCTCATGCCTCA	CAAGGTGGGAAGATCACCTG
TLR3	GATGGCTGTGAGGGGTAAAA	CCACCTTGGCCTTCTGAGTA
C5orf33	CCAGTTTTTGCCCATTCAGT	TGCAAGGCTGGTTCAACATA
BAMBI	TGCCCAGCTAACAGTGATTG	GCTCATGTGTCCATGGTTG
USP2	GGCTTCGGAAGATGAGTCAG	CTCCAGAGCACAGTCAGCAA

Supplementary Table II. RT-PCR primer sequences

	Fw (5'→3')	Rv (5'→3')
CTBP1	TGGCCACTGTGGCCTTCT	CGTTCAGGACCTTCTCATGGA
CTBP1-AS	AACCTGGCAGCACGGAAGT	GAGCACAACCACCACCTCATC
TMPRSS2	TCAACCCCTCTAACTGGTGTGA	AGGCGAACACACCGATTCTC
NEDD4L	TCTTTCCGGTGCAATATCTATCAA	AGGCTCAGCTTTACATCCAGGTT
UGT2B15	TGGCAAGAAGCCAAATACTTTAGG	TGGGATGACCAAGAAGGTCATT
SMAD3	CCCCAGAGCAATATTCCAGA	GGCTCGCAGTAGGTAACTGG
HDAC1	GGAAATCTATCGCCCTCACA	AACAGGCCATCGAATACTGG
HDAC2	ATAAAGCCACTGCCGAAGAA	CACAGCTCCAGCAACTGAAC
HDAC3	CTGGTCCTGCATTACGGTCT	CAGTCATCGCCTACGTTGAA
HDAC8	GGCCAGTATGGTGCATTCTT	GGCTGGGCAGTCATAACCTA
p53	CCCCTCTGAGTCAGGAAACA	TCATCTGGACCTGGGTCTTC
(ncRNA1) NR_027420	ACTAAACCACCCACCACTGC	ACAGCTTACCCGCTTCTCAA
(ncRNA2) AK095225.1	ATCCATCCATCCAACCTTCA	GTCAACTGAATGCGCAAAGA
(ncRNA3) AK097109.1	GCTATGCACCCGTTTTGTT	GCAGGGGATGGTAATGAGAA
(ncRNA4)AK098314.1	TCTCGATCTCCTGACCTCGT	CTAACCACGGCAACAGGTTT

Supplementary Table III. Univariate and multivariate analyses of cancer specific survival in 105 prostate cancer patients examined

	Univariate	Multivariate	
Variable	*P*	*P*	Relative risk (95% CI)
Gleason score (8-10 / 2-7)	0.01*	0.003	2.5 (1.9-24.1)
CTBP1 IR (low / high)	0.047*	0.04	9.0 (1.1-71.2)
pN (pN1 / pN0)	0.10		
pT (pT3-4 / pT2)	0.12		
Adjuvant therapy (No / Yes)	0.57		
PSA (≦ 10 / > 10 ng / ml)	0.71		

*; Data were considered significant in the univariate analyses, and were examined in the multivariate analyses.

Supplementary Table IV. Association between CTBP1 immunoreactivity and clinicopathological parameters in 105 human prostate carcinomas

Value	CTBP1 status		*P* value
	High (*n* = 53)	Low (*n* = 52)	
Age* (years)	66.7±6.4	66.9±5.6	0.82
PSA* (ng / ml)	16.6±20.2	16.6±18.1	0.98
Stage (Jewett Staging Sysyem)			
B	20	13	
C	28	31	
D1	5	8	0.31
pT			
pT1-2	20	14	
pT3-4	33	38	0.24
pN			
pN0	48	44	
N1	5	8	0.35
Gleason score			
2-6	13	9	
7	21	20	
8-10	19	23	0.57
AR status			
-	5	10	
+	48	42	0.15

*; The association was statistically evaluated utilizing a correlation coefficient (r) and regression equation. Other data were statistically analyzed utilizing Mann-Whitney's U test or Kruskal-Wallis test.

Supplementary Table V. Association of clinicopathological parameters with *CTBP1-AS* expression status in 105 prostate cancer patients

Value	CTBP-1 AS			*P* value
	negative (n = 48)	weak (n = 42)	strong (n = 15)	
Age* (years)	66.9±6.5	66.3±5.3	67.7±6.4	0.74
PSA* (ng / ml)	16.6±22.6	14.9±13.6	21.3±20.7	0.55
Stage (Jewett Staging Sysyem)				
B	19	11	3	
C	25	26	8	
D1	4	5	4	0.24
pT				
pT1-2	20	12	2	
pT3-4	28	30	13	0.08
pN				
pN0	44	37	11	
N1	4	5	4	0.17
Gleason score				
2-6	14	7	1	
7-8	24	13	4	
9-10	10	22	10	**0.004**
AR status				
-	6	9	0	
+	42	33	15	0.11
				0.009 (strong *vs* the others)

*; The association was statistically evaluated utilizing a correlation coefficient (r) and regression equation. Other data were statistically analyzed utilizing Mann-Whitney's U test or Kruskal-Wallis test.

Supplementary Methods

Establishment of castration-resistant prostate cancer cell lines

LTAD cells were developed and maintained in phenol red-free RPMI medium supplemented with 10% dextran charcoal-stripped FBS, 50 units/ml penicillin, and 50 μg/ml streptomycin. The cells were passaged once a week and maintained for at least 6 months before the experiments. We observed increased AR expression and hypersensitivity to low level of DHT in LTAD cells (Takayama K et al., 2012).

RNA-fluorescence *in situ* hybridization (FISH) and immunofluorescence analysis

To detect *CTBP1-AS*, a digoxigenin (DIG)-labeled antisense *CTBP1-AS* probe was made by using an RNA labeling kit (Roche), according to the manufacturer's protocol. For colocalization studies, LNCaP cells and VCaP cells were cultured on cover glasses in 24-well culture dishes. Twenty-four hours after androgen treatment, the cells were briefly rinsed with phosphate-buffered saline (PBS), fixed in a solution of 3.6% formaldehyde and 10% acetic acid in PBS for 20 min at room temperature, and then permeabilized with 0.5% Triton X-100 in PBS for 5 min at room temperature. After several washes with PBS, hybridization with the probe was performed in a moist chamber at 42°C overnight. The probe was detected with Alexa555-conjugated donkey anti-DIG antibody (Invitrogen). Endogenous PSF was detected with rabbit anti-PSF antibody and Alexa488-conjugated anti-rabbit IgG antibody. Results were visualized by confocal microscopy. We used a commercially available rabbit polyclonal antibody (Novus Biologicals) in this study. This antibody reacts specifically with the carboxyl terminal of PSF protein (657-707 amino acids), which region has only 30% similarity with PTB. We confirmed that this antibody detects a 100-kDa protein with the clear single band, indicating that this antibody does not cross react with PTB. Cells were mounted on slides using mounting media after staining with DAPI solution and visualized on a Fluoview FV10i (Olympus) with an attached camera. Images were analyzed using

FV10i review system (FV10-ASW ver 2.0, Olympus).

RNA immunoprecipitation (RIP)

Confluent LNCaP and VCaP cells in 15-cm dishes were harvested and resuspended in 1 ml of hypotonic lysis buffer (10 mM HEPES pH 8.0, 10 mM KCl, 1 mM DTT, 0.1 mM EDTA, 0.1 mM EGTA and 1% NP-40) to collect nuclear fraction. Nuclear fractions were lysed in RIP buffer (Rinn et al., 2007). Specific antibody was added to the supernatant, and the mixture incubated overnight with rotation at 4°C. Thirty microliters of protein G beads was added, followed by incubation at 4°C for 2 h with gentle rotation. The beads were washed 3 times with lysis buffer and resuspended in 1 ml of ISOGEN (Nippon Gene). Co-precipitated RNA was isolated and used for qRT-PCR analysis of *CTBP1-AS* mRNA levels.

RNA pull-down assay

Biotin-labeled *CTBP1-AS* fragments and antisense *CTBP1-AS* fragments were prepared with Biotin RNA Labeling Mix (Roche) and T7 RNA polymerase. Biotinylated RNAs were treated with RNase-free DNase, and 10 pmol biotinylated RNA was heated to 60°C for 10 min and slowly cooled to 4°C. RNA pull down was performed as described (Rinn et al., 2007) with some modification. The RNA was mixed with 100 μg of pre-cleared LNCaP nuclear extract in RIP buffer supplemented with tRNA (0.1 μg/μl) and incubated at 4°C overnight. Sixty microliters of washed Streptavidin Agarose beads (Invitrogen) was added and incubated at 4°C for an additional 1 h. Beads were washed 5 times in RIP buffer and boiled in SDS buffer, and retrieved proteins were visualized by western blot analysis.

ChIP, Re-ChIP, and quantitative PCR

ChIP and qPCR were performed as previously described (Takayama et al., 2007) . For the Re-ChIP assay, DNA immunoprecipitated by anti-AR antibodies was eluted with elution buffer containing 10 mM DTT. After dilution with ChIP dilution buffer, secondary ChIP was performed using an

anti-CTBP1 antibody. Fold enrichments relative to IgG-IP control or input were quantified by real-time PCR using SYBR green PCR master mix (Applied Biosystems) and the ABI Prism 7000 or Step one system (Applied Biosystems) based on SYBR green I fluorescence. The primer sequences for detection of ARBSs by qPCR are listed in Supplementary Table I or described previously (Takayama K et al., 2007, 2009, 2011). For RNA pull-down following ChIP, DNA-protein complexes were eluted after ChIP for PSF, and then, an RNA probe consisting of biotinylated *CTBP1-AS* was added and incubated overnight with rotation. Biotinylated RNA probes were collected with avidin beads. Protein-DNA complexes were eluted by RNase treatment to obtain RNA probe-precipitated pure DNA-protein complexes. After de-crosslinking by heating at 65°C overnight, DNA was collected by ethanol precipitation.

Plasmids and *CTBP1-AS* cloning

pcDNA3-FLAG AR has been described previously (Takayama K et al., 2007, 2009, 2011). To generate pcDNA3 plasmids expressing N-terminally Myc- and FLAG-tagged CTBP1 (pcDNA3-FLAG CTBP1, pcDNA3-Myc CTBP1), CTBP1 fragments were amplified by PCR and subcloned into the *XhoI* and *EcoRI* sites of pcDNA3 plasmids containing N-terminal FLAG or Myc tags. For construction of the pcDNA3 plasmid containing *CTBP1-AS*, *CTBP1-AS* was amplified by 3′ RACE PCR using the RACE PCR kit (Roche) with cDNA derived from LNCaP cells as the template and inserted into the *EcoRI* and *XhoI* sites of pcDNA3. To generate pcDNA3 plasmids expressing N-terminally FLAG-tagged PSF and NONO (pcDNA3-FLAG PSF, pcDNA3-FLAG NONO), PSF and NONO fragments were amplified by PCR and subcloned into the *XhoI* and *EcoRV* sites of pcDNA3 plasmids containing N-terminal FLAG tag. LNCaP cells were transfected with expression vectors by using FUGENE HD reagent (Roche). To generate stable cell lines carrying pcDNA3 *CTBP1-AS* or pcDNA3-FLAG CTBP1, transfected cells were selected with 0.5 mg /ml G418.

ChIP-sequence

ChIP-sequence was performed using an Illumina Genome Analyzer (Illumina). Libraries were prepared according to Illumina's instructions. Unfiltered 36 bp sequence reads were aligned against the human reference genome (hg18) using CASAVA v1.7 (Illumina). Signal scores of PSF bindings were calculated using in-house program. Shortly, rates of ChIP counts / control counts in 300bps window were used for calculation as described previously (Wakabayashi et al., 2009). Integrative genome browser (IGB) (Affymetrix) was used for visualization. The data discussed in this publication have been deposited in NCBI's Gene Expression Omnibus and are accessible through GEO Series accession number GSE45124.

Gel shift assay

Gel shift assay was performed by using the DIG Gel shift kit (Roche) with DIG labeled oligonucleotides containing PSF binding peak positions identified by ChIP-sequence. Nuclear extracts were prepared from LNCaP cells by using the PARIS kit (Ambion) according to the manufacturer’s protocol. After mixing probes with nuclear extracts at room temperature, samples were analyzed on a 6% PAGE gel. After transferring to positive-charged nylon membrane (Roche), signals were detected by using anti-DIG antibody. For the supershift experiments, 1 μg anti-PSF specific antibody was added before electrophoresis and incubated for 15 min. Oligonucleotide sequences for preparing probes were as follows: CTBP1 5’ upstream (PSFBS#0): 5’-AGGGACCGCGCCTGCCCGGCCCGGGAAGTTCAGGGCTGG-3’,

5’-TTCTCCAGCCCTGAACTTCCCGGGCCGGGCAGGCGCGGT-3’;

CTBP1 Intron 1 (PSFBS#1) :5’-ACCAGGACATGGCAGGGGGCCGGCTCTGCGCCAA

GCCCACAGG-3’,5’-AGTCCTGTGGGCTTGGCGCAGAGCCGGCCCCCTGCCATGTCC-3’

Microarray and data analysis

For expression microarrays, the GeneChip Human Exon 1.0 ST Array (Affymetrix) was used according to the manufacturer's protocol. Data analysis was performed using Affymetrix Microarray Suite software. To compare arrays, normalization was performed on data from all probe sets. For microarray analysis, LNCaP cells were transfected with siControl, siPSF#1, or si*CTBP1-AS* 48 h before androgen treatment. Twenty-four hours after androgen treatment, total RNA was extracted with ISOGEN reagent. We confirmed that the RIN (RNA integrity number) was > 8.0 using an Agilent Bioanalyzer. Two independent microarray analyses each were performed for si*CTBP1-AS* and siPSF. In experiments using siPSF#1, 1331 genes were repressed (fold change < 0.7) by androgen. Among them, 718 genes (53%) were considered putative PSF target genes (de-repressed more than 20% by siPSF transfection). For these gene sets, DAVID was used to assess the biological functions of these target genes, and KEGG was used for pathway analysis. In independent control samples from both studies, 369 genes were identified as common repressed genes (fold change < 0.75). Among them, 190 genes (52%) were de-repressed by siPSF#1, and 265 (72%) by si*CTBP1-AS* (de-repressed more than 20% by si*CTBP1-AS* transfection). We observed that 316 of the common repressed genes (86%) were de-repressed more than 20% by siPSF or si*CTBP1-AS* transfection. Similar results were obtained in microarray analyses in VCaP cells (Supplementary Figure 16). The data discussed in this publication have been deposited in NCBI's Gene Expression Omnibus and are accessible through GEO Series accession number GSE44264.

Luciferase assay

LNCaP cells were incubated with phenol red-free medium supplemented with 5% dextran charcoal-stripped FBS for 24 h before transfection. Cells were transfected with pGL3 vectors expressing ARBSs and tk-pRL by using FuGENE HD reagent. Twenty-four hours after transfection, cells were treated with 10 nM R1881 or vehicle for 24 h, and luciferase activities

were determined as previously described (Takayama K et al., 2007). For luciferase assays with siRNA, cells were transfected with siRNA 72 h before androgen stimulation.

Quantitative reverse transcriptase PCR (qRT-PCR)

Total RNA was isolated using ISOGEN reagent. First strand cDNA was generated using the Primescript RT reagent kit (TAKARA).The primer sequences were listed in Supplementary Table II or described previously. mRNA expression levels were quantified by real-time PCR as previously described (Takayama K et al., 2007, 2009, 2011).

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