Supplementary data

Supplementary Materials and Methods

Quantitative Chromosome Conformation Capture Assay (3C)

Briefly, nuclei were cross-linked using formaldehyde, digested with Bgl II (NEB), and ligated under extra diluted conditions. After reversing of the crosslinks, DNA was purified by phenol-chloroform extraction followed by ethanol precipitation. Real time PCR was performed using TaqMan® Universal PCR Master Mix (Applied Biosystems). Taqman probe and primer sequences are listed in Supplemental Table I. The data was normalized for primer efficiency difference using BAC RP11-75C3 that covers the UBE2C locus. GADPH loading control was used to normalize DNA concentration. In addition, two regions in UBE2C locus were selected to correct for difference in possible amplification between different cell lines. The interaction of two Bgl II sites in the GADPH locus was also used to allow comparison between different 3C assays.

Reporter gene assays

The pGL4.10-E4TATA-Luc and pBEC22 (a Renilla luciferase vector) were kindly provided by Eric Bolton (University of Illinois at Urbana-Champaign) (Bolton et al, 2007). Putative UBE2C enhancer regions (~ 400 bp) were PCR amplified from either LNCaP or PC-3 genomic DNA (primer sequences are listed in Supplemental Table I) and subcloned into pGL4.10-E4TATA-Luc. For reporter gene assays, transfection was performed using Lipofectamine 2000 (Invitrogen, Carlsbad, CA). Cells were treated with DHT or vehicle for 24 h and then harvested. Luciferase activity was measured using a Dual-Luciferase Reporter Assay kit and GloMax™ Systems (Promega).

Chromatin Immunoprecipitation (ChIP) and serial ChIP (re-ChIP)

Antibodies used were anti-GATA2 (H116), anti-Oct1 (C21), anti-Ets-1 (C-20), anti-MED1 (M255), anti-SRC1 (M341), anti-p300 (C20), anti-TFIID (N-12), and anti-CRSP77 (H-285) from Santa Cruz Biotechnology (Santa Cruz, CA), anti-FoxA1 (ab23738), anti-H3K4me1 (ab8895), anti-H3K4me2 (ab7766), anti-H3K4me3 (ab8580), anti-RNA pol II (8WG16) and anti-phosphorylated RNA pol II (phospho S5) from Abcam (Cambridge, MA). An anti-MED1 (NR box) has been previously described (Zhang et al, 2005).

An antibody against phosphorylated MED1 (p-MED1) was produced by YenZym Antibodies (South San Francisco, CA). Rabbits were immunized with a synthetic phosphopeptide corresponding to residues surrounding Thr1032 of human MED1. The antibody was obtained by affinity purification and affinity absorption using phosphor-peptide conjugated affinity matrix. Antibody specificity was further confirmed by antigen-specific ELISA assay.

Western Blots and Immunoprecipitation

Antibodies used were anti-AR (441), anti-GATA2 (H116), anti-Oct1 (C21), anti-Ets-1 (C-20), anti-MED1 (M255), anti-MED1 (C-19), anti-SRC1 (M341), anti-p300 (C20), anti-TFIID (N-12), and anti-CRSP77 (H-285) from Santa Cruz Biotechnology, anti-FoxA1 (ab5095) from abcam, anti-UBE2C (A650) from Boston Biochem (Cambridge, MA), anti-FLAG (M2) from Sigma, and anti-AKT (C67E7), anti-phospho-Akt (Ser473),

anti-Phospho-Threonine (42H4) from Cell Signaling Technology. Anti-p-MED was produced by YenZym Antibodies (South San Francisco, CA).

Supplementary Figure legends

Supplementary Figure S1. Increased UBE2C expression is necessary for PC-3 cell growth. **(A)** The UBE2C mRNA level is higher in PC-3 cells than in LNCaP cells in the presence of DHT. LNCaP and PC-3 cells were treated with 10 nM DHT for 4 h. Total RNA was isolated and amplified with primers recognizing AR mRNA and UBE2C mRNA (mean [n=3] ± SD). **(B)** Effects of DHT on PSA and TMPRSS2 mRNA expression in LNCaP cells. LNCaP cells were treated with vehicle or 10 nM DHT for 4 h, and RNA was isolated to perform real-time RT-PCR using transcript-specific primers (mean [n=3] ± SD). **(C)** Steady-state levels of UBE2C mRNA are similar in LNCaP and PC-3 cells. Top panel: LNCaP and PC-3 cells were incubated in the presence of either 5 μg/ml or 20 μg/ml of actinomycin D for 5-20 h to block mRNA synthesis. Total RNA was extracted from each sample at the indicated time points, and analyzed by real-time RT-PCR. Bottom panel: Half-life of UBE2C mRNA in LNCaP and PC-3 cells was determined. **(D)** The UBE2C protein expression level is higher in PC-3 cells than in LNCaP cells. Western blots analyses were performed in presence of 10 nM DHT for 4 h. **(E)** UBE2C silencing delayed cell-cycle progression from G2/M phase to G1 phase. PC-3 cells were synchronized to G2/M phase by using a thymidine-nocodazole block (Fang et al, 1998) and released for 1.5 h. FACS analyses were then performed.

Supplementary Figure S2. Identification and characterization of UBE2C and TMPRSS2 enhancers. **(A)** 3C analysis of the UBE2C locus in DHT-treated LNCaP and PC-3 cells. 3C assays were performed in LNCaP and PC-3 cells in the presence of 10 nM DHT for 4 h. **(B)** 3C analysis of the TMPRSS2 locus in LNCaP cells. 3C assays were performed using HindIII enzyme in LNCaP cells treated with vehicle or 10 nM DHT for 4 h. The four AR binding regions (-12 kb, -14 kb, -20 kb and -73 kb) (Wang et al, 2007; Yu et al, 2010) were indicated. **(C)** Three distal regions (-20 kb, -14 kb and +2 kb regions) function as enhancers. The E1, E2 and E3 constructs were transiently transfected into LNCaP and PC-3 cells. The luciferase activities were determined after 24 h vehicle or DHT stimulation and results were presented as the mean±SD of the triplicated transfections. **(D)** DHT treatment significantly increases transcriptional activation of the PSA enhancer reporter gene in LNCaP cells. The PSA enhancer construct (Wang et al, 2005) and empty vector were transfected into LNCaP cells stimulated with vehicle or 10 nM DHT for 24 h. **(E)** Functional analysis of UBE2C enhancers in DU-145 cells. The constructs shown on Figure 2B were transfected into DU-145 cells, and luciferase activities were measured. **(F)** 3C analysis of the UBE2C locus in LNCaP and DU-145 cells. 3C assays were performed in LNCaP and DU-145 cells in the absence of DHT. **(G)** Comparison of AR and UBE2C mRNA expression in LNCaP and DU-145 cells treated with vehicle or 10 nM DHT for 4 h. RT-PCR analyses were performed using gene-specific primers (mean [n=3] ± SD). **(H)** Comparison of AR and UBE2C protein expression in LNCaP and DU-145 cells stimulated with vehicle or 10 nM DHT for 4 h. Western blot analyses were performed using the indicated antibodies.

Supplementary Figure S3. Increased recruitment of FoxA1 and the Mediator complex to the UBE2C enhancers in PC-3 versus LNCaP cells. **(A)** Comparison of protein expression levels of transcription factors, transcription coactivators and basal transcription factors in LNCaP and PC-3 cells. Western blot analyses were performed using the antibodies indicated in the presence (+) or absence (-) of 10 nM DHT. **(B)** ChIP analysis for FoxA1, GATA2, Oct1 and ETS1 binding at the UBE2C enhancers in LNCaP and PC-3 cells treated with 10 nM DHT for 4 h. ChIP assays were performed using antibodies indicated. **(C)** ChIP analysis for occupancy of H3K4me1, H3K4me2 and H3K4me3 at the UBE2C enhancers in LNCaP and PC-3 cells treated with 10 nM DHT for 4 h. ChIP assays were performed using antibodies indicated. **(D)** ChIP analysis for SRC1, p300, MED1 and MED17 binding at the UBE2C enhancers in LNCaP and PC-3 cells treated with 10 nM DHT for 4 h. ChIP assays were performed using antibodies indicated. **(E)** ChIP analysis for occupancy of Pol II, p-Pol II and TBP at the UBE2C enhancers in LNCaP and PC-3 cells treated with 10 nM DHT for 4 h. ChIP assays were performed using antibodies indicated.

Supplementary Figure S4: Phosphorylated MED1 interacts with FoxA1, Pol II, TBP and MED17 on chromatin in PC-3 cells. **(A)** Densitometric analysis of p-MED1 and total MED1 western blots. The band intensities in LNCaP and PC-3 cells were quantified using Image J (V1.43, NIH) and normalized to the degree of PC-3. **(B)** Inhibition of MAPK kinase pathway has no effect on MED1 phosphorylation at T1032 in PC-3 cells. PC-3 cells were treated with 30 μM U0126 or vehicle for 24 h, and western blot analyses were performed using antibodies indicated. **(C)** Peptide competition assays in ChIP

conditions. The crosslinked chromatin from PC-3 cells was sonicated and diluted, and 1032T phospho-peptide or 1032T non-phospho-peptide (cold peptide) of 10-fold, 100-fold or 1000-fold molar ratio antigen-to-antibody was added to ChIP diluted buffer before immunoprecipitation with p-MED1 antibodies. **(D)** ChIP analysis for p-MED1 binding at UBE2C regulatory regions in PC-3 cells treated with 10 nM DHT for 4 h. **(E)** Re-ChIP analysis of FoxA1/PolII and FoxA1/TBP in PC-3 cells in the presence of 10 nM DHT. Re-ChIP assays were performed using antibodies against FoxA1 (for first ChIP) and Pol II/TBP (for second ChIP).

Supplementary Figure S5: Flow cytometric analysis of PC-3 cells stably expressing either a wild-type (WT) MED1 or a double mutated (DM) MED1. Cells were collected for FACS analysis using IgG (red) or a HA-tag (6E2) mouse monoclonal antibody (Alexa Fluor® 488 Conjugate) (blue).

Supplementary Figure S6: Functional roles of MED1 and phosphorylated MED1 in LNCaP-abl cells. **(A)** Increased interactions between the UBE2C enhancers (E1, E2, E3 and Enhancer-2) and the UBE2C promoter in LNCaP-abl compared to LNCaP cells. The results of 3C assays were presented as fold changes in relative frequencies (** $P<0.01$). **(B)** Silencing of MED1 decreases UBE2C enhancer/promoter interactions in LNCaP-abl cells. The results of 3C assays were presented as fold changes in relative frequencies (** $P<0.01$). **(C)** Effect of LY294002 on MED1 phosphorylation at T1032 and UBE2C mRNA expression in LNCaP-abl cells. LNCaP-abl cells were treated with 50 μM LY294002 or vehicle for 24 h. Left panel: Western blots were performed using antibodies

indicated. Right panel: Real-time RT-PCR was performed using UBE2C-specific primers. **(D)** Comparison of MED1 expression among parental LNCaP-abl, LNCaP-abl WT and LNCaP-abl DM. Western blot analyses were performed using the antibodies indicated.

Supplementary Figure S7: Phosphorylated MED1 is not sufficient alone to accelerate androgen-independent growth of LNCaP cells. **(A)** Comparison of p-MED1 and MED1 protein expression between LNCaP cells stably expressing a MED1 (LNCaP-MED1) and LNCaP cells stably expressing a green fluorescent protein (LNCaP-GFP). Western blot analyses were performed using the antibodies indicated. **(B)** The effect of MED1 overexpression on UBE2C mRNA expression in LNCaP cells. Total RNA was isolated from LNCaP-MED1 and LNCaP-GFP cells and amplified by real-time RT-PCR using gene-specific primers (mean [n=3] ± SD). **(C)** The effect of MED1 overexpression on LNCaP cell growth in the absence of androgen. The cell proliferation of LNCaP-MED1 and LNCaP-GDP was measured by a direct viable cell count assay. **(D)** The effect of silencing of MED1 on endogenous UBE2C expression in LNCaP cells. LNCaP cells were transfected with siControl or siMED1. Seventy-two h after siRNA transfection, real-time RT-PCR was performed (mean [n=3] ± SD).

Supplementary Table I Primers, siRNA and Plasmids sequences

RT-PCR primers	
AR mRNA+ (Yang et al, 2006)	CGGAAGCTGAAGAAACTTGG
AR mRNA- (Yang et al, 2006)	CGTGTCCAGCACACACTACA
UBE2C mRNA+ (Okamoto et al, 2003)	TGGTCTGCCCTGTATGATGT
UBE2C mRNA- (Okamoto et al, 2003)	AAAAGCTGTGGGGTTTTTCC
PSA+ (Jia et al, 2003)	GGCAGCATTGAACCAGAGGAG
PSA- (Jia et al, 2003)	GCATGAACTTGGTCACCTTCTG
TMPRSS2+ (Wang et al, 2007)	GGACAGTGTGCACCTCAAAGAC
TMPRSS2- (Wang et al, 2007)	TCCCACGAGGAAGGTCCC
MED1+	TCAGGGGGAAACCGAGGAGTCA
MED1-	GCATGGAGCCGTTCCAGGAGA
siRNA sequences	
siControl (Dharmacon Non-TARGET plus siRNA)	D-001810-10-20
siFoxA1 (Carroll et al, 2005)	GAGAGAAAAAAUCAACAGC
siUBE2C (Dharmacon ON TARGET plus siRNA)	(1)GAACCCAACAUUGAUAGUC (2)UAAAUUAAGCCUCGGUUGA (3)GUAUAGGACUCUUUAUCUU (4)GCAAGAAACCUACUCAAAG
siUBE2C (Reddy et al, 2007)	GGTATAAGCTCTCGCTAGA
siMED1 (Dharmacon siGenome siRNA)	(1)GCAGAGAAAUCUUAUCAGA (2)CCAUUAAGCUUGUGCGUCA (3)CAGCAAUGACUGAUCGUUU (4)GGCCGAAGAGCAAGGCUUA
siMED17 (Dharmacon siGENOME SMART pool siRNA)	(1)GAAAGUGGCAGCAAGAUUA (2)GGGAAGCUGUUCAAAUUAA (3)GCAGAAAGACUGACUAAAU (4)GAUUCGAGUUGUACAUAGA
siMED1 3'UTR	GGACAAACAACGCAGGAUAUU
siAKT1 (Dharmacon ON-TARGET plus SMARTpool)	(1)CAUCACACCACCUGACCAA (2)ACAAGGACGGGCACAUUAA (3)CAAGGGCACUUUCGGCAAG (4)UCACAGCCCUGAAGUACUC
3C primers	
UBE2C-Taqman probe	6FAMCAGGAGGTGGCGGCMGBNFQ
UBE2C-Anchor	TAGGCATTGGTACCCAGAGCA
U1	AGCAATCTCCTTTACAATAGCTACAAA
U2	GGCTCTCTGACCGACTCCTTCT
U3	CAGGAGCTATCAGGAAAGGAGATC
U4	GGTCACCTATGGGTAGGGAGATC
U5	AAAGAATATACGTGATGACATGAAAGATC

U6	CTAAGATTTCATTTGGGGCAGG
U7 (E1)	TGGCTTGCATGGCAGATTT
U8	AGATGAACAAGTTCTGGGCAATC
U9 (E2)	CCTGGGGTACTCTACCCTTAACTC
U10	TGAGGCAGGAGAATCGCTTA
U11 (E3)	GGACAGACAGCAAGGAAATGG
U12	GGAAGAGTAGGTTGAAGTGATAGGG
U13	GCAGGAGGAGGAAGAAGGAAG
U14	CCAGGAGTGGCTAGAGTATTGTCA
U15	ATTGCCAGCCAGCCCAG
U16	CCACGAGTGGCTAGAGACTACCTT
U17	TGACACCTGGCTGGGCA
U18	AGCCATGTTCGTGCCACTG
U19	CCATATGAAAATAAAGATCTCCCGC
U20	GTGGTGGGCACCTATAATCCC
U21	AGGCGTCAGCCACTGTGC
U22	CAGGAGGCGGAGGTTACAGTG
3C Control +	TGTGCGGTGTGGGATTGTC
3C Control -	CCTTCTCCCCATTCCGTCTT
Loading Control 1+	ACAGTCCATGCCATCACTGCC
Loading Control 1-	GCCTGCTTCACCACCTTCTTG
Loading Control 2+	TGCCATACGTCCCAGATTTCA
Loading Control 2-	GCCTGGAAAAGGGTAGAGGTAAG
Loading Control 3+	TGCGGGTGCAGGAGAAC
Loading Control 3-	GAATCTCACCCGCGCAAG
Digestion Control 1+	TGCCTGTTATCCCAGCTATTCA
Digestion Control 1-	TGCCTTGCTCCAAACTCTATTTT
Digestion Control 2+	GTAAGGCTGGCCCTGCATG
Digestion Control 2-	TTCCTCAGTCTTGTGCTCTTTCT
TMPRSS2-Taqman probe	6FAMCCTTGGCTGGTGGGAMGBNFQ
TMPRSS2-Anchor	TCCCCAGCAGCTTCCCTT
T1	CTGCCCTGCCAAATCCAA
T2	TAATGACACAGAGTAGTGCCCAGG
T3	GATTATTTAGTCACCCAGGTACAAAAG
T4	GAACGAATGTGGATGTGTGCTCT
T5	ACAGATGAGGAGGTGGTGAGAAG
T6	GTAACCGCACCAAACGATAAACT
T7	GTTTAAAAGGTCACCAAATATCAAGGTA
T8	GATTGGCTGAGTTGCTCTGC
T9	AGTGTAAGTGCTGAGGAAACAAGC
T10	CCATGAAATCAATGAAGTTGAAGC
T11	ACTTGTGGATGTGGACCCGT
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T21	CATCATTTATCACTTGCTCTCTTTCC
T22	CGAAGAACAATATCTGGTGGCTG
T23	CGCCATGCTCAGCCTGAC
Primers for plasmid constructions and mutagenesis	
MED1 mutation 1 (T1032A)	TCATAGTTCTTCTAACAGACCTTTT GCCCCACCTACCAG
MED1 mutation 2 (T1457A)	GCCATAGTAAGTCACCAGCATATG CCCCCCAGAATC
E1-1+	AGTGGTACCAGAGGGAAATAGTTTCATCC
E1-1-	AGGCTCGAGTGAATTGGTTTGGTTTGG
E1-2+	AGTGGTACCACACCCAAAGAAGGCCA
E1-2-	AGGCTCGAGATGTGATCTCACAGTAGC
E1-3+	AGTGGTACCGTGCTACTGTGAGATCACAT
E1-3-	AGGCTCGAGTAGAGGTAAGTGCTCCATG
E2-1+	GGGGTACCAGGTGCGTGGTGGATCAAGT
E2-1-	GAAGATCTGGCACTCTAGCCAACAGAGG
E2-2+	GGGGTACCGCTCCTCTGTTGGCTAG
E2-2-	GAAGATCTCCCAGCAGGATCATGGTAAG
E2-3+	GGGGTACCGCCTTACCATGATCCTG
E2-3-	GAAGATCTGGAGTGTCTGTTCATGTCC
E2-4+	GGGGTACCGACATGAACAGACACTCC
E2-4-	GAAGATCTCCAGTCTATCATTGTTGGACA
E2-5+	GGGGTACCGTCCAACAATGATAGACTG
E2-5-	GAAGATCTATCTCCTAATGCTATCCCTCCC
E2-6+	GGGGTACCGGGATAGCATTAGGAGATA
E2-6-	GAAGATCTGAAGGAGCCGGCTTTATAGAG
E3-1+	GGGGTACCTGCGGGTGCAGGAGAACAC
E3-1-	GAAGATCTCCAGATCCACAGGACGATCC
E3-2+	GGGGTACCGTGGATCTGGTGAGTATACC
E3-2-	GAAGATCTGGAGGGACTGCTGGAAG
E3-3+	GGGGTACCTCCAGCAGTCCCTCC
E3-3-	GAAGATCTCCCAGGCTAGAGTGCAGTG
E3-4+	GGGGTACCCACTGCACTCTAGCCTG
E3-4-	GAAGATCTGGGCATAGCAAAGGTCAGTG
E3-5+	GGGGTACCCACTGACCTTTGCTATG
E3-5-	GAAGATCTGTCCTCACTTCATCCATTTCCT T
ChIP primers	
E1+	TGCCATGTGCCCTAGAAACTG

E1-	CAAGCTCAGCAAAATGGTGAAA
Promoter +	GCCCGAGGGAAATTGGAT
Promoter -	TTACTCCGCGTGGGAACACT
E2-a+	GTGCGTGGTGGATCAAGTTATC
E2-a-	GGGTGCTCATCCCCATGA
E2-b+	TCCTTTTTAGGGACATGGATGAAG
E2-b-	GTGTTTGGTTTTTTGTCCTTGTGAT
E3-a+	CCCTGGTGGGCCTAGATGA
E3-a-	CAACTTCTCCCTTCCCCTGTCT
E3-b+	CATCCCCCCACACGAAGTTA
E3-b-	TGGATAGGGAGGGTCTTGTATGA
Control +	CCACAAACTCTTCTCAGCTGGG
Control -	TTCTTTCCTTCCCTGTTACCCC
Plasmids Sequences	
E2-4 LNCaP	GACATGAA**C**AGACACTCCTCAAAAGAAGA CATTTATGCAGCCAAAAGACACATGAAAA AATGCTCATCATCACTGGCCATCAGAGAA ATGCAAATCAAAACCACAATGAGATACCA TCTTACACCAGTTAGAATGGTGATCATTAA AAAGTCAGGAAACAACAGGTGCTGGAGAG GATGTGGAGAAATAGGAACACTTTTACAC TGTTGGTGGGACCGTAAACTAGTTCAACC ATTGTGGAAGTCAGTGTGGCGATTCCTCAG GGATCTAGAACTAGAAATACCATTTGACC CAGCCATCCCATTACTGGGTATATACCCAA AGGAATATAAATCATGCTGCTATAAAGAC ACATGCACACATATGTTTATTGTGGCACTA TTCACAATAGCAAAGACTTGGAACCAACC CAAATGTCCAACAATGATAGACTGG
E2-4 PC-3	GACATGAA**A**AGACACTCCTCAAAAGAAGA CATTTATGCAGCCAAAAGACACATGAAAA AATGCTCATCATCACTGGCCATCAGAGAA ATGCAAATCAAAACCACAATGAGATACCA TCTTACACCAGTTAGAATGGTGATCATTAA AAAGTCAGGAAACAACAGGTGCTGGAGAG GATGTGGAGAAATAGGAACACTTTTACAC TGTTGGTGGGACCGTAAACTAGTTCAACC ATTGTGGAAGTCAGTGTGGCGATTCCTCAG GGATCTAGAACTAGAAATACCATTTGACC CAGCCATCCCATTACTGGGTATATACCCAA AGGAATATAAATCATGCTGCTATAAAGAC ACATGCACACATATGTTTATTGTGGCACTA TTCACAATAGCAAAGACTTGGAACCAACC CAAATGTCCAACAATGATAGACTGG

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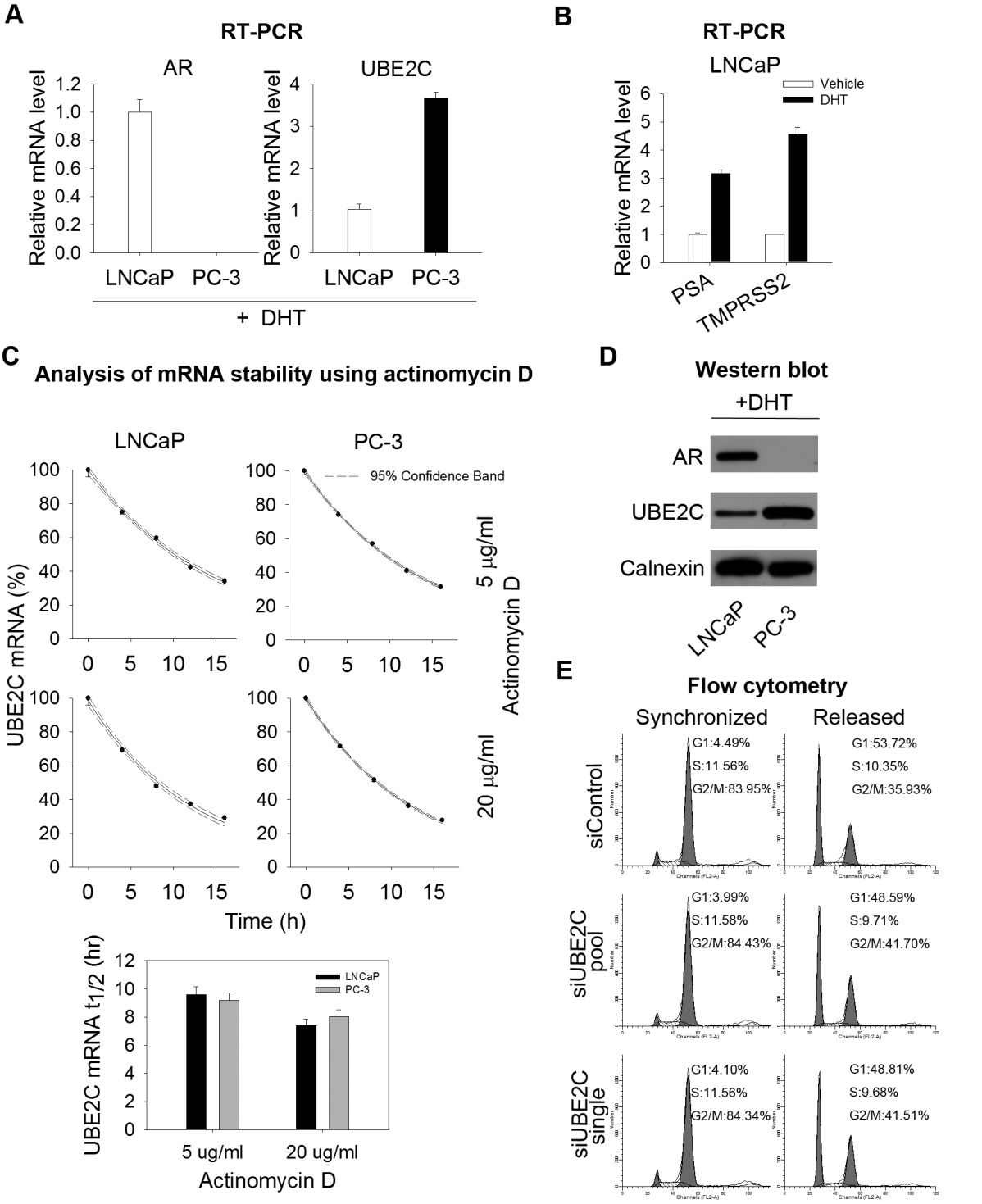
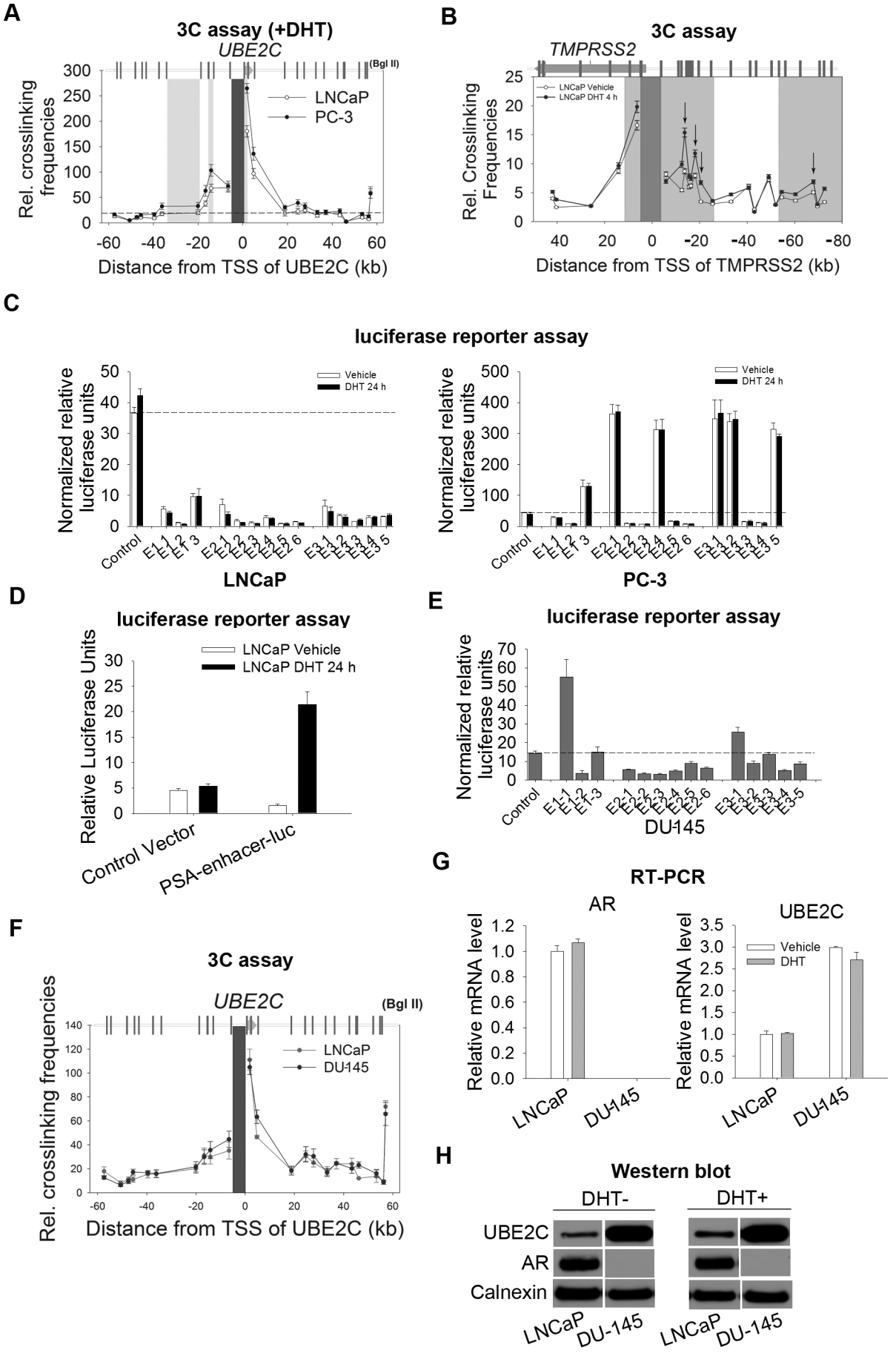
Chen_supplementary Figure 1

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Control Vector
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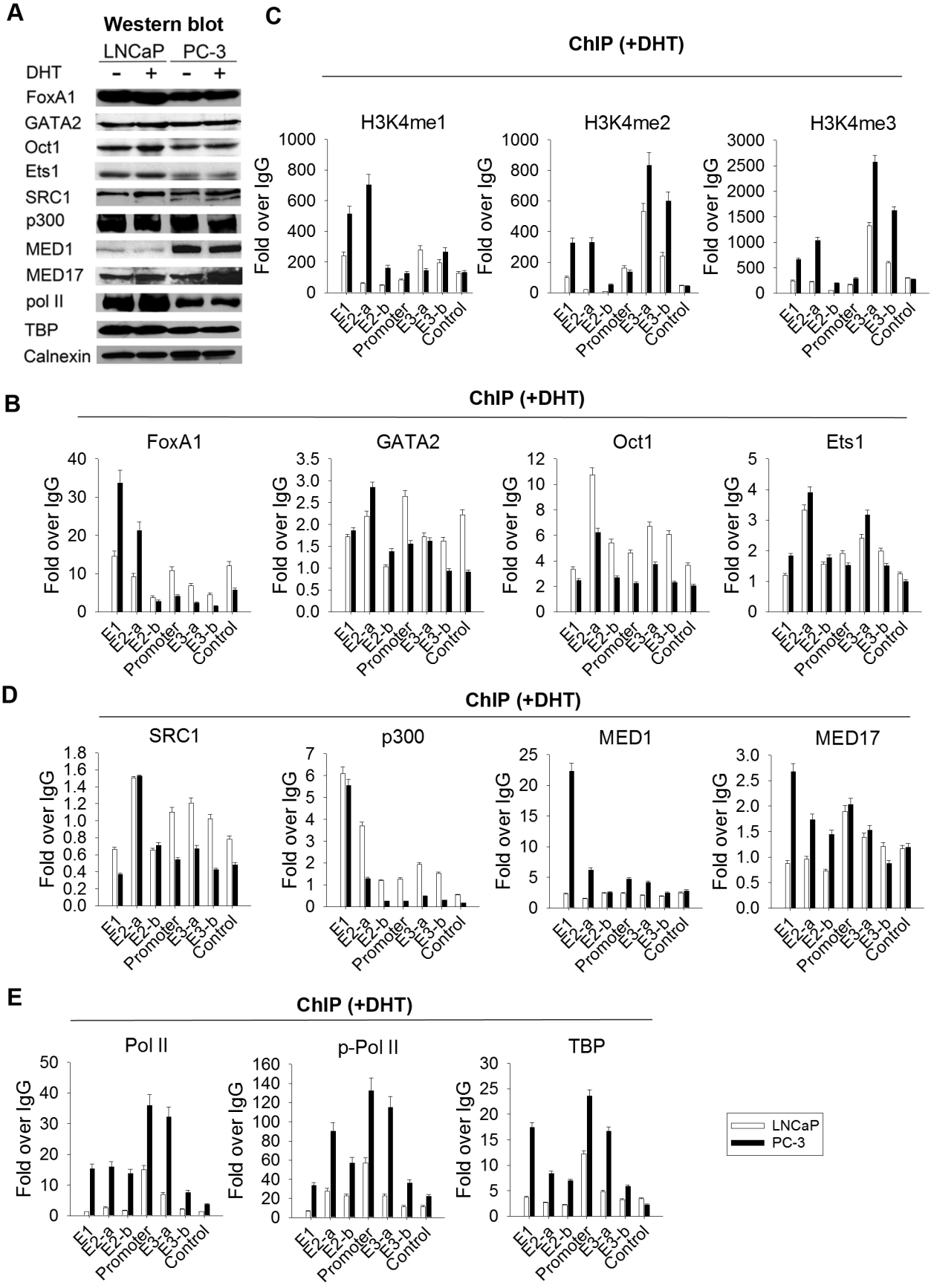
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pol II
TBP
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ChIP (+DHT)
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Fold over IgG
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FoxA1
GATA2
Oct1
Ets1
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p300
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MED17
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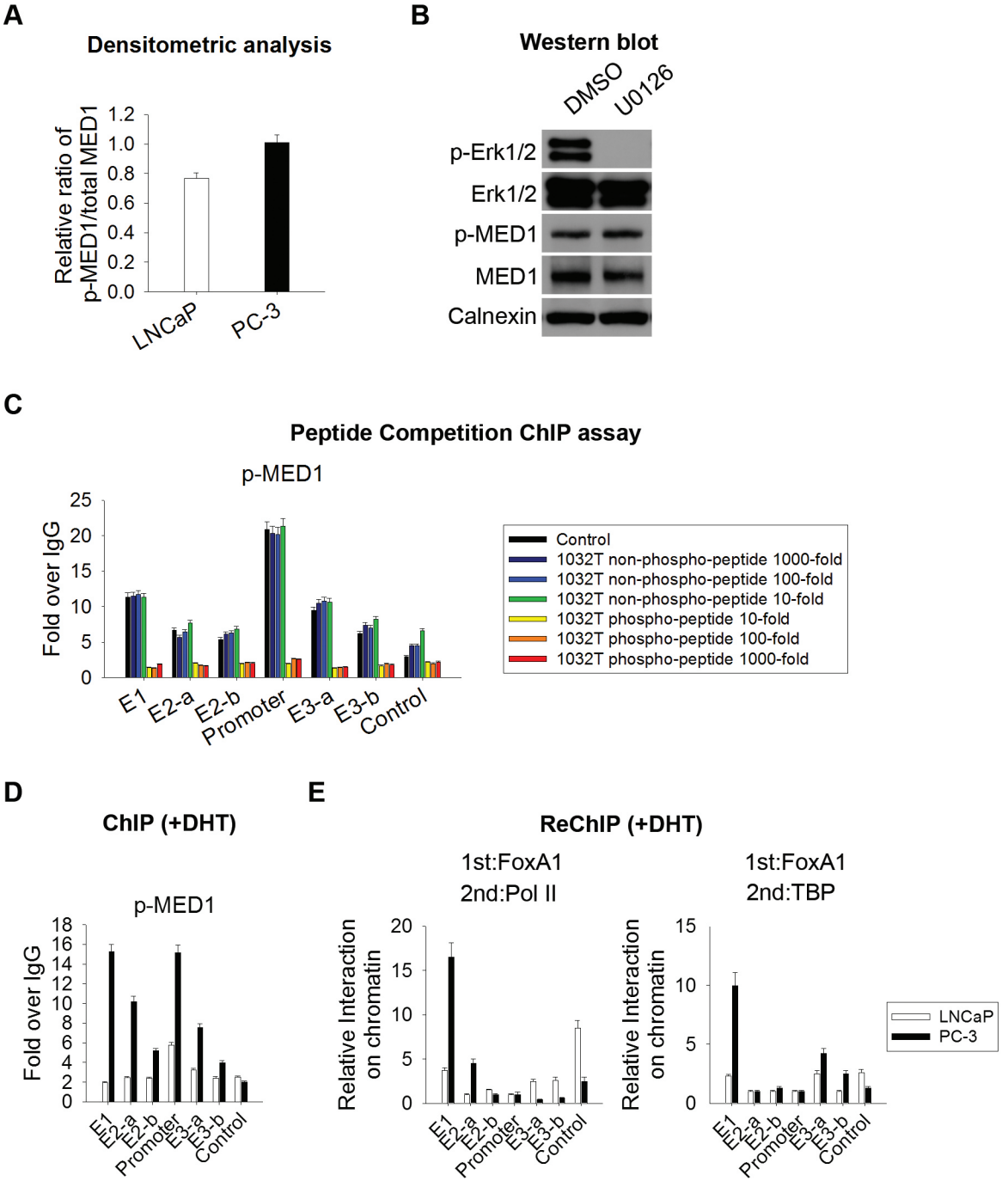
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Densitometric analysis
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1.0
0.8
0.6
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0.2
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Fold over IgG
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E2-b
Promoter
E3-a
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Control
Control
1032T non-phospho-peptide 1000-fold
1032T non-phospho-peptide 100-fold
1032T non-phospho-peptide 10-fold
1032T phospho-peptide 10-fold
1032T phospho-peptide 100-fold
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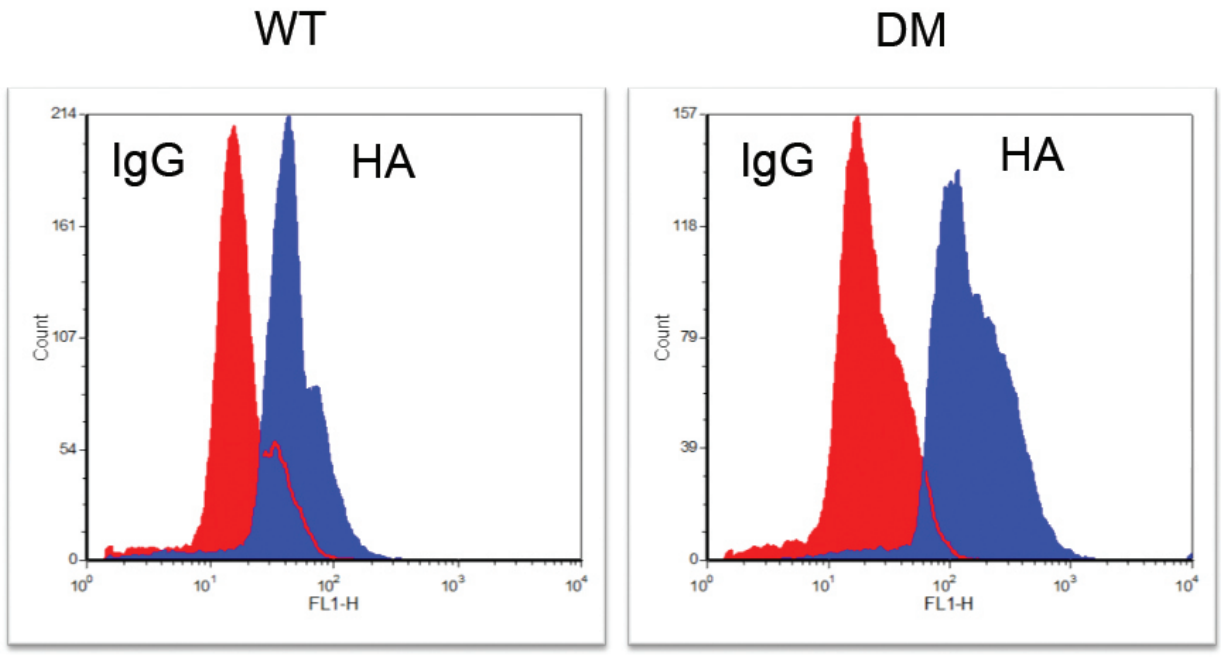
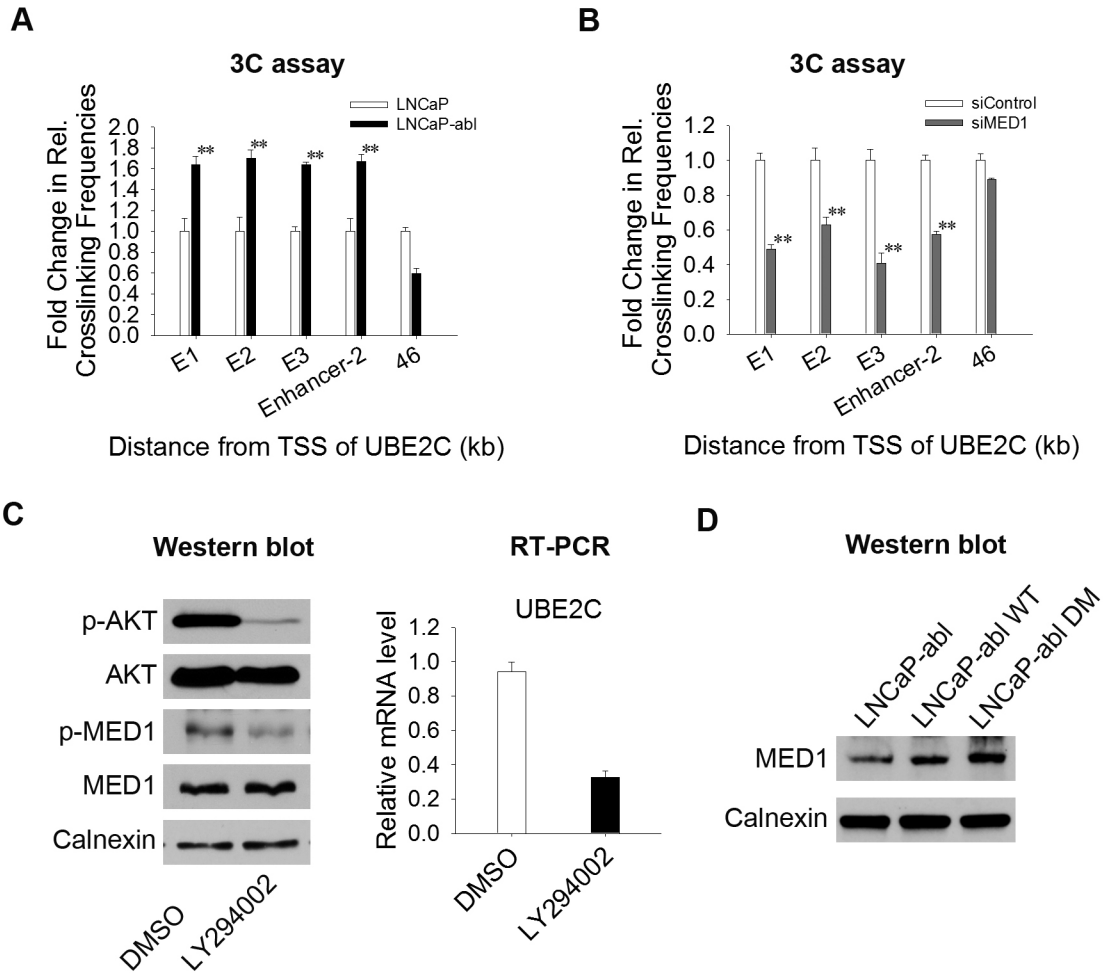
6
4
2
0
E1
E2-a
E2-b
Promoter
E3-a
E3-b
Control
E
ReChIP (+DHT)
1st:FoxA1
2nd:Pol II
Relative Interaction on chromatin
20
15
10
5
0
E1
E2-a
E2-b
Promoter
E3-a
E3-b
Control
1st:FoxA1
2nd:TBP
Relative Interaction on chromatin
15
10
5
0
E1
E2-a
E2-b
Promoter
E3-a
E3-b
Control
LNCaP
PC-3

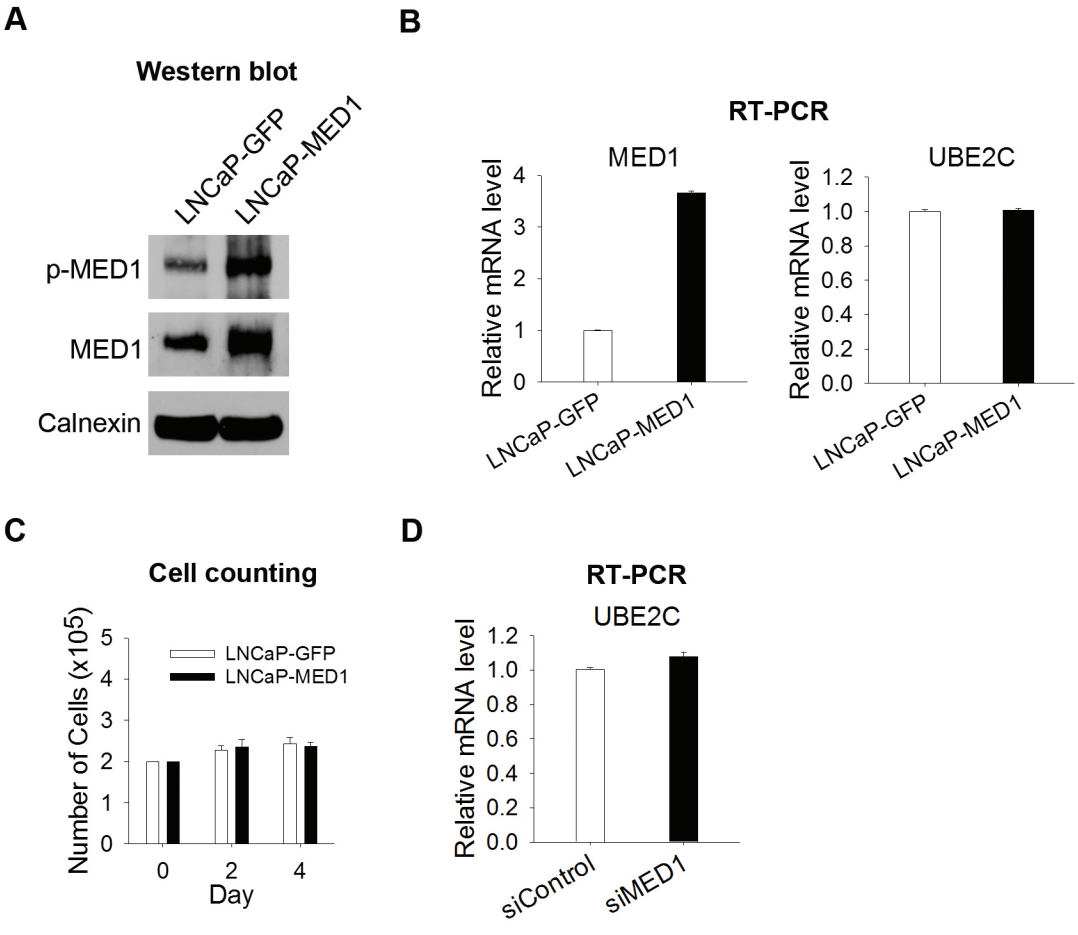
Chen_supplementary Figure 5

Flow cytometry



Chen_supplementaty Figure 6




A
Western blot
LNCaP-GFP
LNCaP-MED1
p-MED1
MED1
Calnexin
B
RT-PCR
MED1
Relative mRNA level
0
1
2
3
4
LNCaP-GFP
LNCaP-MED1
UBE2C
Relative mRNA level
0.0
0.2
0.4
0.6
0.8
1.0
1.2
LNCaP-GFP
LNCaP-MED1
C
Cell counting
Number of Cells (x10^5)
0
1
2
3
4
5
LNCaP-GFP
LNCaP-MED1
0
2
4
Day
D
RT-PCR
UBE2C
Relative mRNA level
0.0
0.2
0.4
0.6
0.8
1.0
1.2
siControl
siMED1