Supplementary Information

Title: Androgen receptor monomers and dimers regulate opposing biological processes in prostate cancer cells

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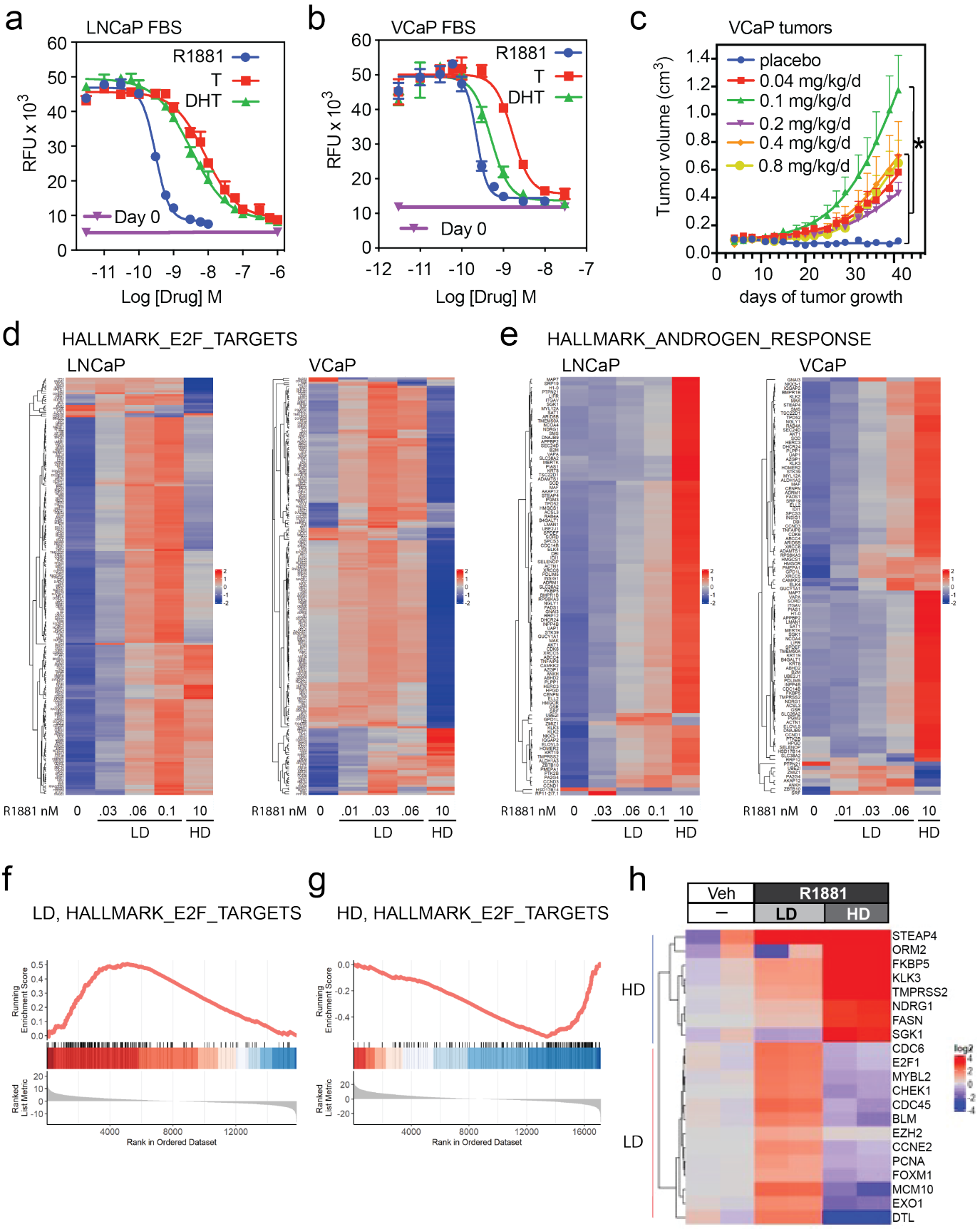
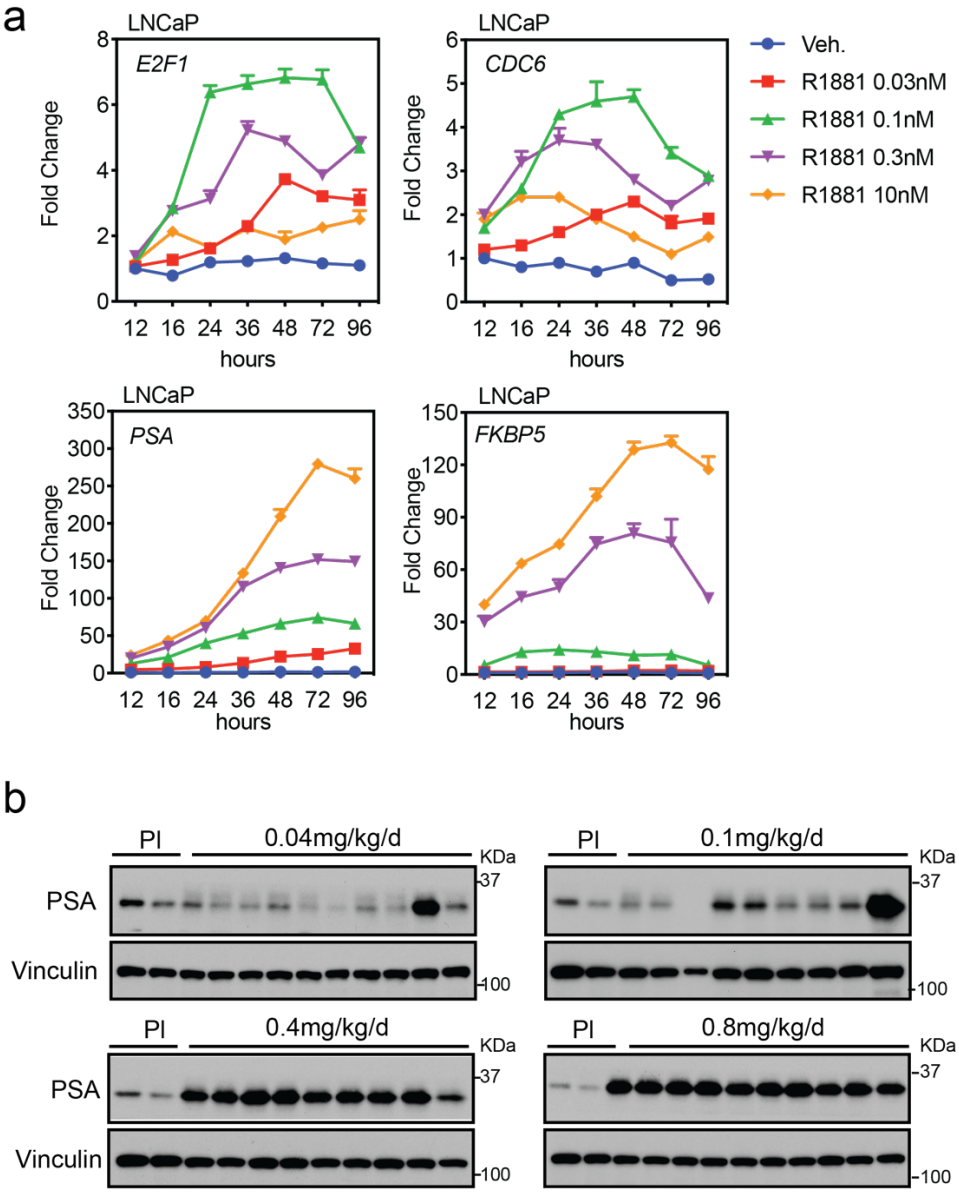
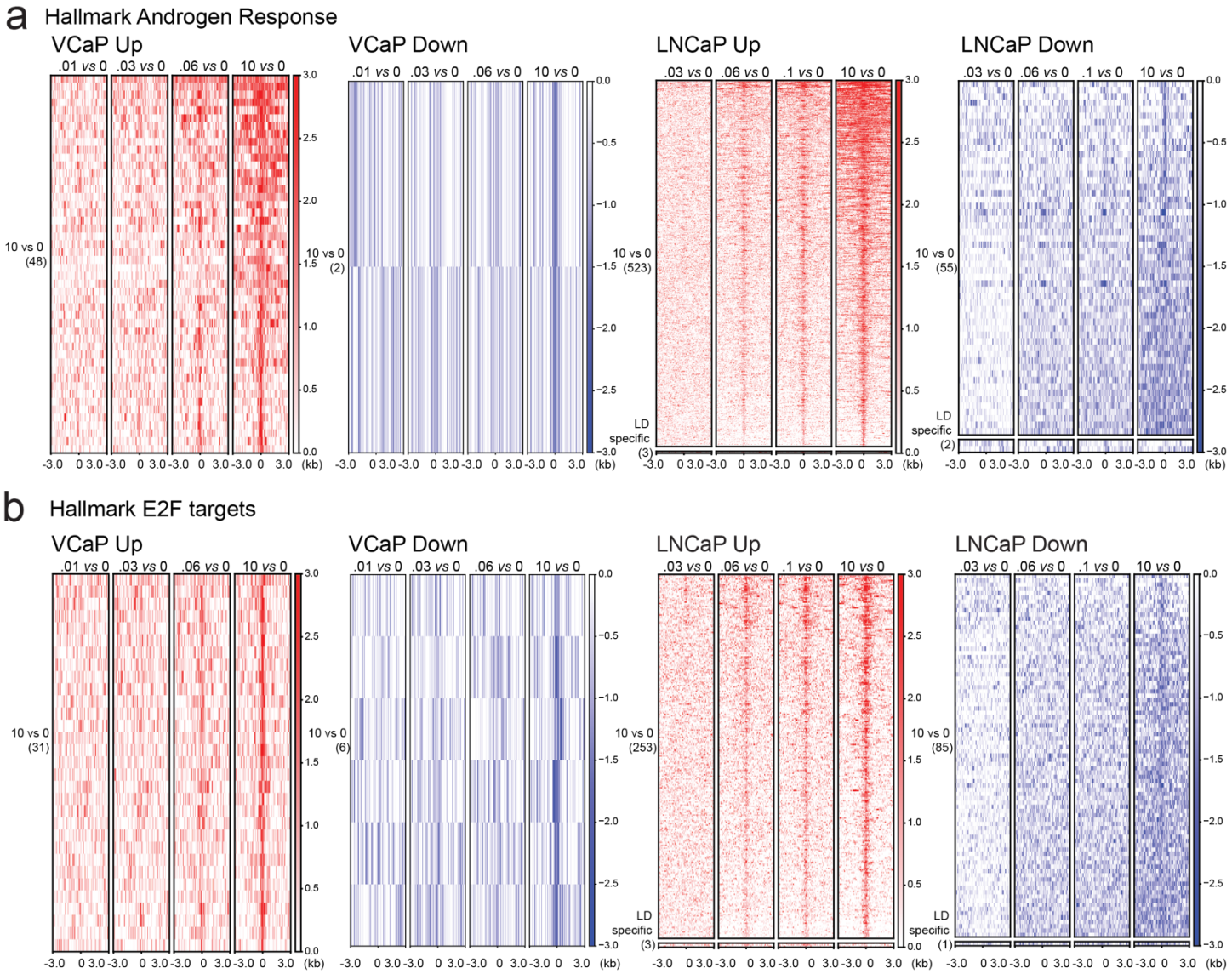

a
LNCaP FBS
RFU x 10^3
R1881
T
DHT
Day 0
Log [Drug] M
b
VCaP FBS
RFU x 10^3
R1881
T
DHT
Day 0
Log [Drug] M
c
VCaP tumors
Tumor volume (cm^3)
placebo
0.04 mg/kg/d
0.1 mg/kg/d
0.2 mg/kg/d
0.4 mg/kg/d
0.8 mg/kg/d
*
days of tumor growth
d
HALLMARK_E2F_TARGETS
LNCaP
VCaP
R1881 nM 0 .03 .06 0.1 10
LD
HD
R1881 nM 0 .01 .03 .06 10
LD
HD
e
HALLMARK_ANDROGEN_RESPONSE
LNCaP
VCaP
R1881 nM 0 .03 .06 0.1 10
LD
HD
R1881 nM 0 .01 .03 .06 10
LD
HD
f
LD, HALLMARK_E2F_TARGETS
Running Enrichment Score
Ranked List Metric
Rank in Ordered Dataset
g
HD, HALLMARK_E2F_TARGETS
Running Enrichment Score
Ranked List Metric
Rank in Ordered Dataset
h
Veh
R1881
–
LD
HD
HD
LD
STEAP4
ORM2
FKBP5
KLK3
TMPRSS2
NDRG1
FASN
SGK1
CDC6
E2F1
MYBL2
CHEK1
CDC45
BLM
EZH2
CCNE2
PCNA
FOXM1
MCM10
EXO1
DTL
log2

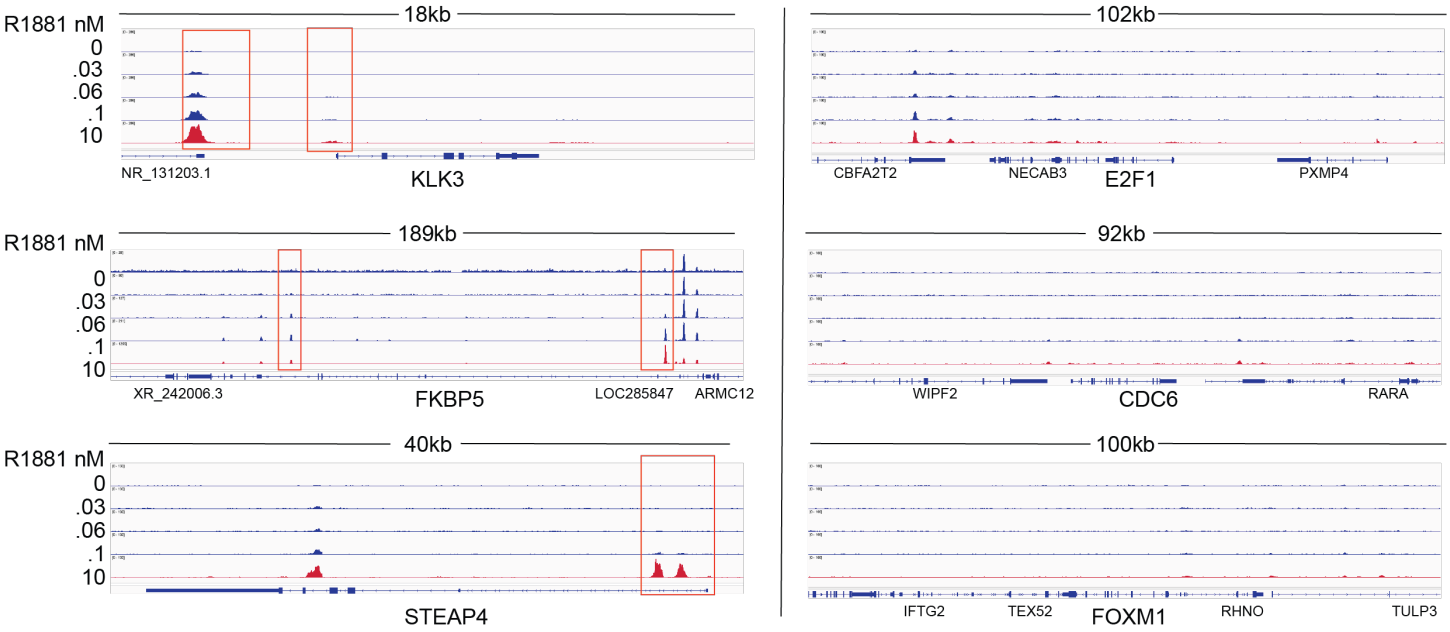
Supplementary Fig. 1 Different doses of the same androgen induce the expression of distinct gene expression programs. LNCaP (**a**) or VCaP (**b**) cells were plated in media containing FBS for 24 hrs. followed by treatment with increasing concentrations of R1881, testosterone (T) or DHT. Cell growth was assessed by DNA quantification at day 7. Data are shown as mean ± SD of representative results from three independent experiments, n=3 wells of cells. Relative Fluorescence Units (RFU). **c** Castrated male NSG mice bearing subcutaneous VCaP tumors were administered increasing doses of DHT. Placebo, n=10 mice; 0.04 mg/kg/d, n=10 mice; 0.1 mg/kg/d, n=9 mice; 0.2 mg/kg/d, n=10mice; 0.4 mg/kg/d, n=9 mice; 0.8 mg/kg/d, n=10 mice. Tumor growth was monitored three times weekly, and Data are shown as mean ± SEM. (*) indicates significant difference between groups. p values were determined by two-way ANOVA followed by Tukey's multiple comparison test ($P \leq 0.0002$). Heatmap presentation of HALLMARK_E2F_TARGTS (**d**) or HALLMARK_ANDROGEN_RESPONSE (**e**) relative gene expression as assessed by RNA-seq from LNCaP or VCaP cells treated in CFS media with vehicle (0) or indicated concentrations of R1881. Gene set enrichment plots of HALLMARK_E2F_TARGTS of VCaP treated with LD 0.06 nM R1881 (**f**) or HD 10 nM R1881 (**g**) are shown. **h** Heatmap of selected mitotic LD and the classic HD androgen genes expression from Fig. 1I are shown. Source data are provided as a Source Data file.



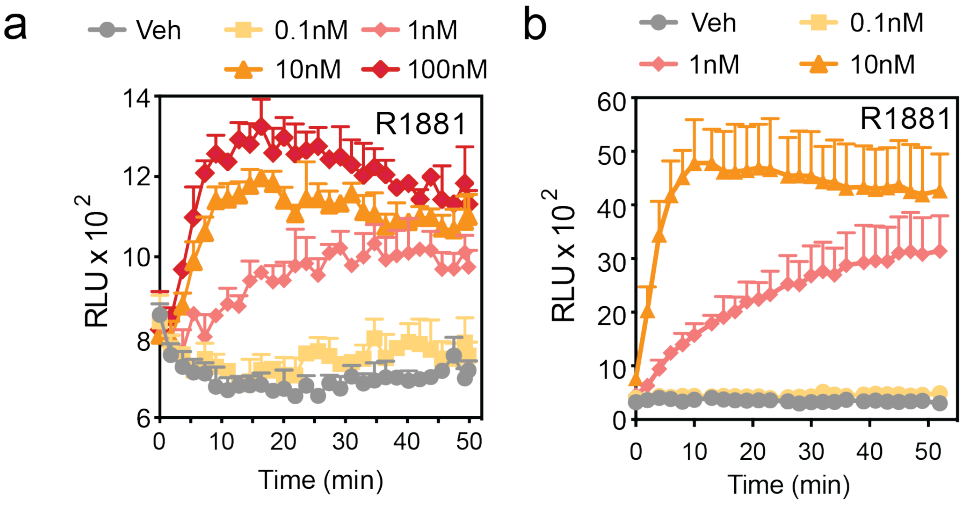
Supplementary Fig. 2 Dose dependent effects of androgens on AR target gene expression. a LNCaP cells were plated in CFS-supplemented media for 3 days and treated with increasing concentrations of R1881 for indicated hours. The mRNA expression for LD genes *E2F1* and *CDC6* or the HD classic genes *PSA* and *FKBP5,* were assessed using qRT-PCR. Data are shown as mean ± SD as representative results from three independent experiments, n=3 technical replicates. **b** Whole cell extracts from VCaP tumors at the end of the study in Fig. S1C were probed for PSA or β-Actin using western blot. Source data are provided as a Source Data file.


a
Hallmark Androgen Response
VCaP Up
VCaP Down
LNCaP Up
LNCaP Down
.01 vs 0
.03 vs 0
.06 vs 0
.1 vs 0
10 vs 0
10 vs 0 (48)
10 vs 0 (2)
10 vs 0 (523)
10 vs 0 (55)
LD specific (3)
LD specific (2)
(kb)
b
Hallmark E2F targets
10 vs 0 (31)
10 vs 0 (6)
10 vs 0 (253)
10 vs 0 (85)
LD specific (3)
LD specific (1)
c
R1881 nM
0
.03
.06
.1
10
18kb
NR_131203.1
KLK3
102kb
CBFA2T2
NECAB3
E2F1
PXMP4
189kb
XR_242006.3
FKBP5
LOC285847
ARMC12
92kb
WIPF2
CDC6
RARA
40kb
STEAP4
100kb
IFTG2
TEX52
FOXM1
RHNO
TULP3

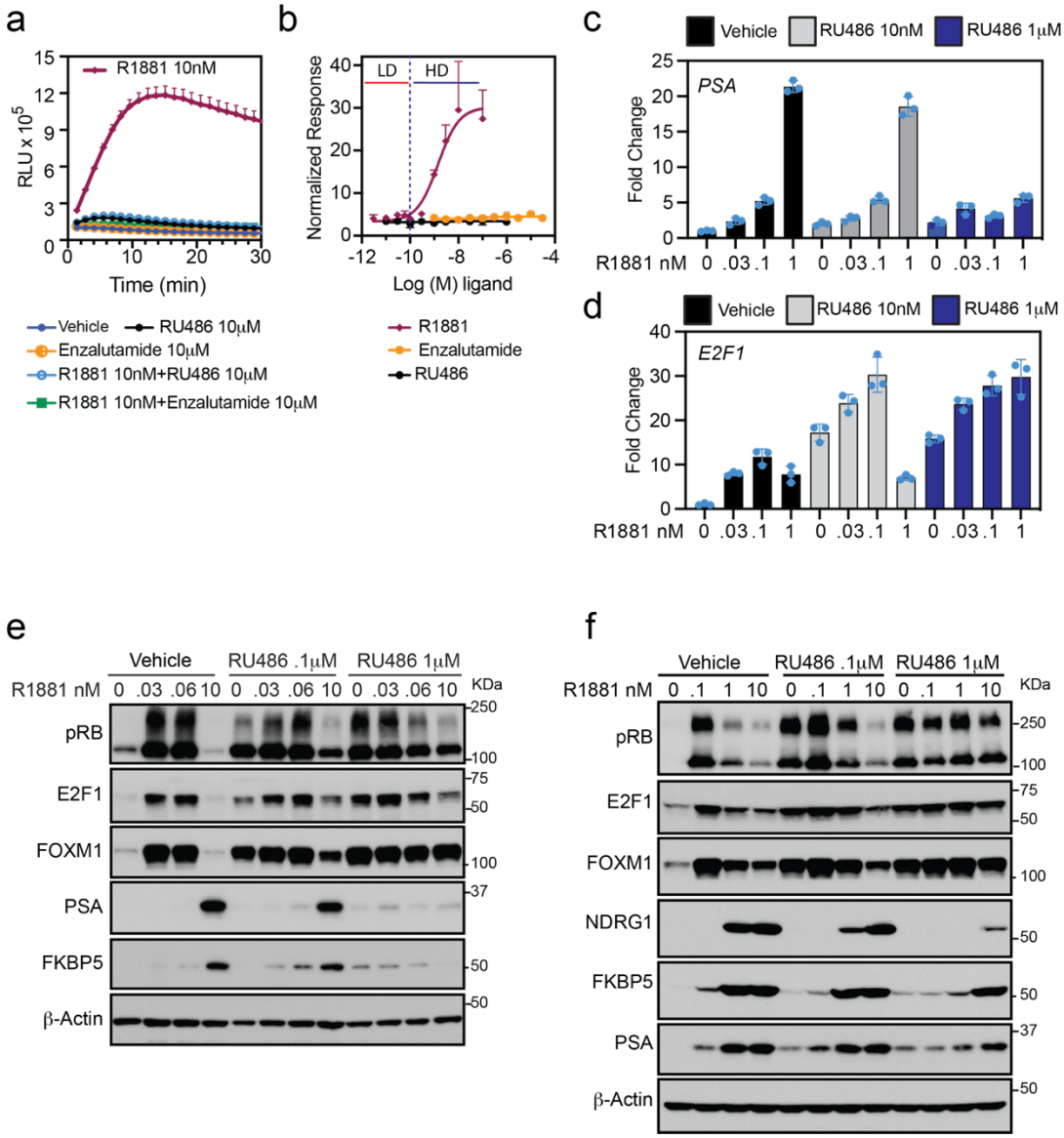
Supplementary Fig. 3 Classical AR action is not detected in response to low dose androgen treatment. Heatmap of ATAC-seq signal in a 6 kb window of high-confidence differentially accessible peaks identified in HALLMARK_ANDROGEN_RESPONSE (**a**) or HALLMARK_E2F_TARGTS (**b**) genes in response to indicated androgen dose compared to vehicle in VCaP or LNCaP cells. Differentially accessible peaks were subdivided based on whether they are significantly increased (VCaP Up or LNCaP Up) or significantly decreased (VCaP down or LNCaP down). **c** Representative genomic tracks of ATAC-seq sites of classical AR regulated genes (i.e., KLK3, FKBP5 and STEAP4) or LD regulated genes (i.e., E2F1, CDC6 and FOXM1) in response to vehicle (0) or increasing androgens in LNCaP cells.



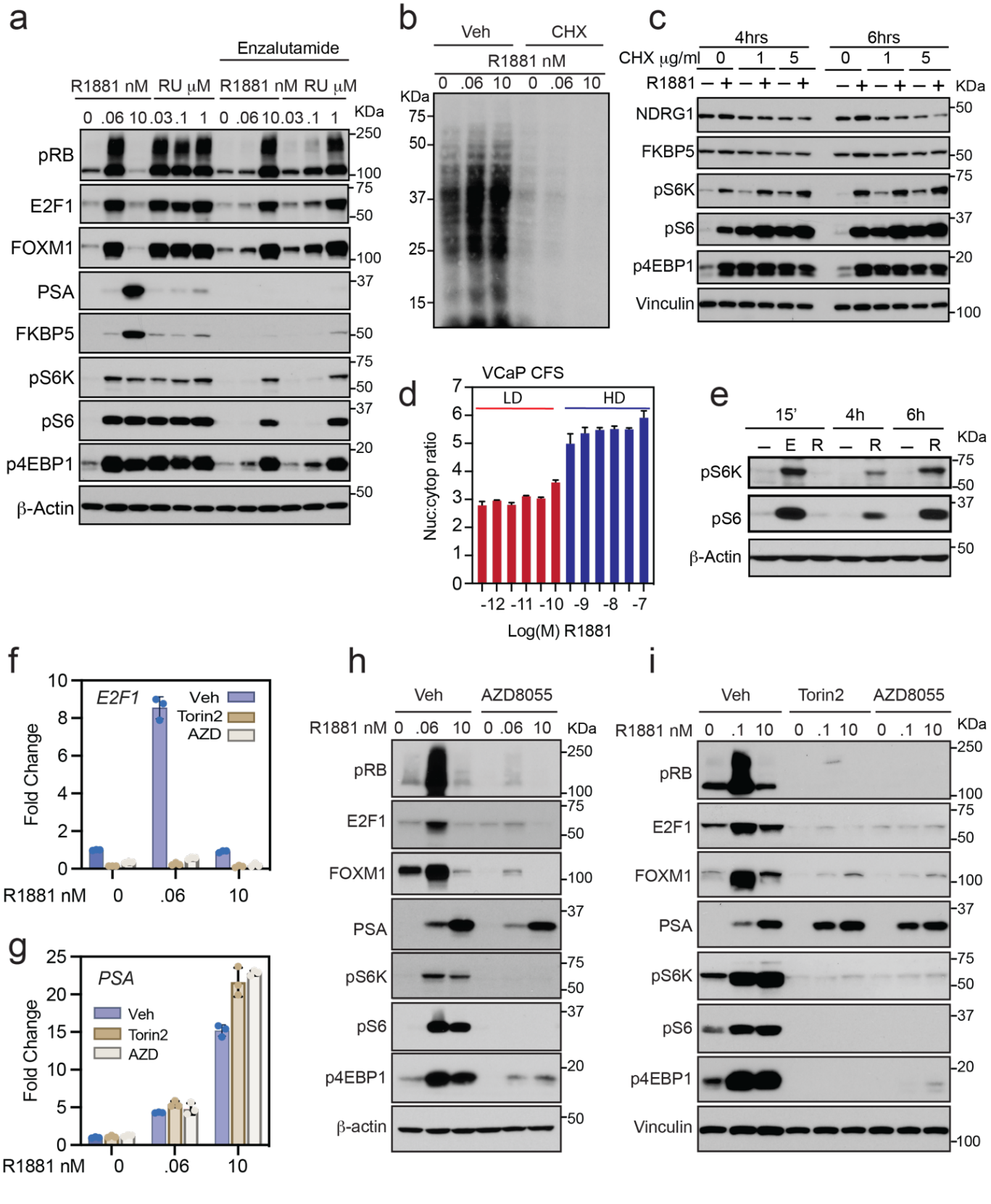
Supplementary Fig. 4 AR-binding to chromatin does not correlate with the expression of genes required for proliferation. Representative genomic tracks of AR bound ChIP-seq sites of classical AR regulated genes (i.e., KLK3, FKBP5 and STEAP4) or LD regulated genes (i.e., E2F1, CDC6 and FOXM1) in response to vehicle (0) or increasing androgens in LNCaP cells.



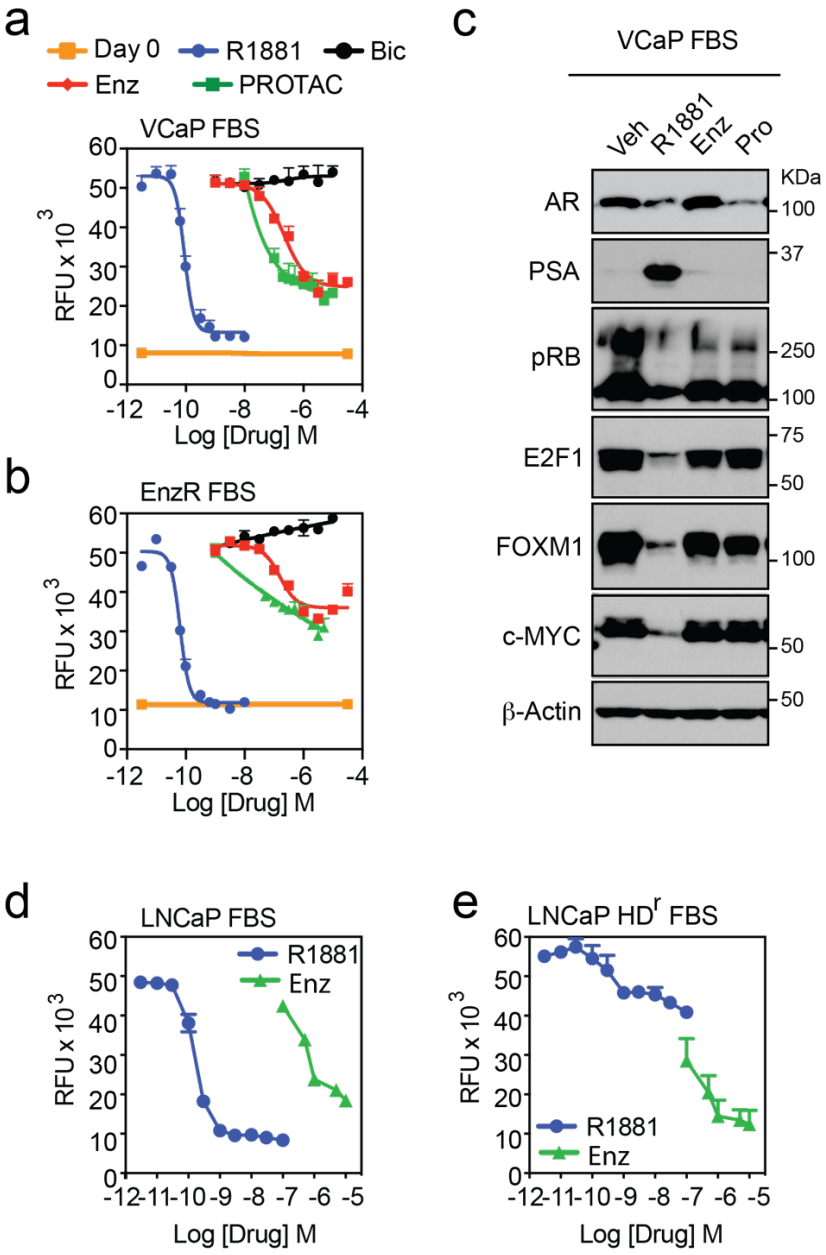
Supplementary Fig. 5 AR-dependent proliferation and dimerization can be uncoupled by dose. **a** LNCaP cells were transfected in CFS-supplemented media or (**b**) HEK293 in FBS-supplemented media with plasmids expressing AR fused to the small- (SmBiT AR) and large-bit (LgBiT AR) of a Nanoluciferase. 48 hrs later, cells were treated with increasing concentrations of R1881 at the same time the Nanoluciferase reagent was added, and light emission measured using a plate reader. Data are plotted as mean ± SD of representative results from three independent experiments, n=3 wells of cells. RLU: relative light units. Source data are provided as a Source Data file.



Supplementary Fig. 6 AR dimerization is required to inhibit the proliferation of prostate cancer cells. a HEK293 cells were transfected in CFS-supplemented media with plasmids expressing AR fused to the small- (SmBiT AR) and large-bit (LgBiT AR) of a Nanoluciferase. 48 hrs later, cells were treated with either Vehicle, RU486 or Enzalutamide alone or in combination with 10nM R1881. At the same time Nanoluciferase reagent was added and light emission measured using a plate reader. Data are plotted as mean ± SD of representative results from three independent experiments. n=3 wells of cells. RLU: relative light units. **b** Mammalian two-hybrid assay was performed in CV-1 cells to test the androgens dependent interaction between a full-length AR-VP16 and Gal4-DBD-AR (507-919) fusion proteins. VCaP cells were plated in CFS-supplemented media for 2 days and treated with increasing concentrations of R1881 alone or in combination with RU486 for an additional 24hr. Data are plotted as mean ± SD of representative results from three independent experiments, n=3 wells of cells. The mRNA expression for *PSA* (**c**) and *E2F1* (**d**) were assessed using qRT-PCR. Data are shown as mean ± SD as representative results from three independent experiments, n=3 technical replicates. VCaP (**e**) or LNCaP (**f**) cells were plated in CFS-supplemented media for 2 days and treated with increasing concentrations of R1881 alone or in combination with RU486 for an additional 48 hrs. Whole cell extracts were probed for RB phosphorylation (p-RB), E2F1, FOXM1, NDRG1, FKBP5, PSA, or β-Actin using western blot. Representative images are shown from n=2 biologically independent experiments. Source data are provided as a Source Data file.


a
Enzalutamide
R1881 nM
RU μM
R1881 nM
RU μM
0 .06 10 .03 .1 1 0 .06 10 .03 .1 1
KDa
pRB
E2F1
FOXM1
PSA
FKBP5
pS6K
pS6
p4EBP1
β-Actin
250
100
75
50
100
37
50
75
50
37
20
50
b
Veh
CHX
R1881 nM
0 .06 10 0 .06 10
KDa
75
50
37
25
15
c
4hrs
6hrs
CHX μg/ml 0 1 5 0 1 5
R1881 – + – + – + – + – + – +
KDa
NDRG1
FKBP5
pS6K
pS6
p4EBP1
Vinculin
50
50
75
37
20
100
d
VCaP CFS
LD
HD
Nuc:cytop ratio
7
6
5
4
3
2
1
0
-12 -11 -10 -9 -8 -7
Log(M) R1881
e
15’
4h
6h
– E R – R – R
KDa
pS6K
pS6
β-Actin
75
50
37
50
f
E2F1
Veh
Torin2
AZD
Fold Change
10
8
6
4
2
0
R1881 nM 0 .06 10
g
PSA
Veh
Torin2
AZD
Fold Change
25
20
15
10
5
0
R1881 nM 0 .06 10
h
Veh
AZD8055
R1881 nM 0 .06 10 0 .06 10
KDa
pRB
E2F1
FOXM1
PSA
pS6K
pS6
p4EBP1
β-actin
250
100
75
50
100
37
75
50
37
20
50
i
Veh
Torin2
AZD8055
R1881 nM 0 .1 10 0 .1 10 0 .1 10
KDa
pRB
E2F1
FOXM1
PSA
pS6K
pS6
p4EBP1
Vinculin
250
100
75
50
100
37
75
50
37
20
100

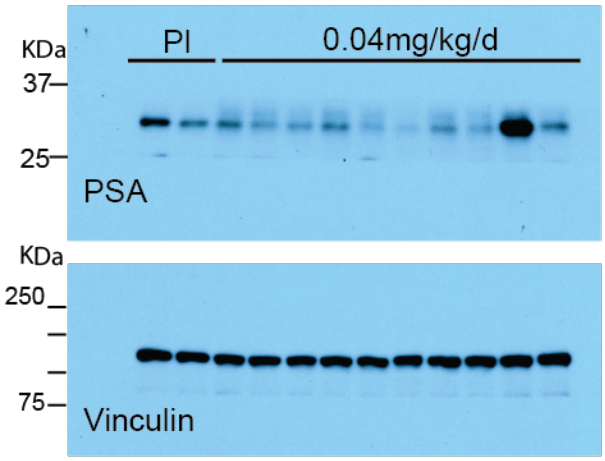
Supplementary Fig. 7 AR-dependent activation of mTOR occurs in a non-genomic manner. a VCaP cells grown in CFS media were treated with increasing concentrations of R1881 or RU486 alone or in combination with Enzalutamide for 48 hrs. WCE were probed for pRB, E2F1, FOXM1, PSA, FKBP5, pS6K, pS6, p4EBP1 and β-Actin. **b** VCaP cells grown in CFS media were treated with vehicle or indicated concentrations of R1881 alone or in the presence of 1μg/ml cycloheximide (CHX) for 24hrs. Prior to collection, cells were treated with 10μM puromycin for 30 min and WCE were probed with an anti-puromycin antibody to allow the immunological detection of puromycin-labelled peptides. **c** VCaP cells grown in CFS media were treated with 10 nM R1881 alone or in the presence of 1 or 5 μg cycloheximide (CHX) for indicated time. WCE were probed for NDRG1, FKBP5, pS6K, pS6, p4EBP1 and β-Actin. **d** VCaP cells grown in CFS media were treated with increasing doses of R1881 and cells were stained for AR, DAPI, and Phalloidin. Nuclear *vs*. Cytoplasmic (Nuc:cytop) ratios were quantified using high content imaging (ArrayScan). Data are shown as mean ± SD of a representative results from two independent experiments, n=2 wells of cells. **e** VCaP cells grown in CFS media were treated with either EGF (100ng/ml) or 10 nM R1881 for indicated time. WCE were probed for pS6K, pS6 and β-Actin. VCaP cells grown in CFS media were treated with vehicle (0), .06nM or 10nM R1881 for 24hr. The mRNA expression for *E2F1* (**f**) and *PSA* (**g**) were assessed using qRT-PCR. Data are shown as mean ± SD as representative results from three independent experiments, n=3 technical replicates. VCaP (**h**) or LNCaP (**i**) cells grown in CFS media were treated with increasing concentrations of R1881 alone or in combination with 100nM of Torin2 or AZD8055 (AZD). WCE were probed for pRB, E2F1, FOXM1, PSA, pS6K, pS6, p4EBP1 and β-Actin. For A, E, H and I, representative images are shown from n=2 biologically independent experiments and n=3 biologically independent experiments for B and C. WCE: Whole Cell Extract. Source data are provided as a Source Data file.

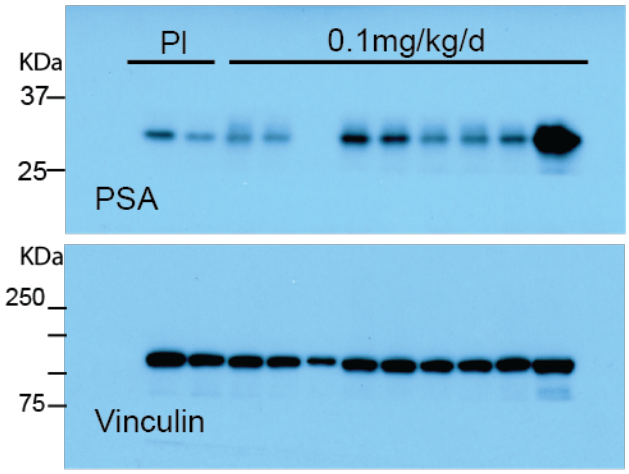


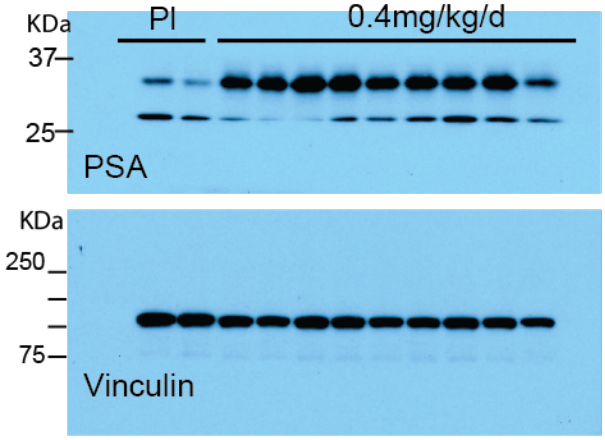
Supplementary Fig. 8 AR dependent proliferation requires c-MYC. VCaP (**a**) or EnzR (**b**) cells were plated in FBS-supplemented media for 24 hrs. and treated with increasing concentrations of either R1881 or AR antagonists as indicated. Bic: Bicalutamide, Enz: Enzalutamide or the AR degrader PROTAC (Pro). Cell growth was assessed by DNA quantification at day 7. Data are shown as mean ± SD of representative results from three independent experiments. n=3 wells of cells. **c** EnzR cells were plated in FBS-supplemented media for 24 hrs. and treated for an additional 48 hrs. with either Vehicle (Veh), 10nM R1881 or AR antagonists as indicated. Enz: Enzalutamide (10mM) or Pro: AR degrader PROTAC (2μM). Whole cell extracts were probed for AR, PSA, RB phosphorylation (p-RB), E2F1, FOXM1, c-MYC or β-Actin using western blot. Representative images are shown from n = 3 biologically independent experiments. **d** LNCaP control (CTRL) or (**e**) over-expressing c-MYC (c-MYC) cells were plated in FBS-supplemented media and treated with increasing concentrations of R1881 or Enzalutamide (Enz). Cell growth was assessed by DNA quantification at day 7. Data are plotted as mean ± SD of representative results from three independent experiments, n=3 wells of cells. RFU: Relative Fluorescence Units. Source data are provided as a Source Data file.

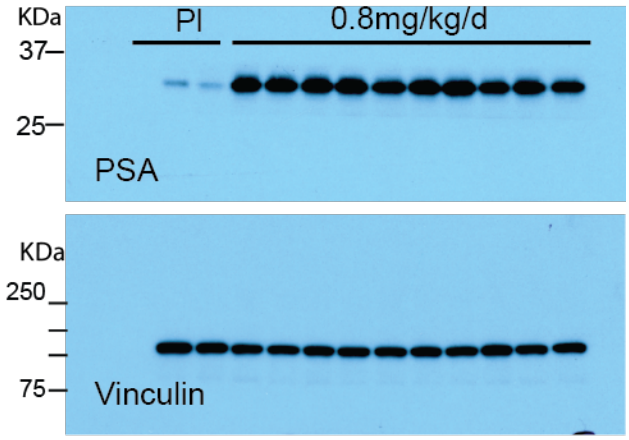
Supplementary Data 1 and Supplementary Data 2. Top motifs of differentially accessible chromatin sites in response to different doses of androgens. Motif enrichment analysis of differentially accessible gained or lost peaks in VCaP (**Table 1**) or LNCaP (**Table 2**) cells. Motifs are ordered in descending rank according to p values. The p-values for were calculated using a one-sided binomial test to determine the probability of observing more target sequences with a motif compared to background sequences. FDR was controlled for multiple hypothesis testing using the Benjamini-Hochberg procedure, as implemented in the findMotifsGenome program in the HOMER suite [98].

Supplementary Fig. 2b

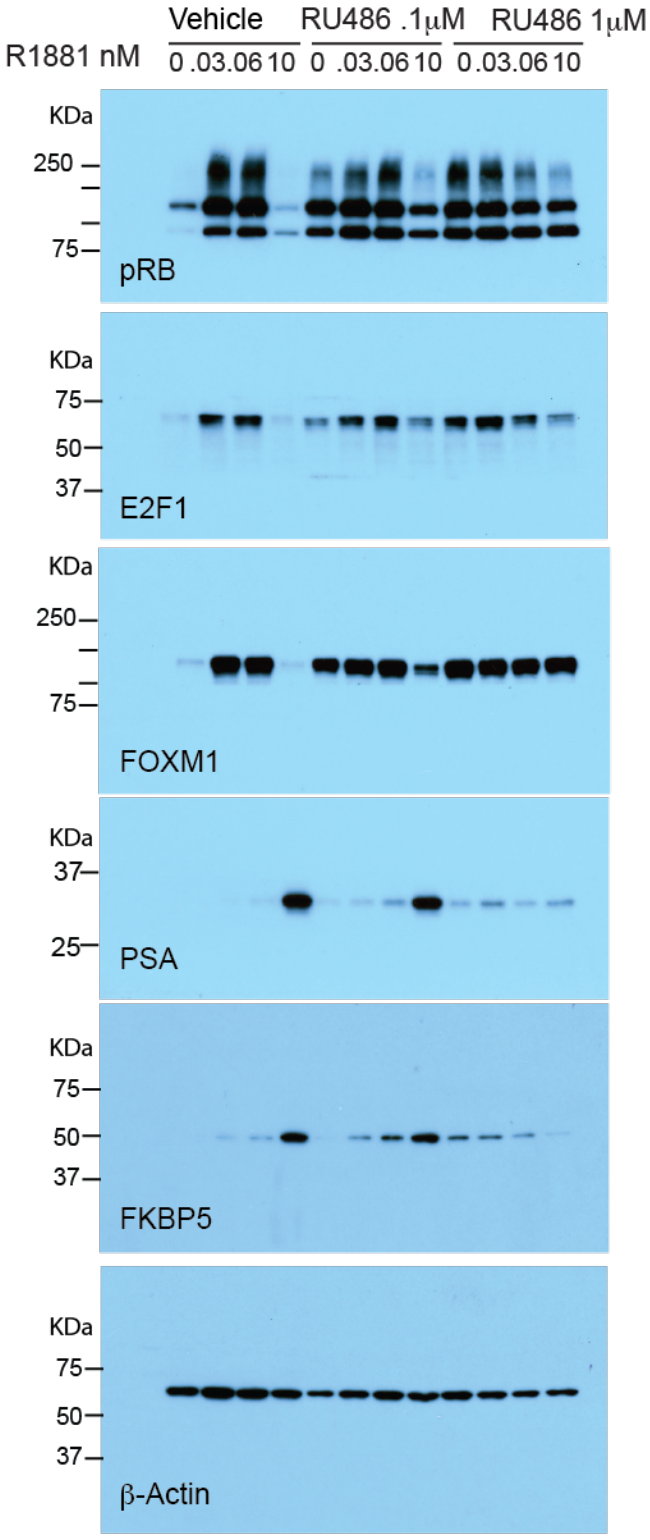


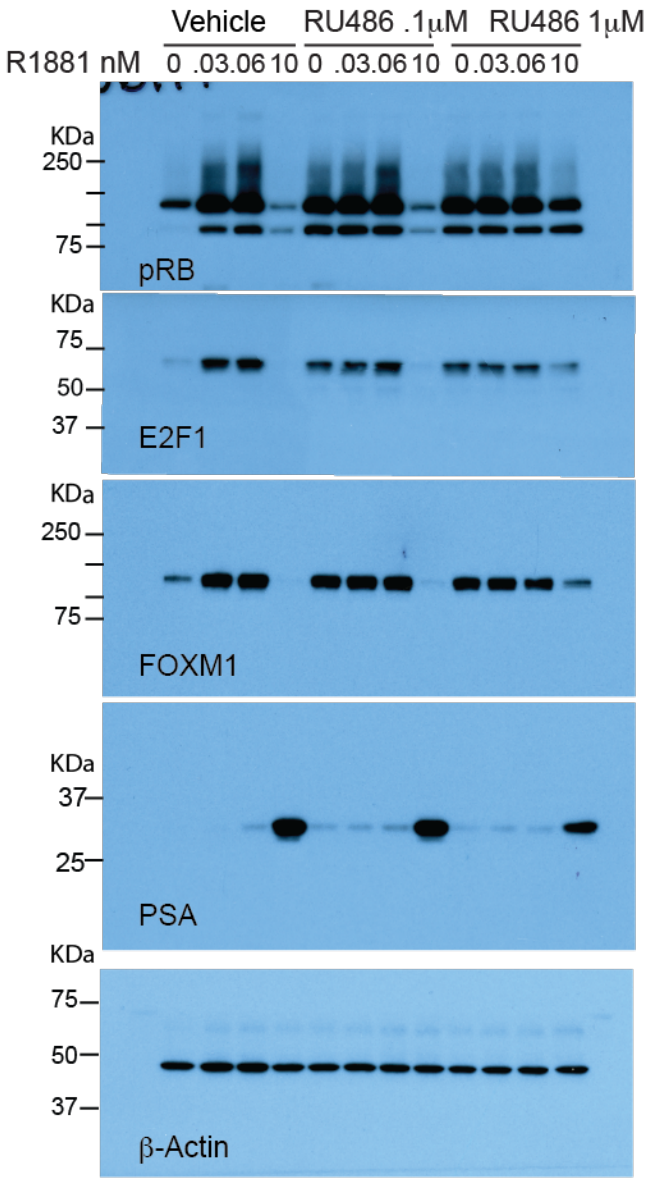




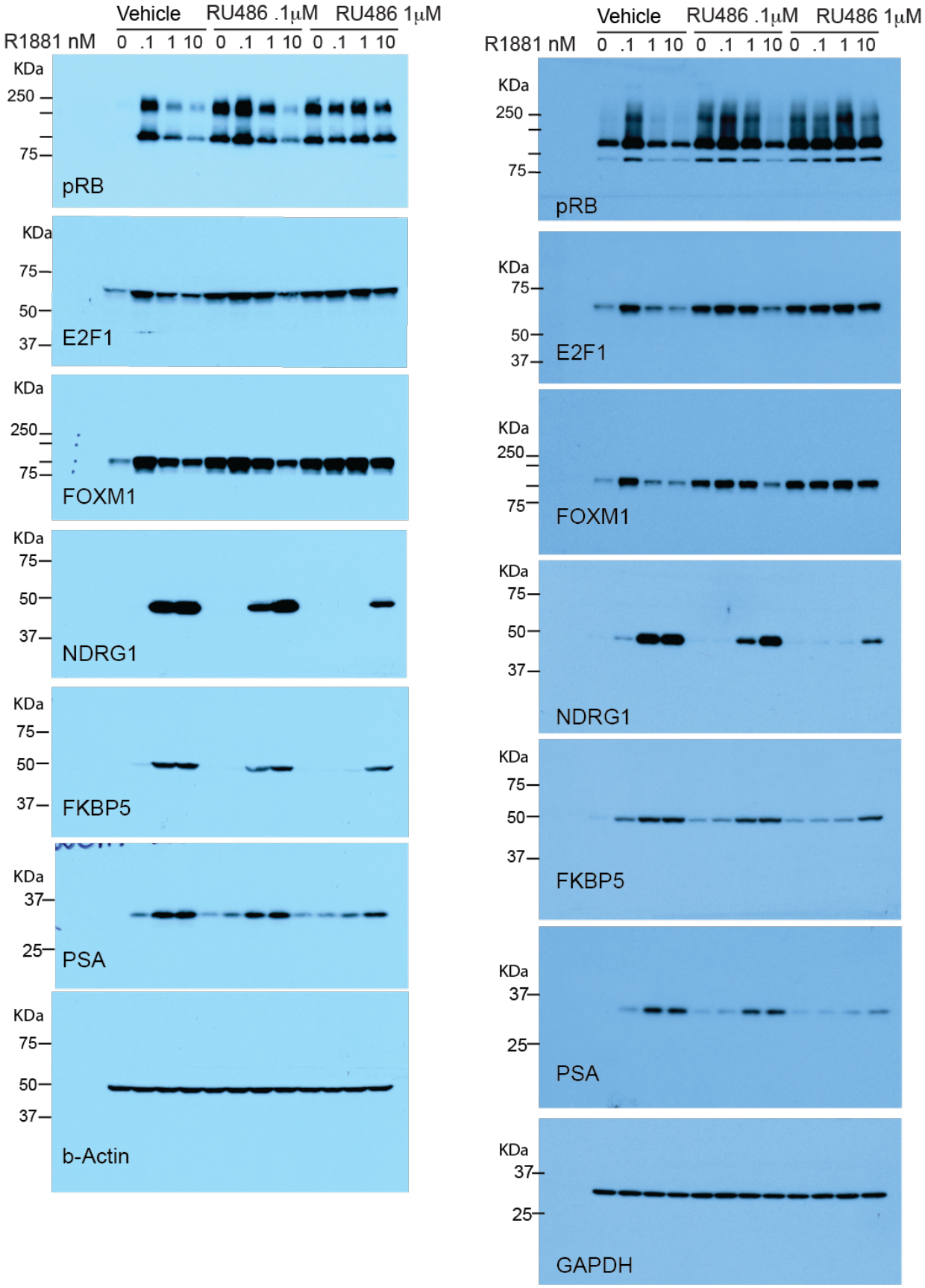


Supplementary Fig. 6e

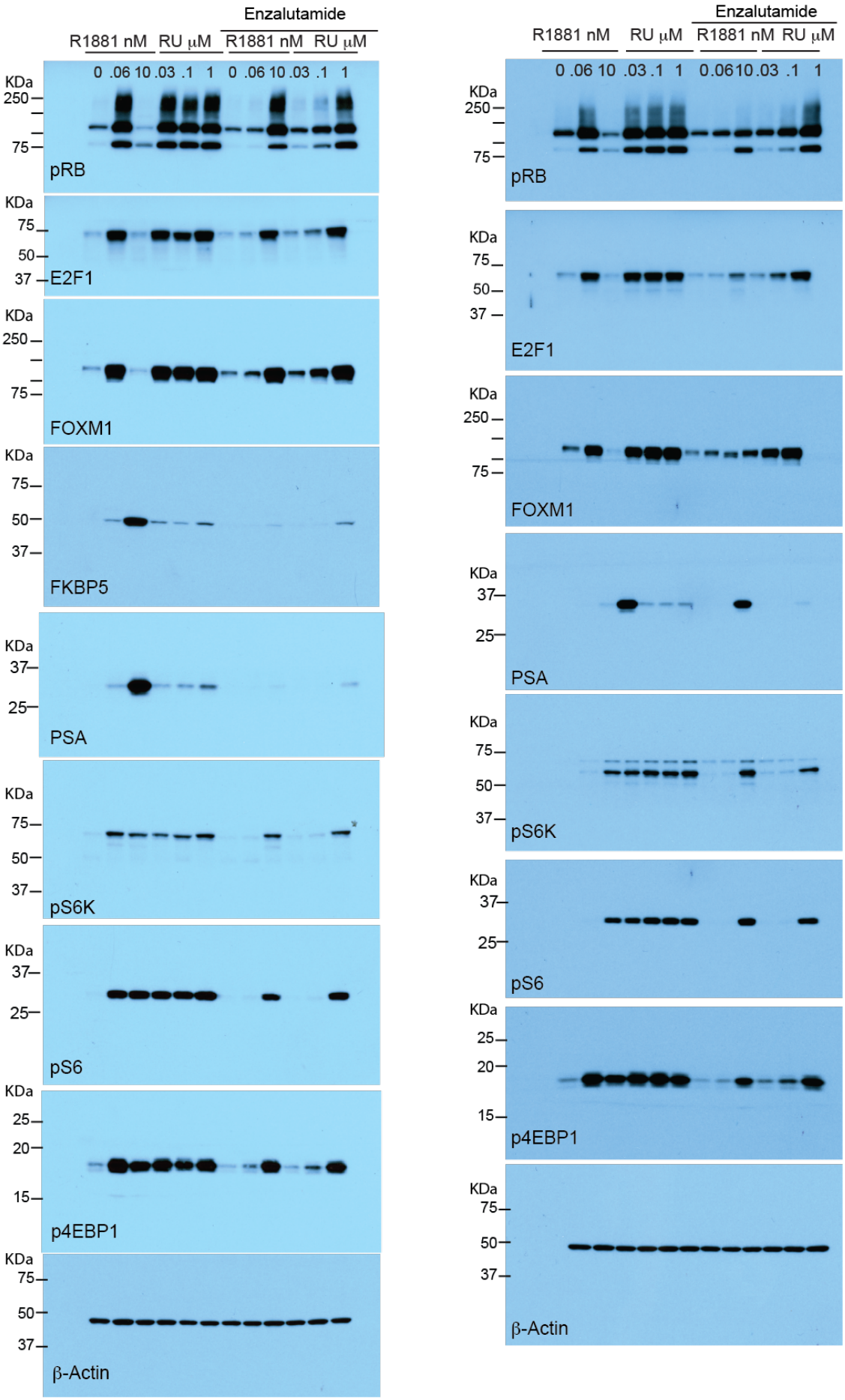




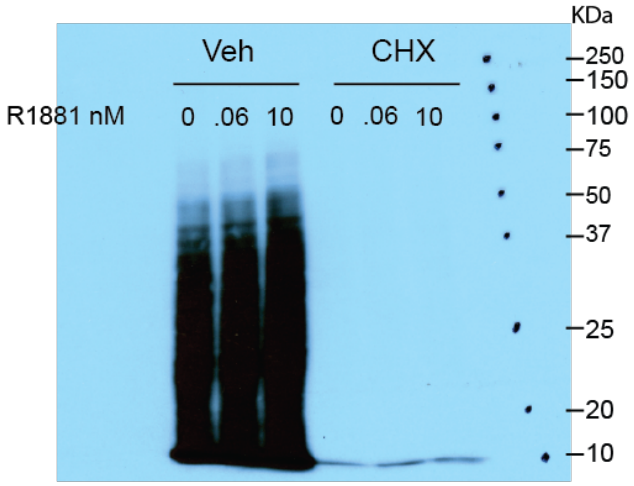
Supplementary Fig. 6f

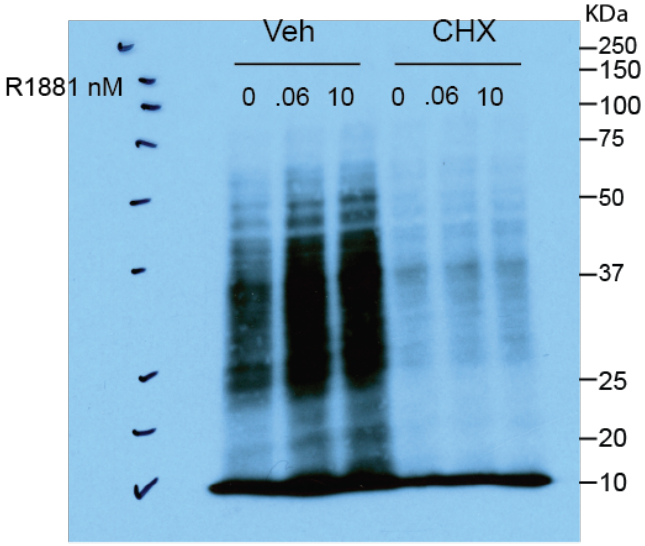


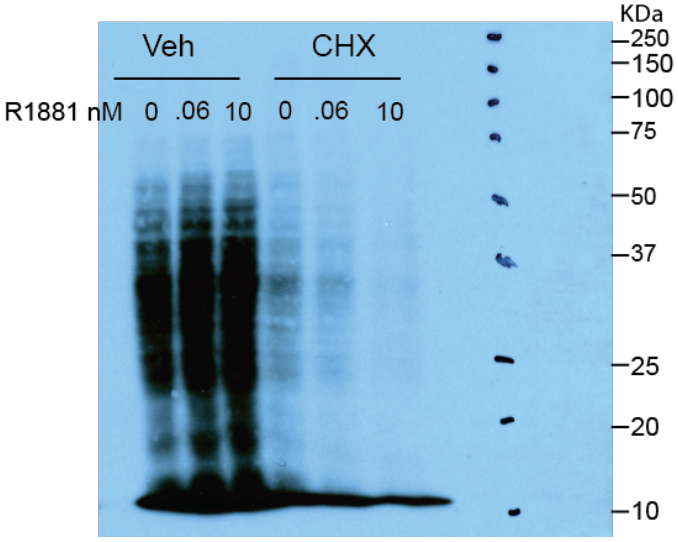
Supplementary Fig. 7a



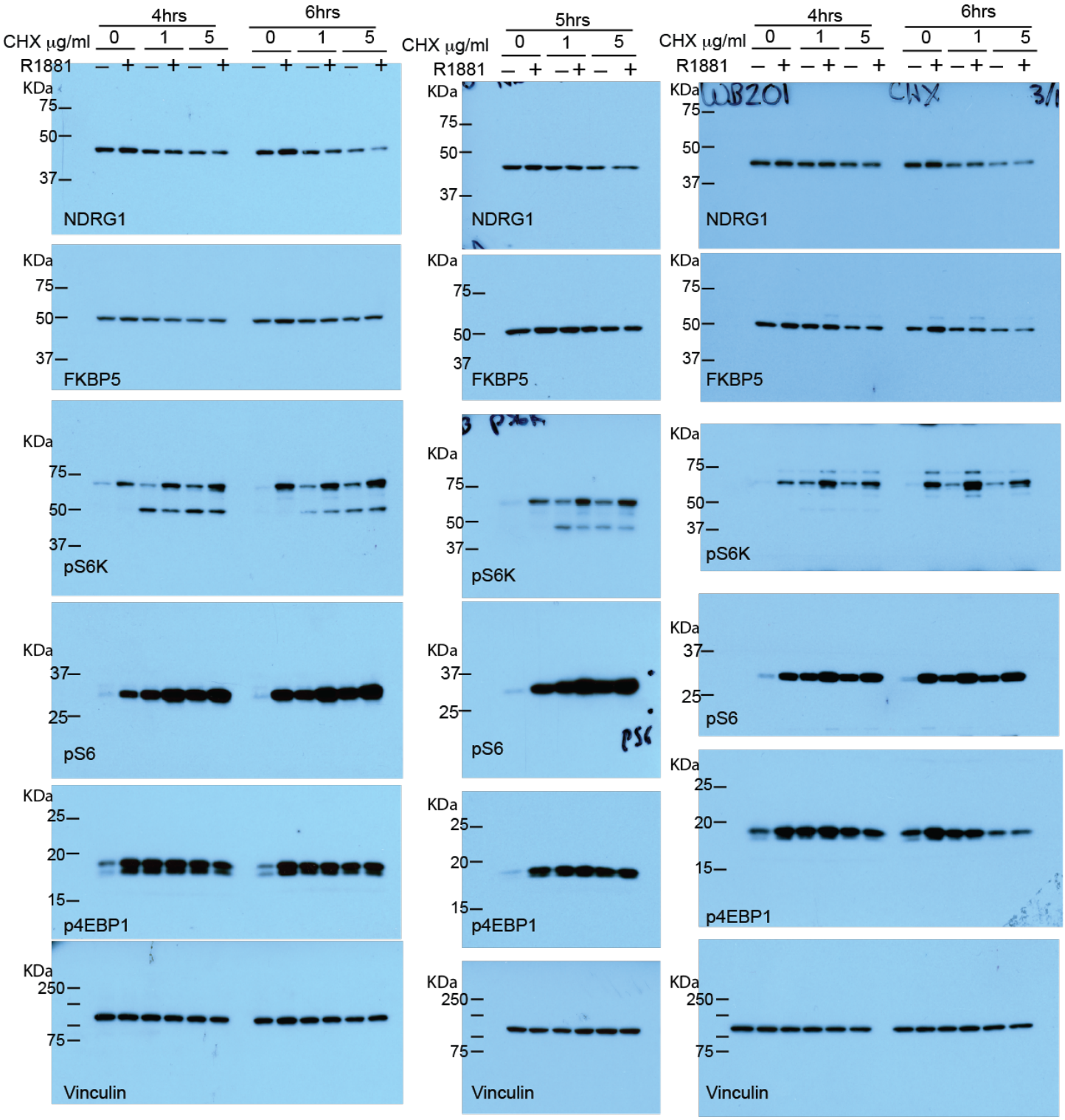
Supplementary Fig. 7b



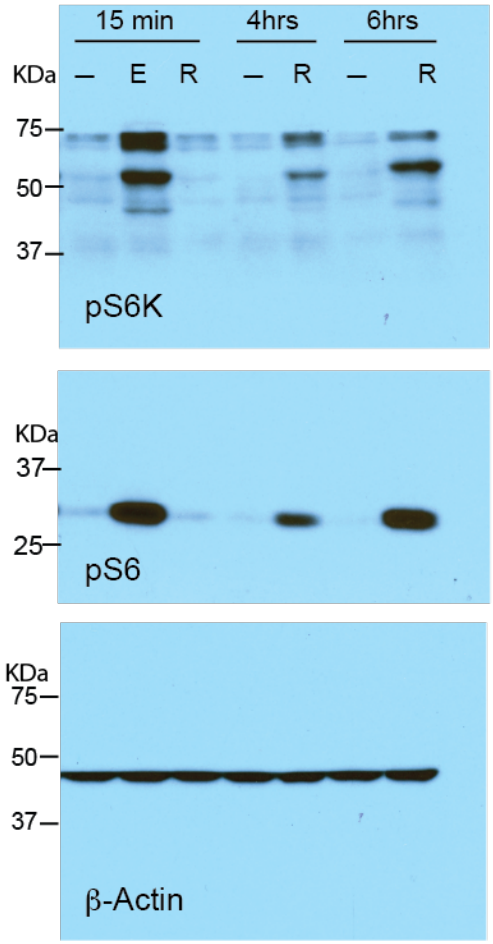


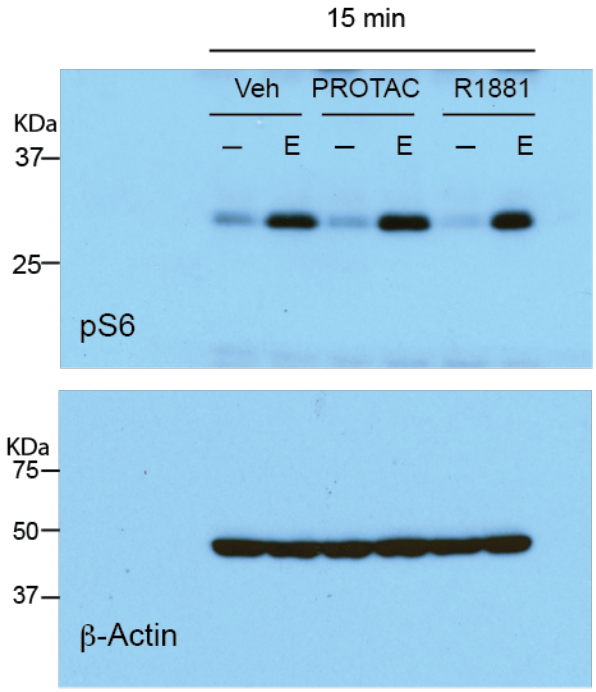


Supplementary Fig. 7c



Supplementary Fig. 7e





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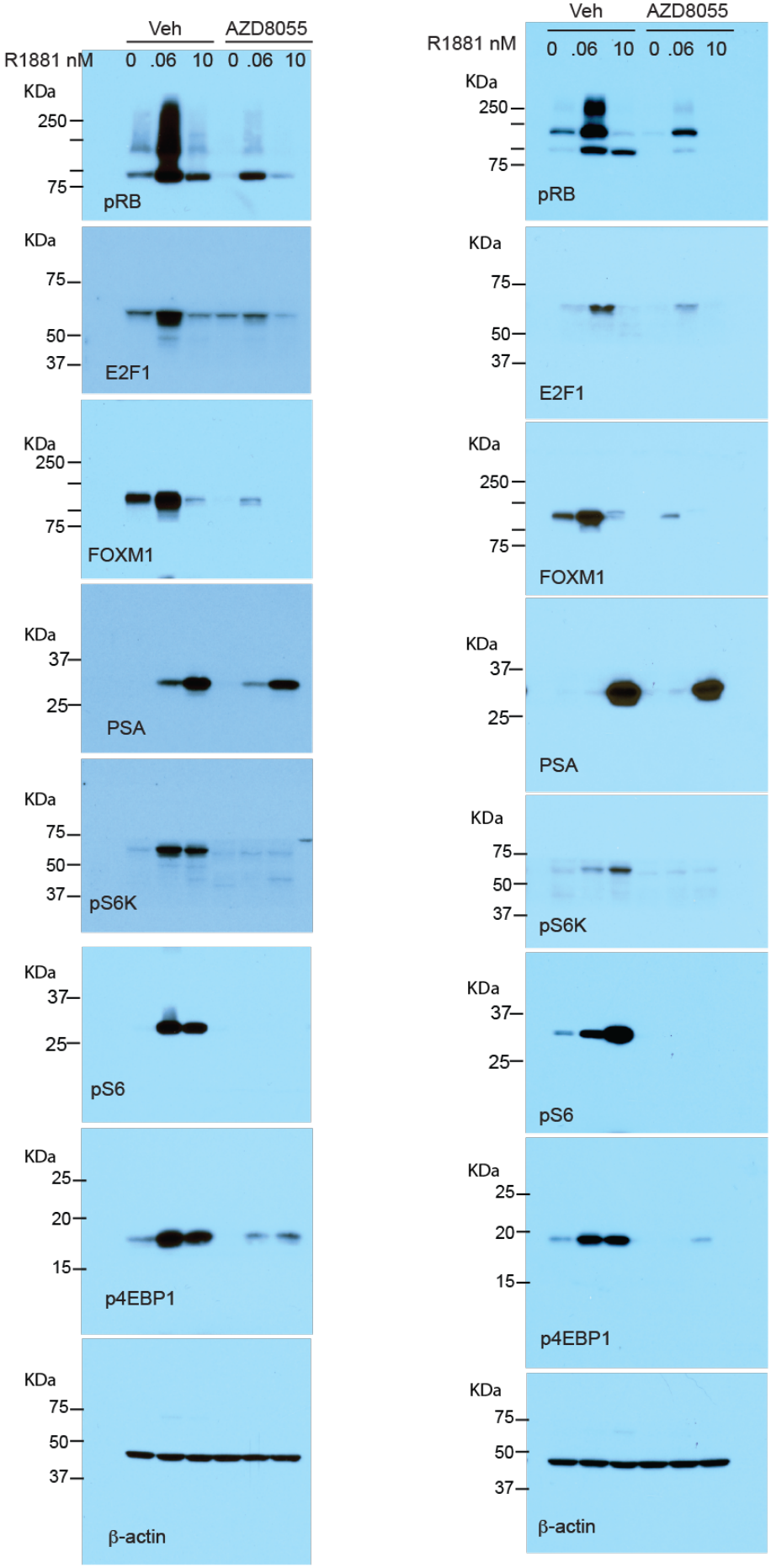
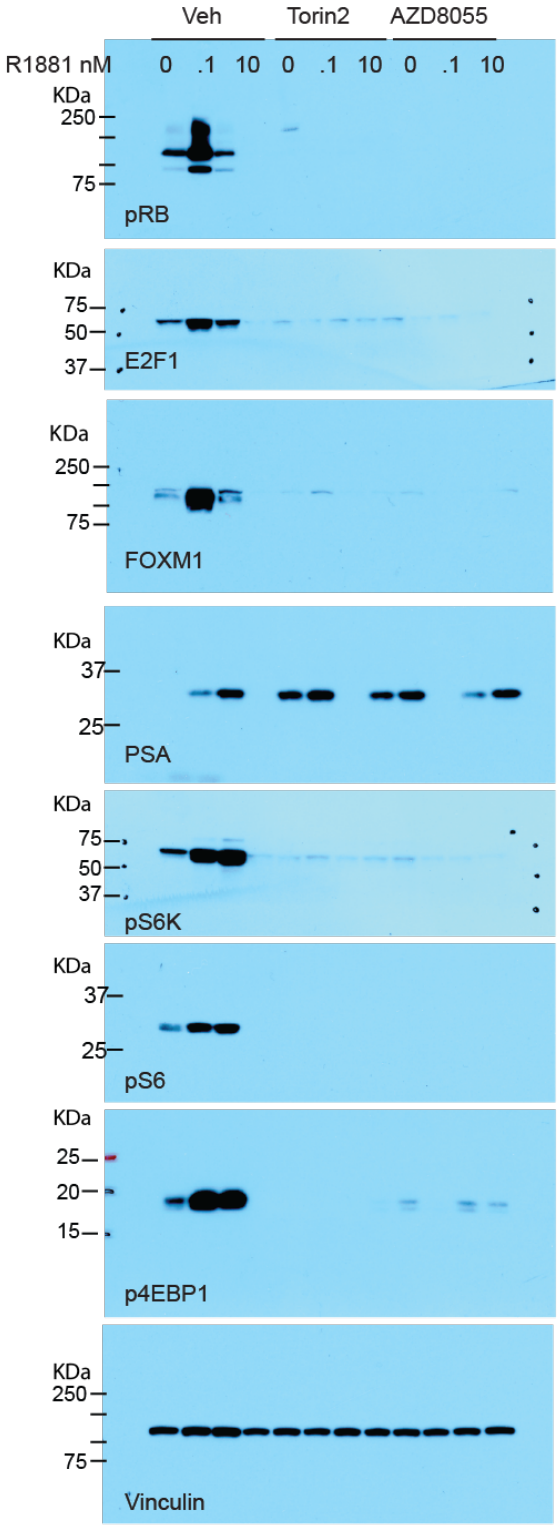
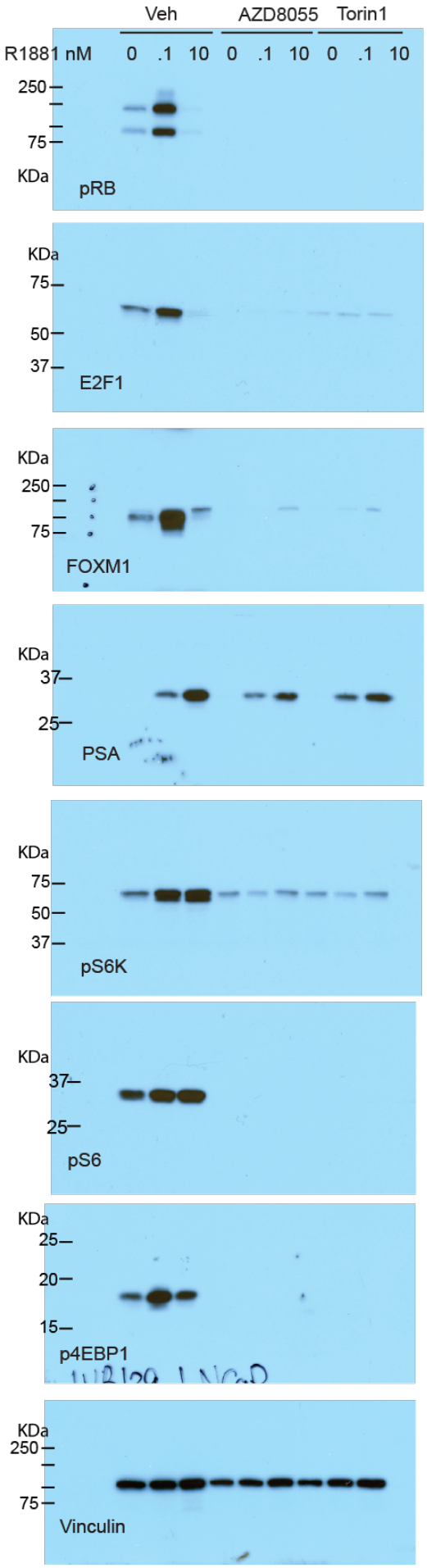


Supplementary Fig. 7i





Supplementary Fig. 8c