FIG. 1

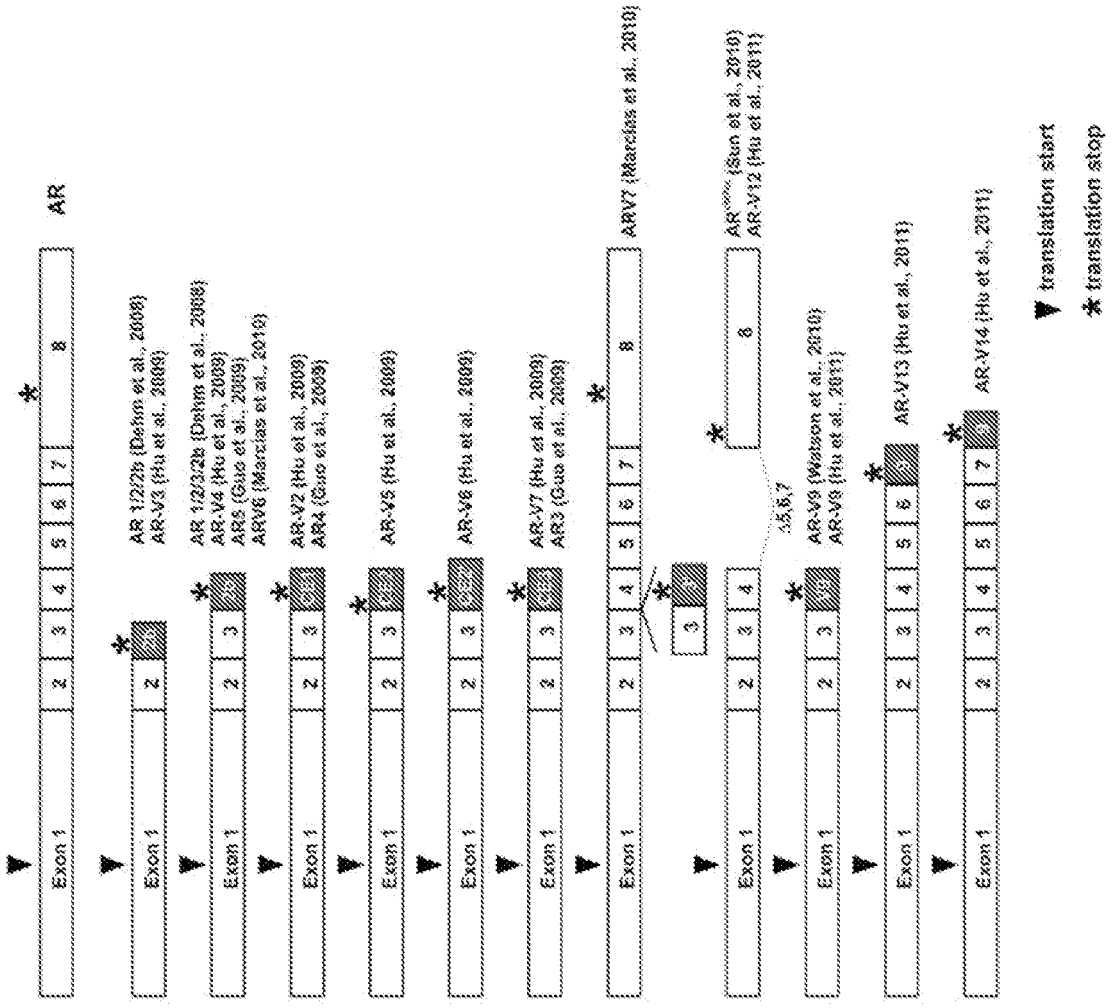

AR
AR 1/2/2b (Dehm et al., 2008)
AR-V3 (Hu et al., 2009)
AR 1/2/3/2b (Dehm et al., 2008)
AR-V4 (Hu et al., 2009)
AR3 (Guo et al., 2009)
ARV6 (Marcias et al., 2010)
AR-V2 (Hu et al., 2009)
AR4 (Guo et al., 2009)
AR-V5 (Hu et al., 2009)
AR-V6 (Hu et al., 2009)
AR-V7 (Hu et al., 2009)
AR3 (Guo et al., 2009)
ARV7 (Marcias et al., 2010)
AR (Sun et al., 2010)
AR-V12 (Hu et al., 2011)
AR-V9 (Watson et al., 2010)
AR-V9 (Hu et al., 2011)
AR-V13 (Hu et al., 2011)
AR-V14 (Hu et al., 2011)
translation start
translation stop

FIG. 2

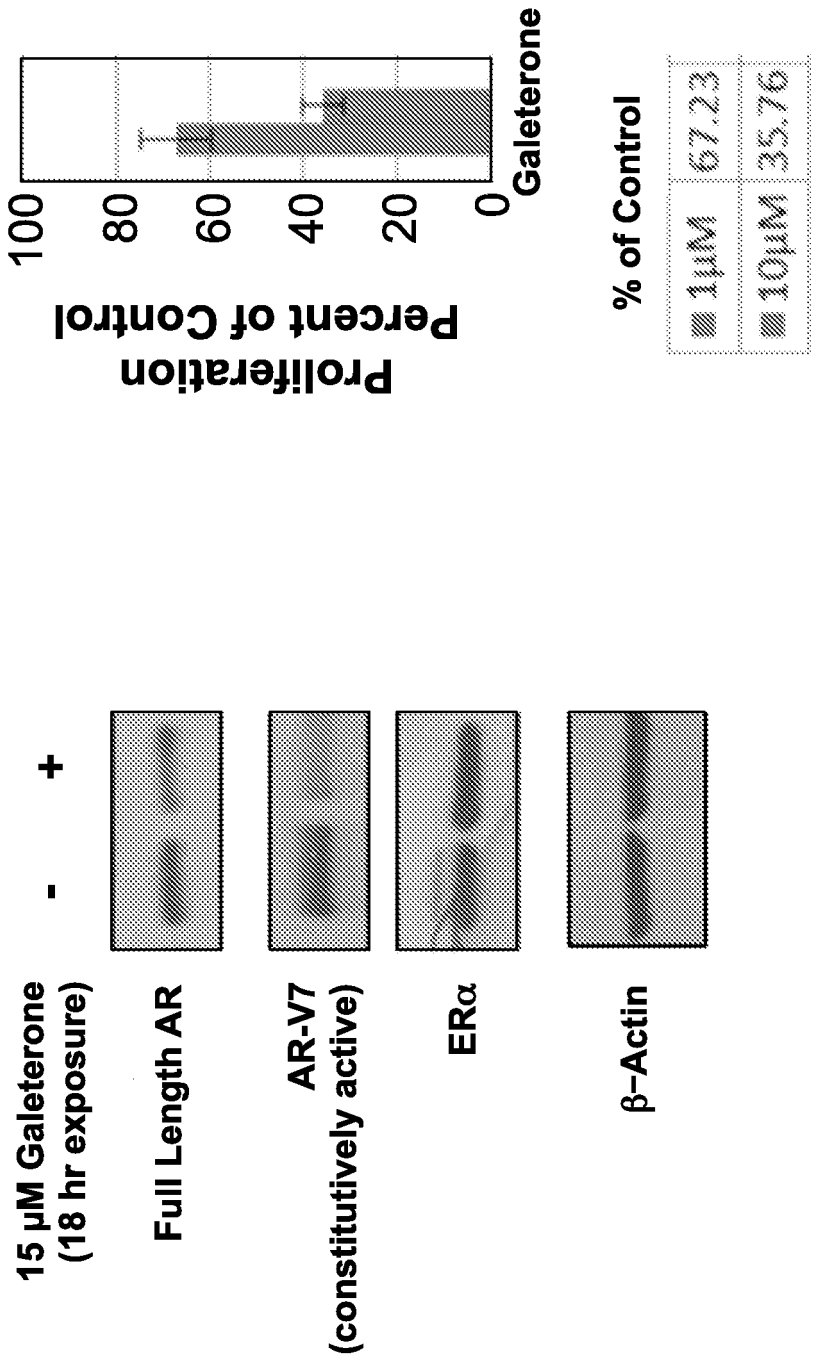
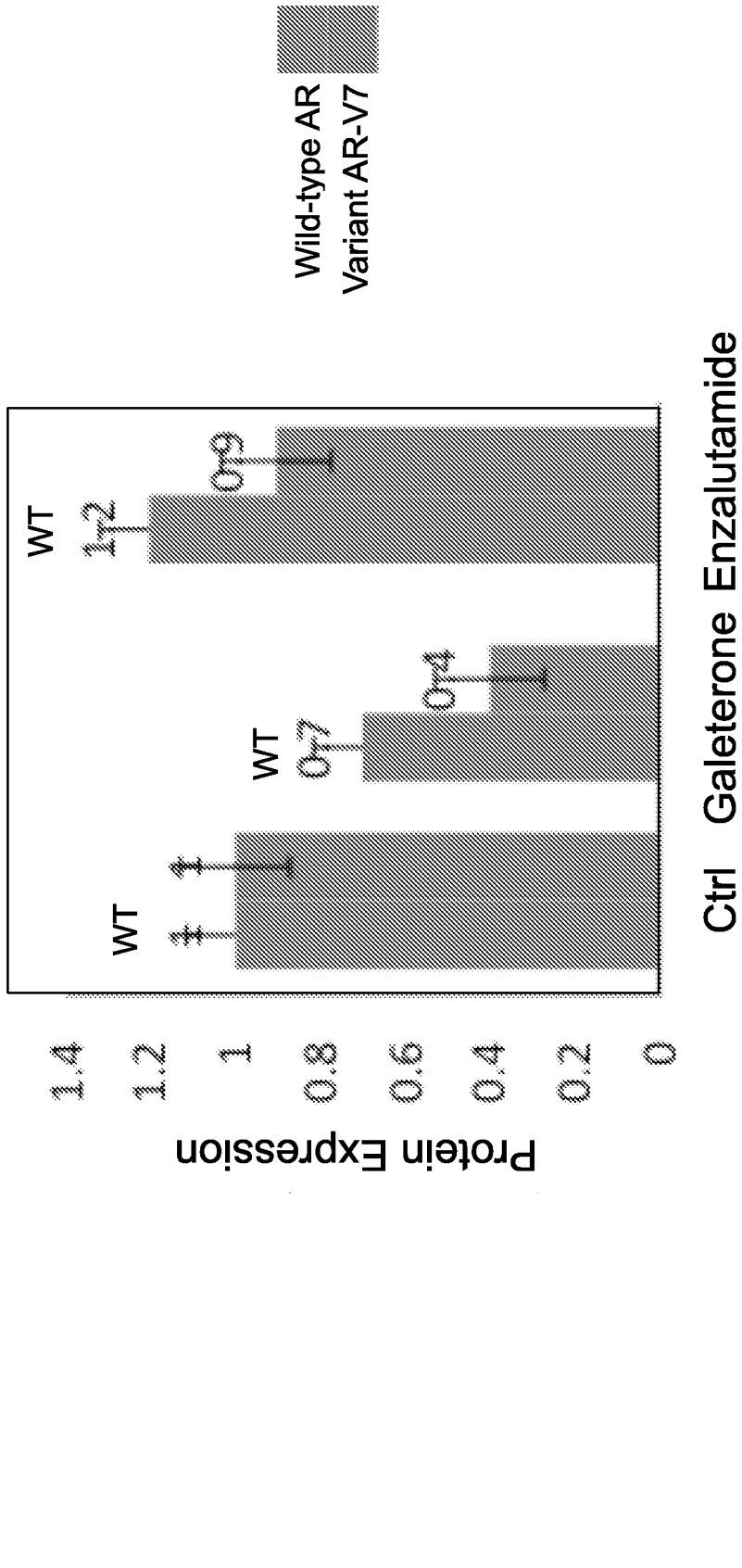

15 µM Galeterone
(18 hr exposure)
-
+
Full Length AR
AR-V7
(constitutively active)
ERα
β-Actin
Proliferation
Percent of Control
100
80
60
40
20
0
Galeterone
% of Control
1µM 67.23
10µM 35.76

FIG. 3



CWR22rv1 Cells; 15 μM; 24 hours; in triplicate

FIG. 4

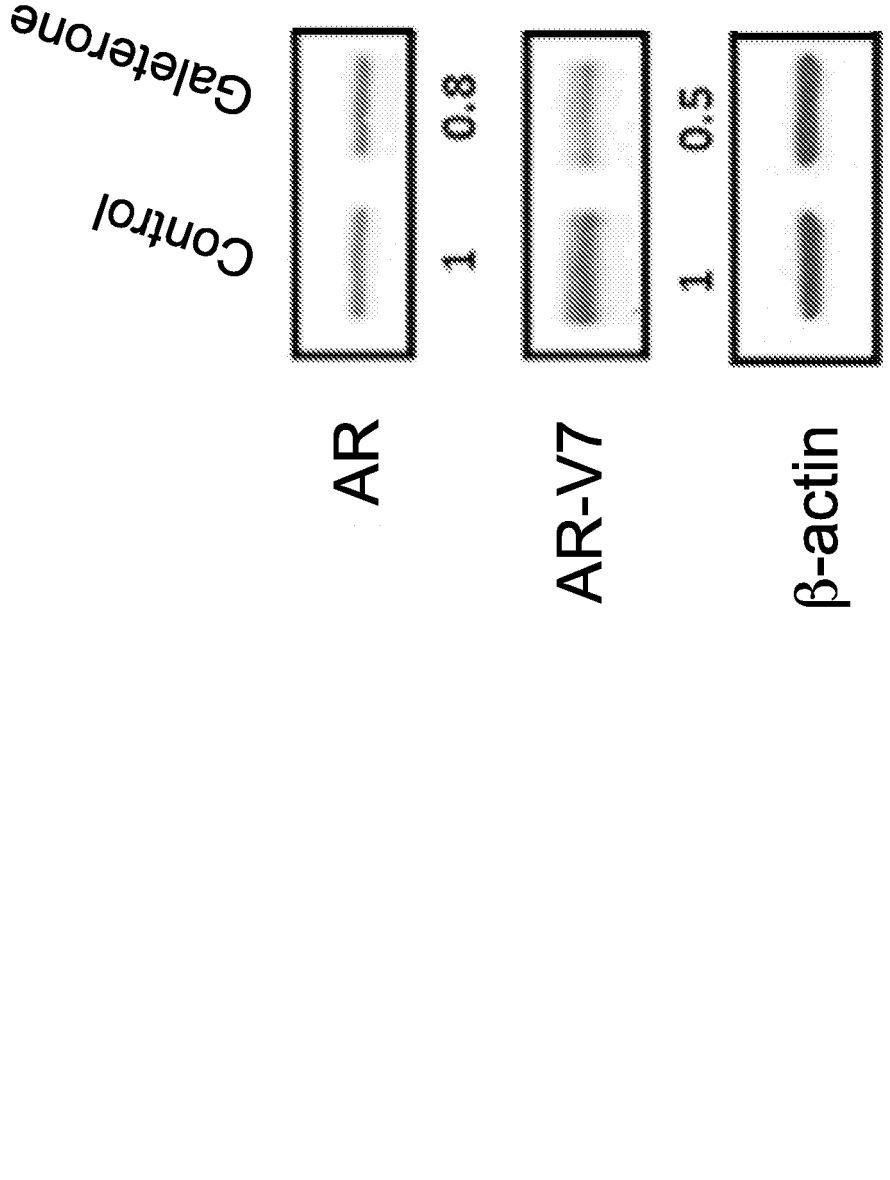

Galeterone
Control
AR
1
0.8
AR-V7
1
0.5
β-actin
CWR22rv1 Cells; 15 μM; 24 hours

FIG. 5

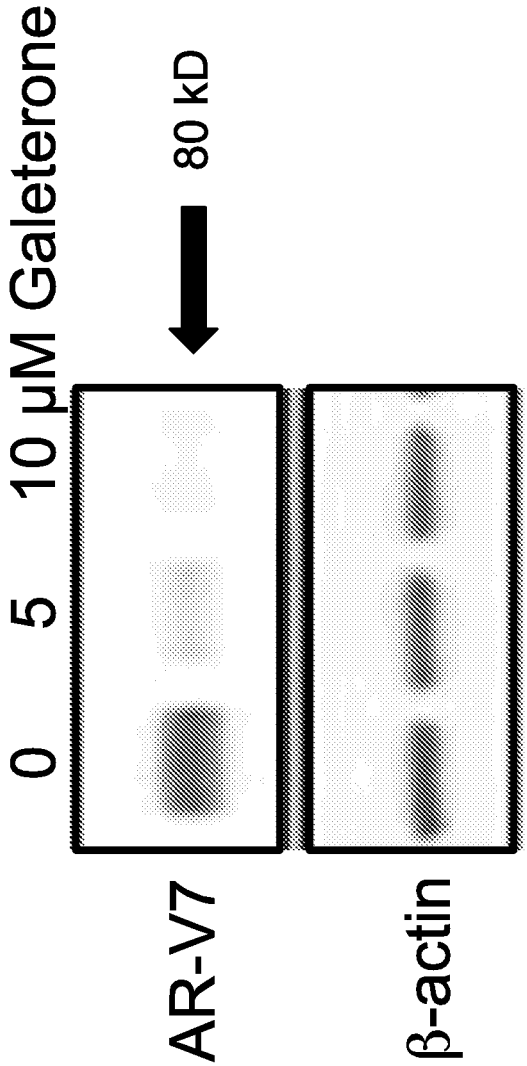


FIG. 6

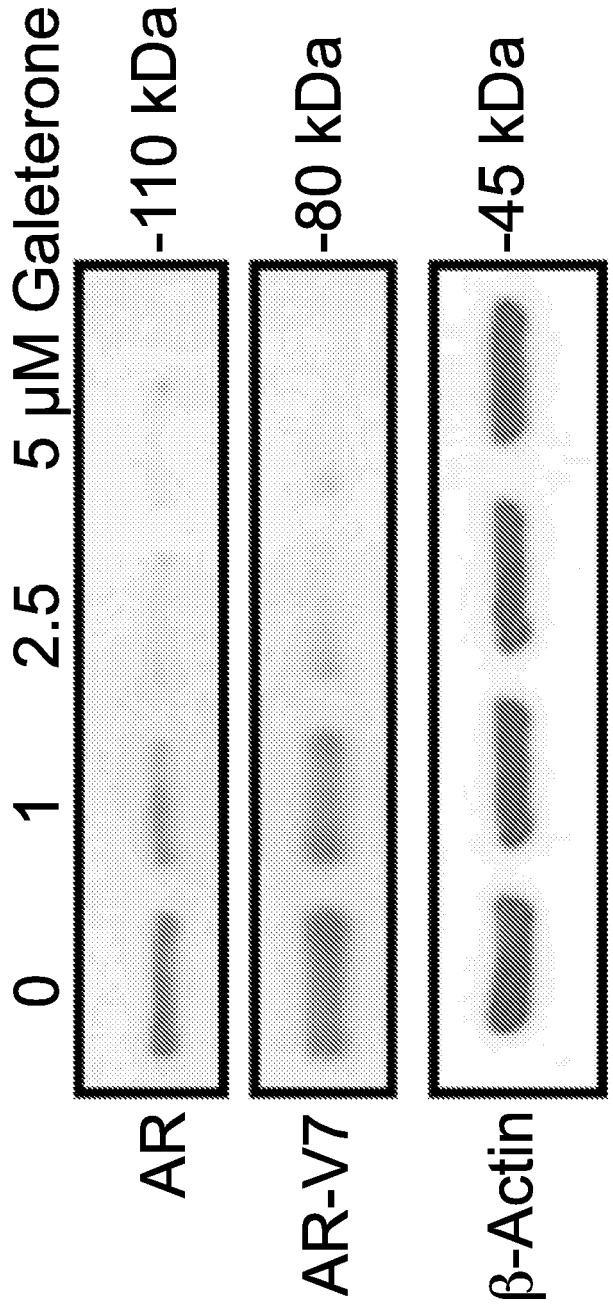


FIG. 7

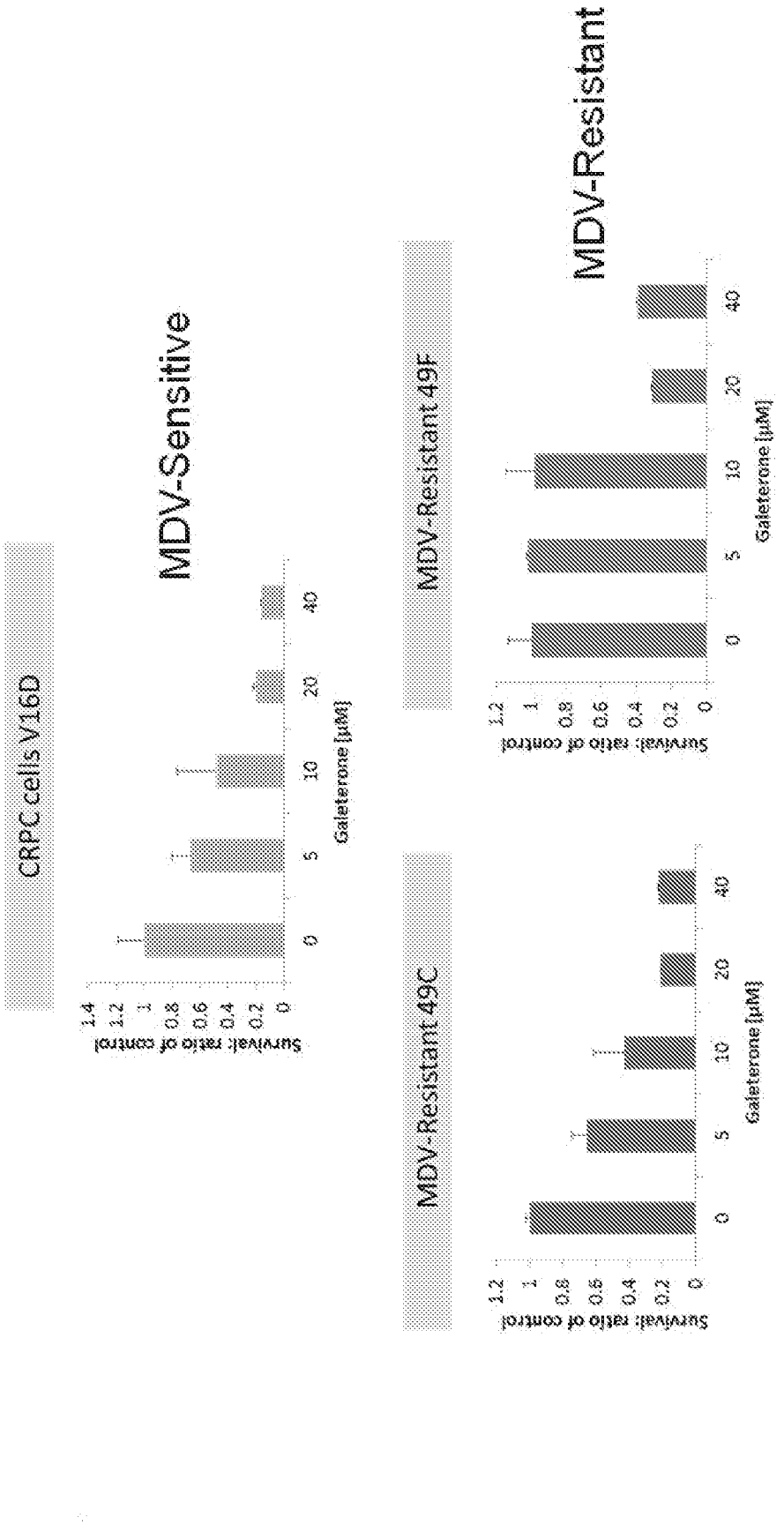

CRPC cells V16D
MDV-Sensitive
MDV-Resistant 49C
MDV-Resistant 49F
MDV-Resistant
Survival: ratio of control
Galeterone [μM]
2 DAY Exposure CSS /No DHT

FIG. 8

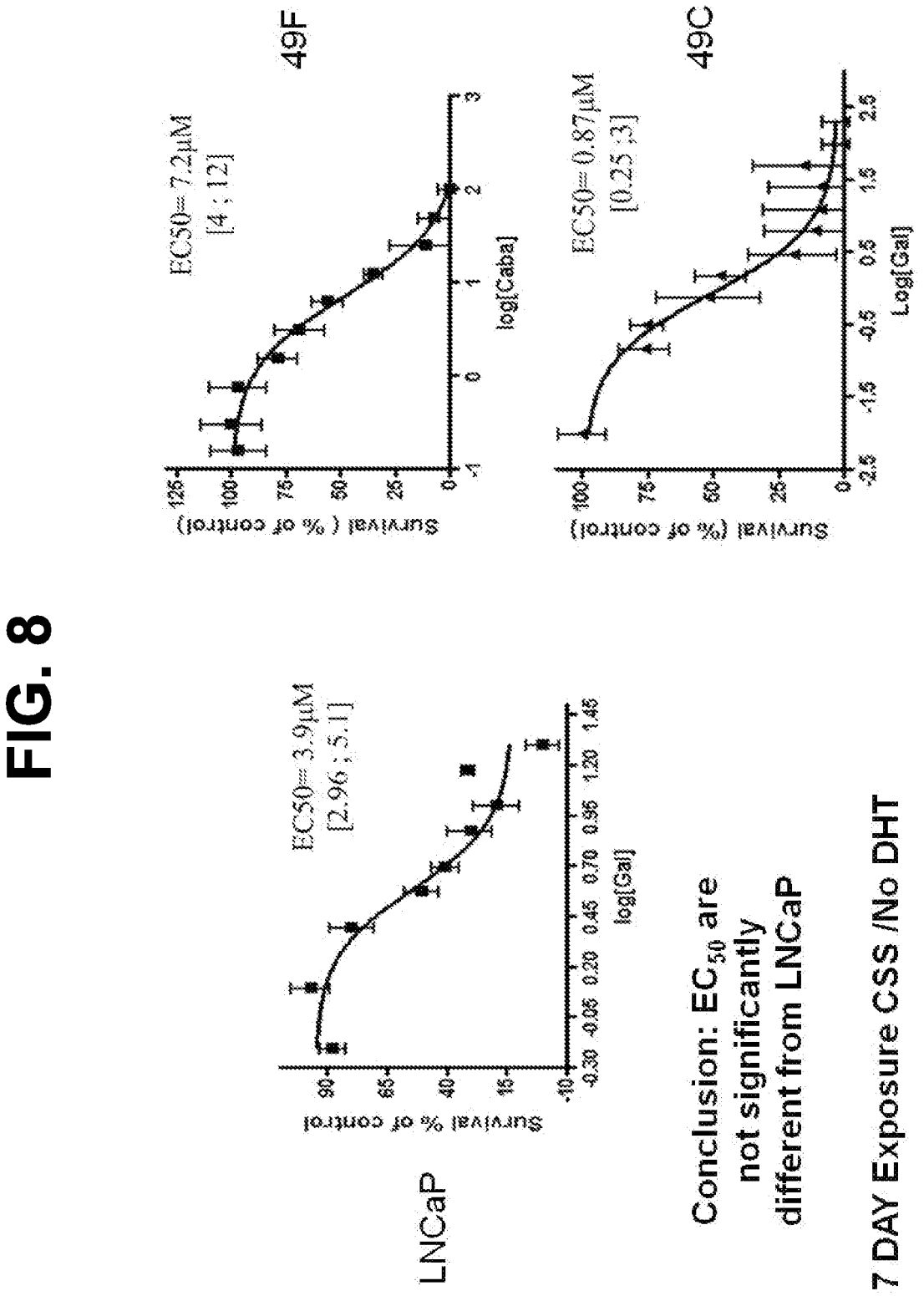

49F
EC50= 7.2µM
[4 ; 12]
Survival (% of control)
log[Caba]
49C
EC50= 0.87µM
[0.25 ;3]
Survival (% of control)
Log[Gal]
LNCaP
EC50= 3.9µM
[2.96 ; 5.1]
Survival % of control
log[Gal]
Conclusion: EC50 are not significantly different from LNCaP
7 DAY Exposure CSS /No DHT

FIG. 9A

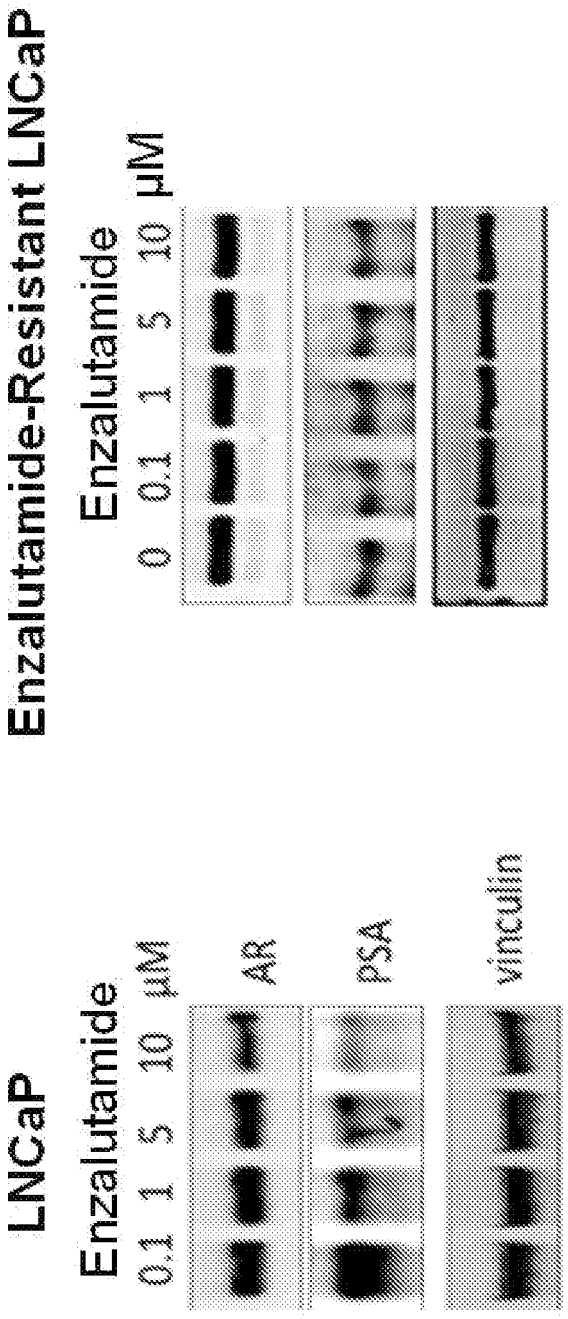

LNCaP
Enzalutamide
0.1 1 5 10 μM
AR
PSA
vinculin
Enzalutamide-Resistant LNCaP
Enzalutamide
0 0.1 1 5 10 μM

FIG. 9B

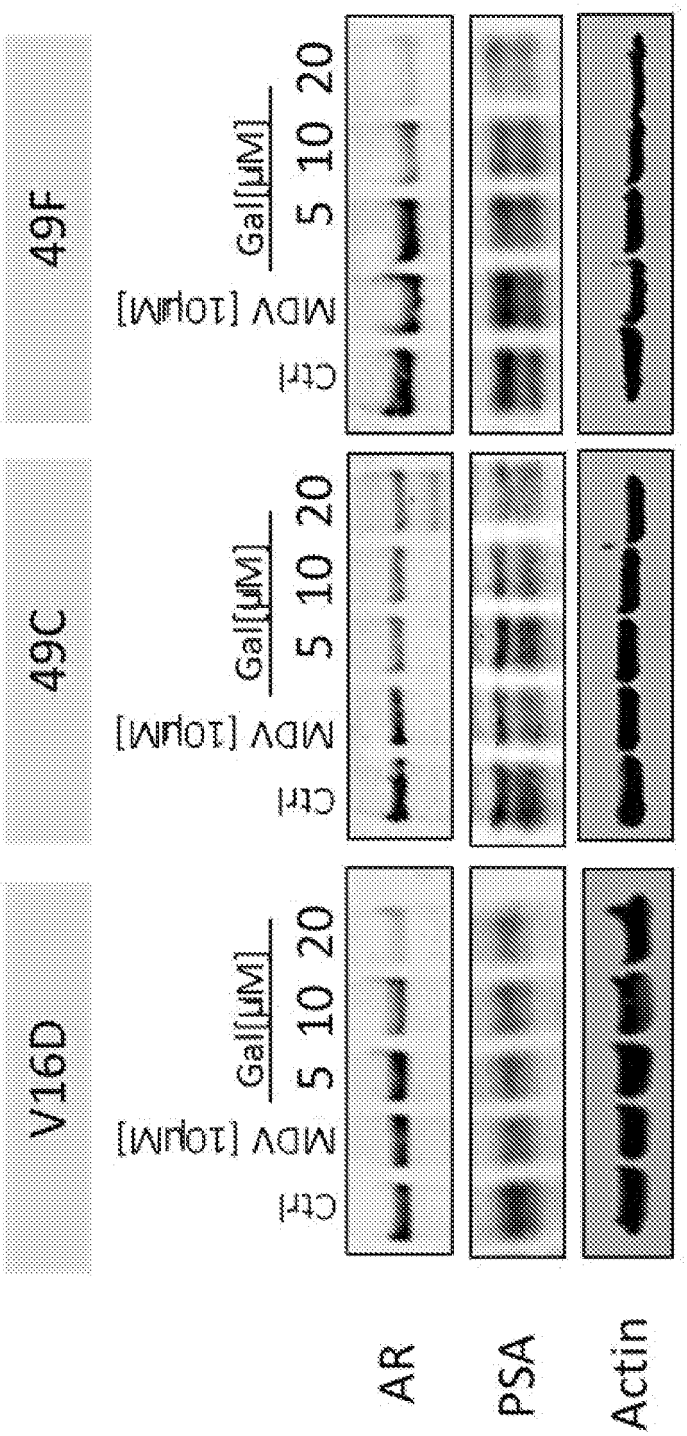


FIG. 10

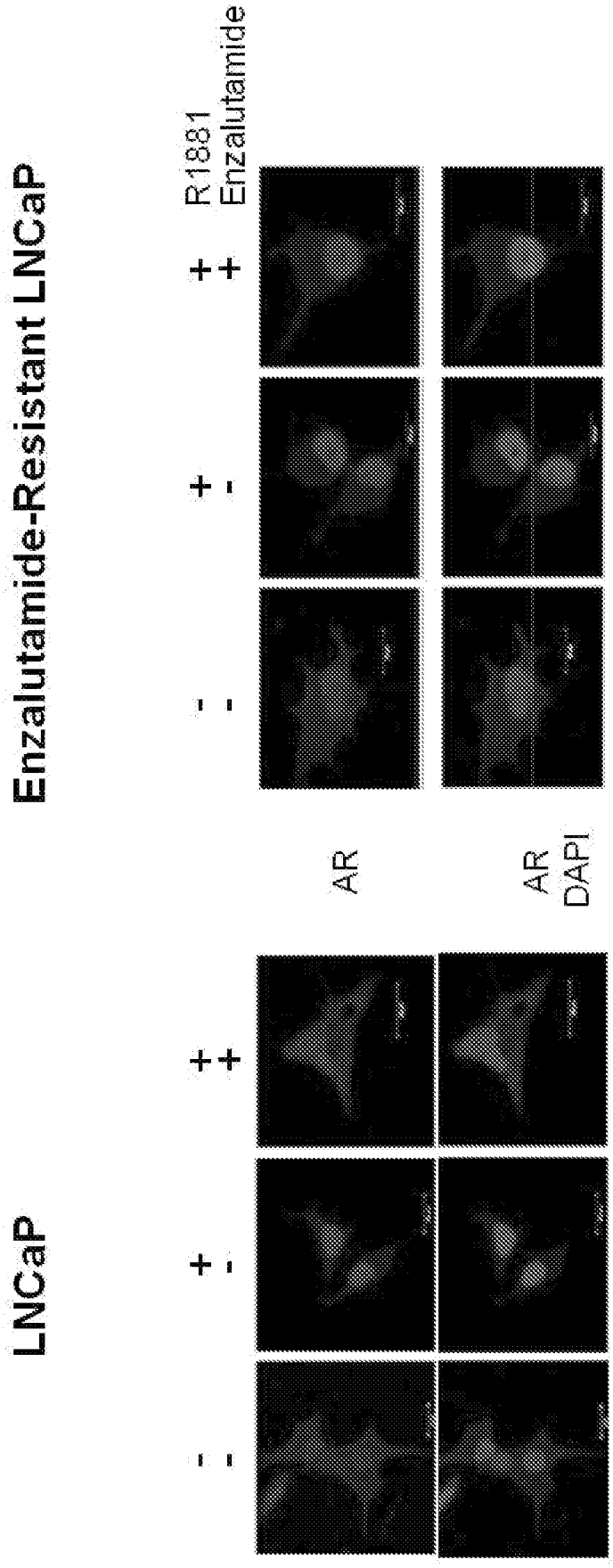

Enzalutamide-Resistant LNCaP
LNCaP
R1881
Enzalutamide
+ +
+ -
- -
AR
AR
DAPI

FIG. 11

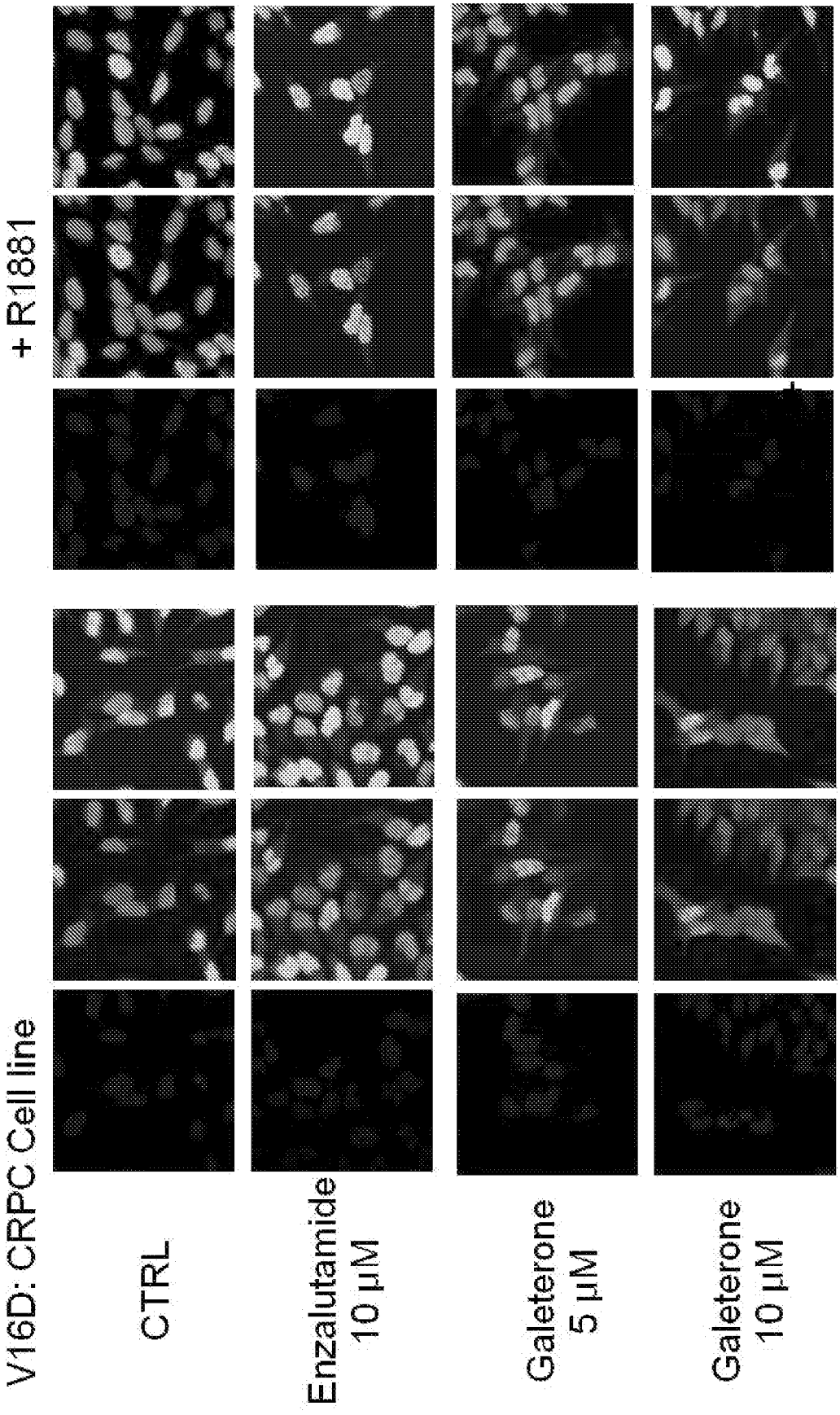

V16D: CRPC Cell line
CTRL
Enzalutamide 10 μM
Galeterone 5 μM
Galeterone 10 μM
+ R1881

FIG. 12

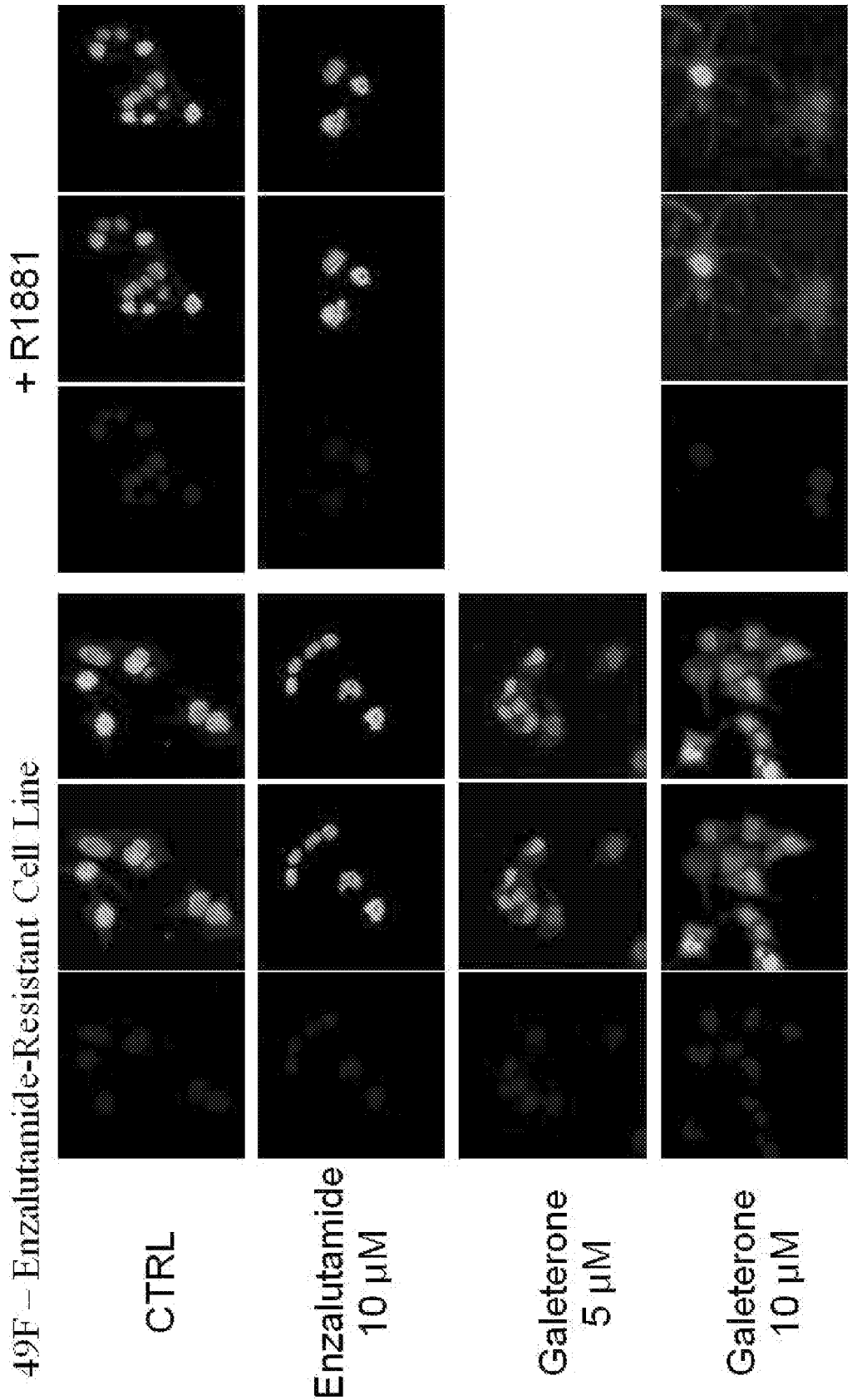

49F – Enzalutamide-Resistant Cell Line
+ R1881
CTRL
Enzalutamide 10 µM
Galeterone 5 µM
Galeterone 10 µM

FIG. 13

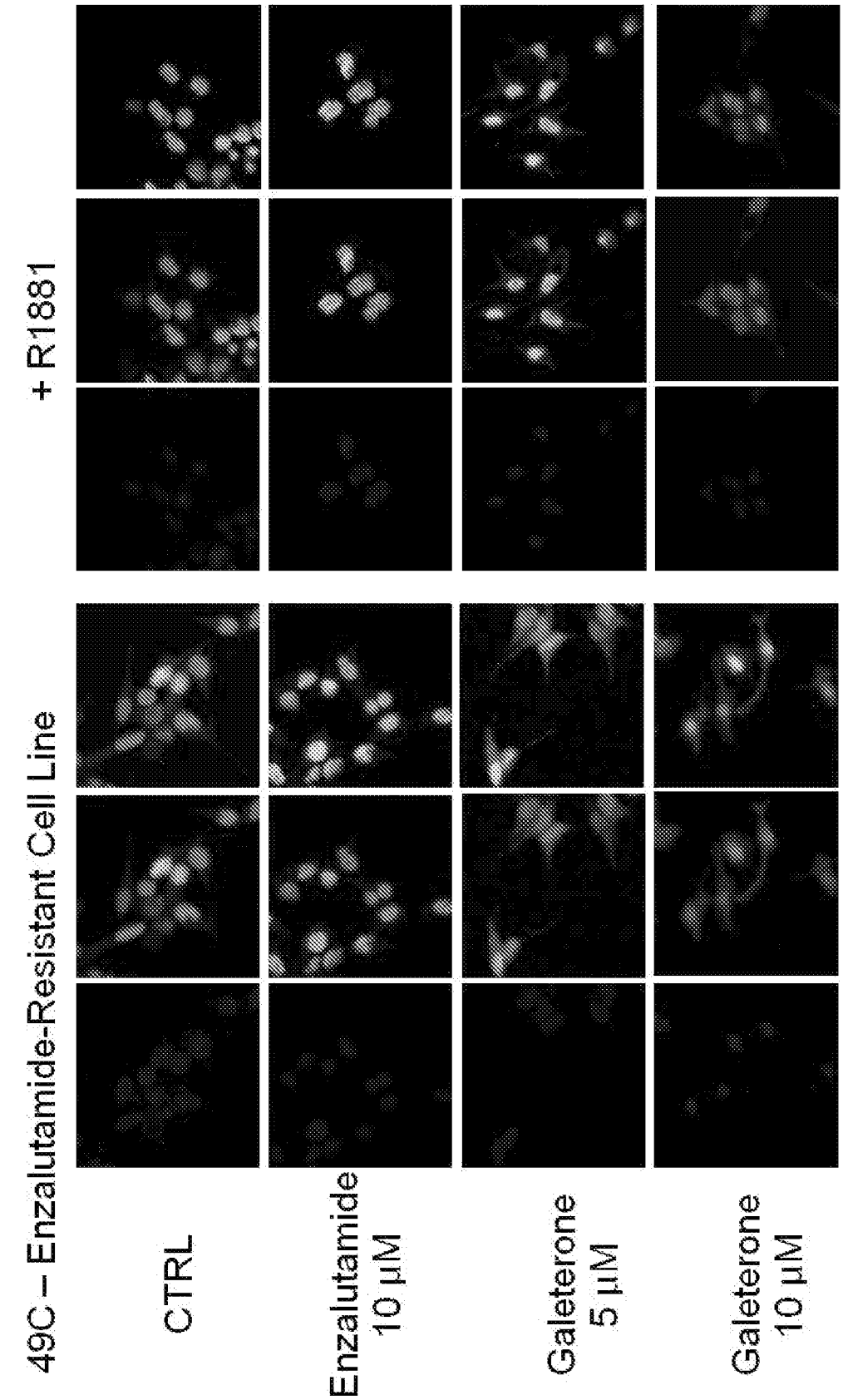

49C – Enzalutamide-Resistant Cell Line
+ R1881
CTRL
Enzalutamide 10 μM
Galeterone 5 μM
Galeterone 10 μM

FIG. 14

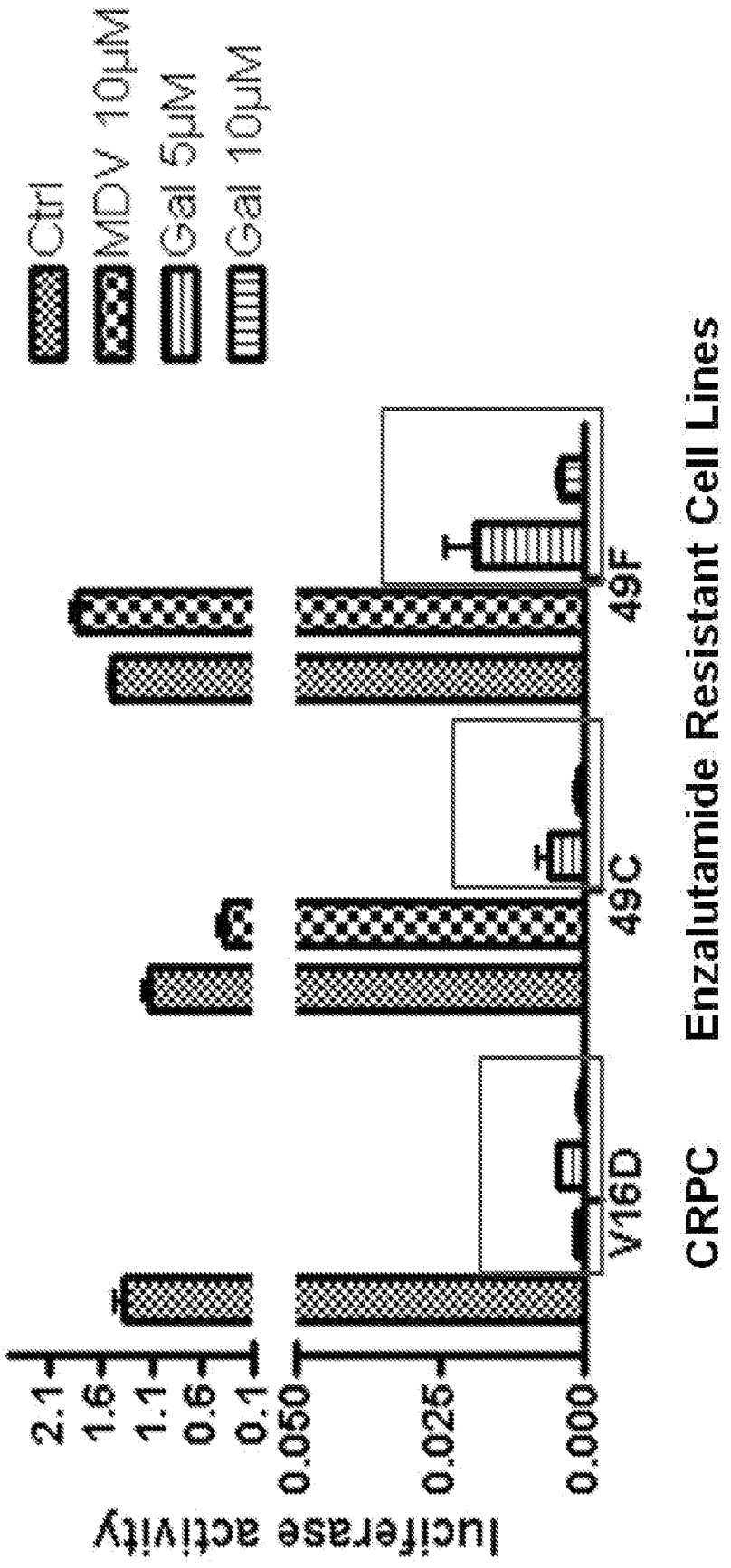

Ctrl
MDV 10μM
Gal 5μM
Gal 10μM
luciferase activity
2.1
1.6
1.1
0.6
0.1
0.050
0.025
0.000
V16D
49C
49F
CRPC
Enzalutamide Resistant Cell Lines

FIG. 15A

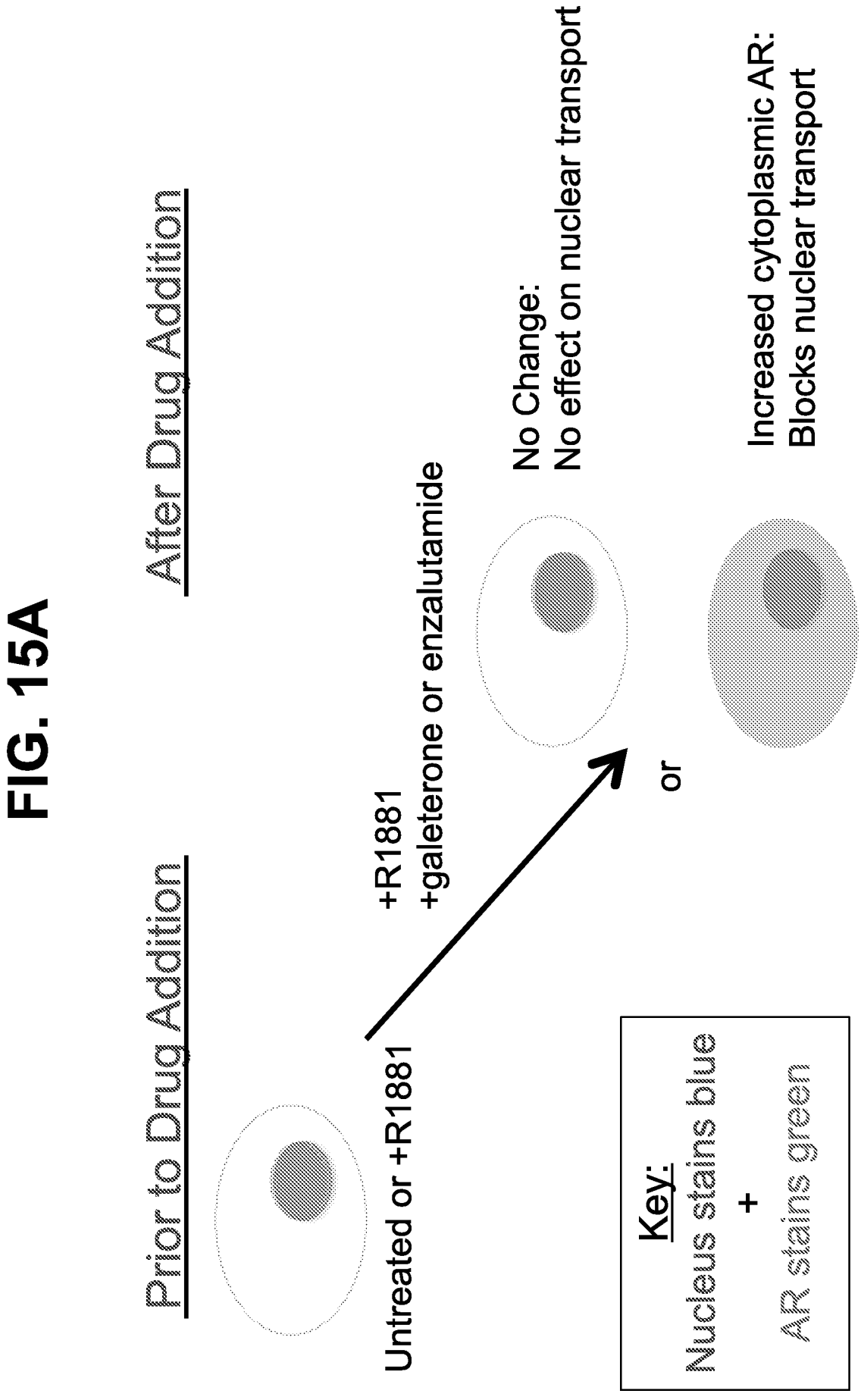

Prior to Drug Addition
After Drug Addition
Untreated or +R1881
+R1881
+galeterone or enzalutamide
No Change:
No effect on nuclear transport
or
Increased cytoplasmic AR:
Blocks nuclear transport
Key:
Nucleus stains blue
+
AR stains green

FIG. 15B

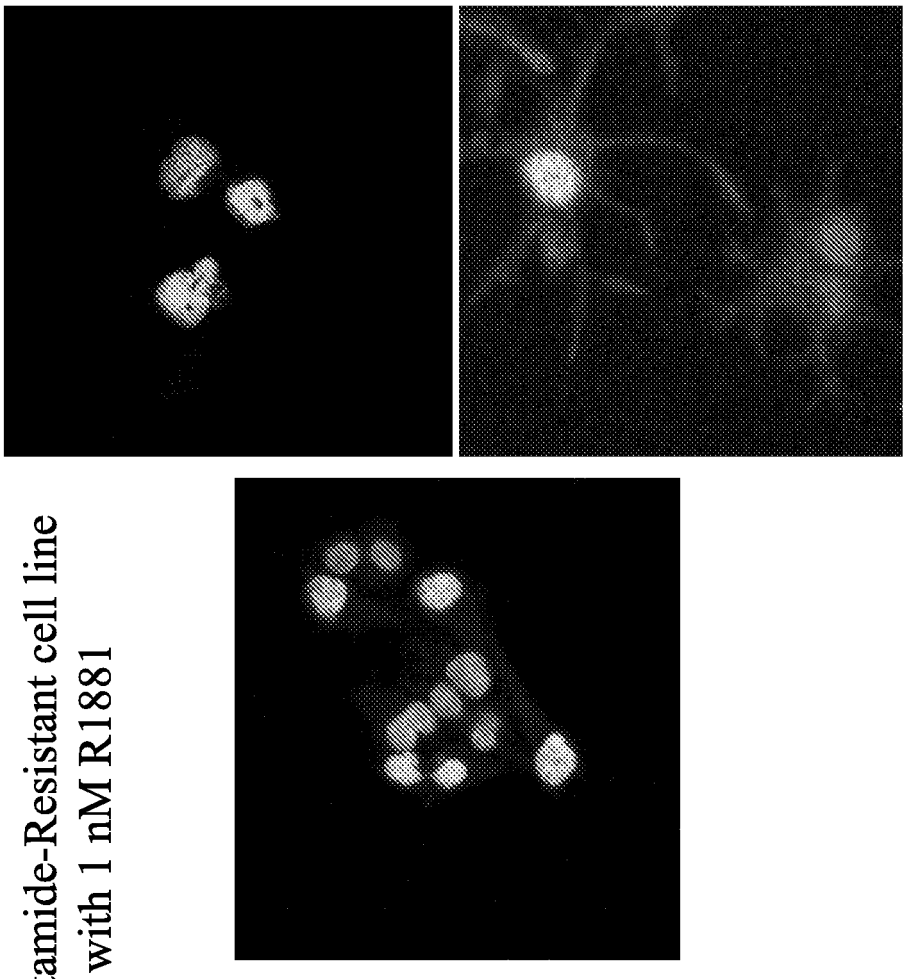

49F – Enzalutamide-Resistant cell line
treated with 1 nM R1881
Control
Enzalutamide
10 µM
Galeterone
10 µM
Nucleus
+
AR

FIG. 16

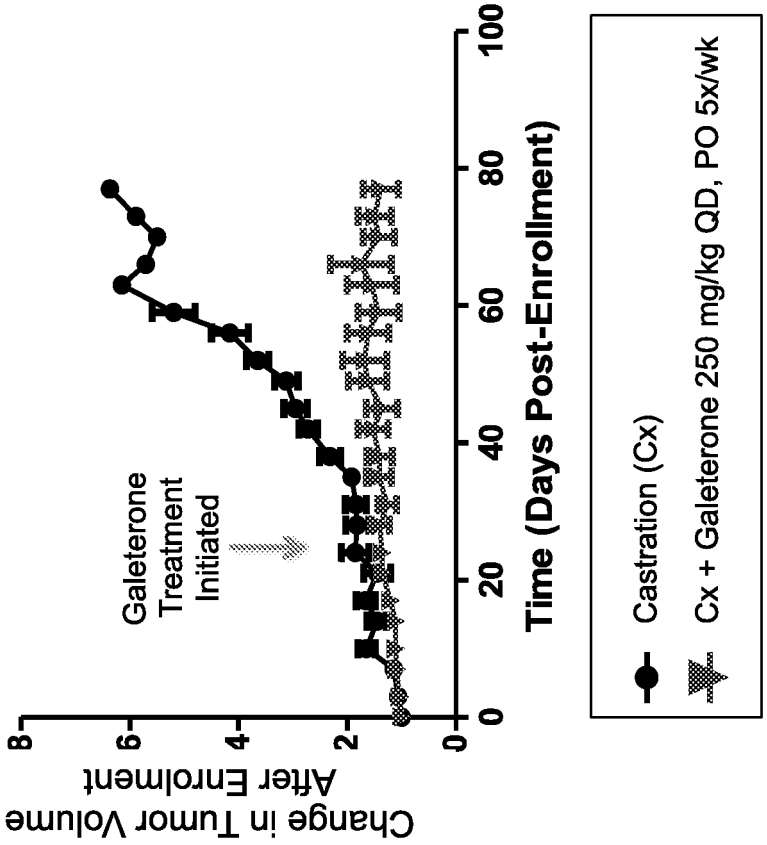

Change in Tumor Volume After Enrollment
0
2
4
6
8
0
20
40
60
80
100
Time (Days Post-Enrollment)
Galeterone Treatment Initiated
Castration (Cx)
Cx + Galeterone 250 mg/kg QD, PO 5x/wk

FIG. 17

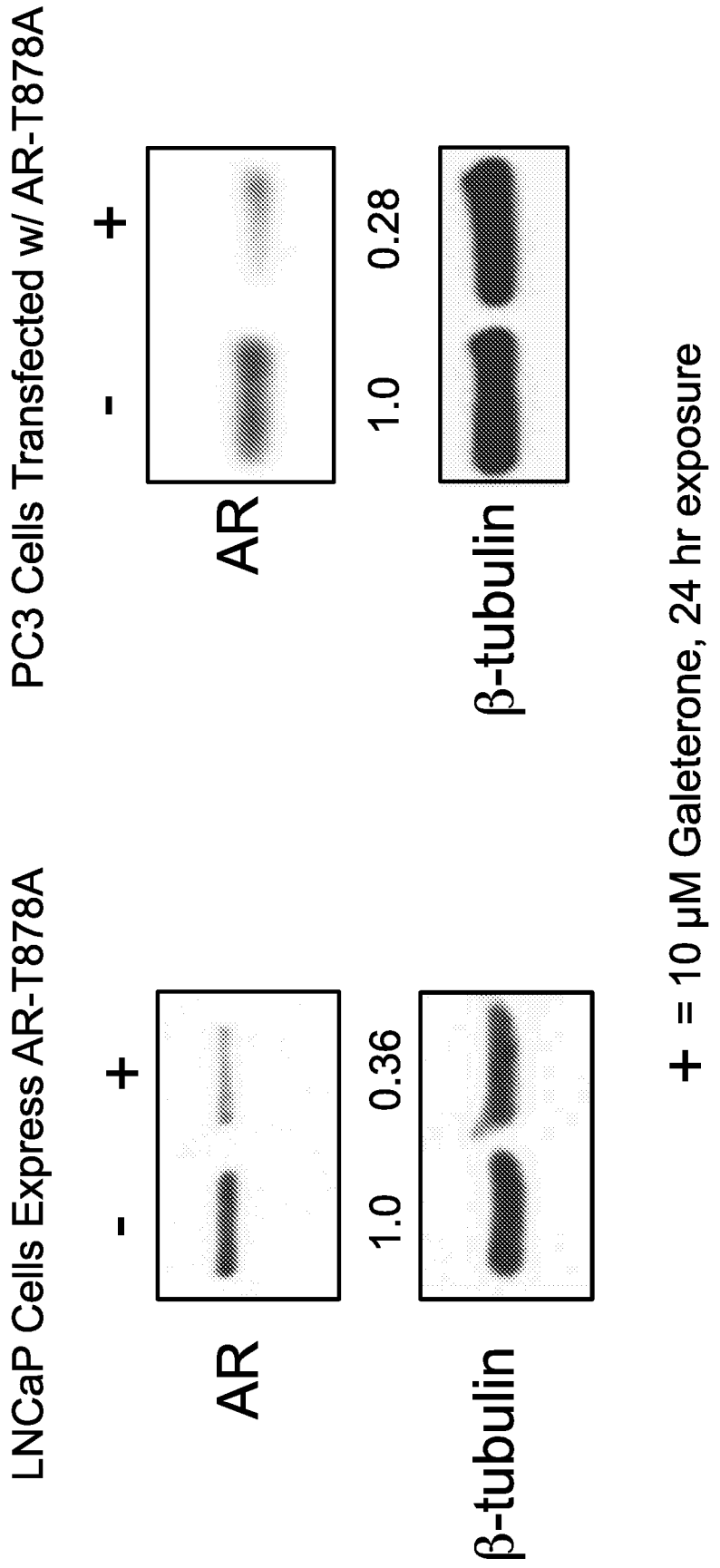

LNCaP Cells Express AR-T878A
PC3 Cells Transfected w/ AR-T878A
- +
AR
1.0 0.36
β-tubulin
- +
AR
1.0 0.28
β-tubulin
+ = 10 μM Galeterone, 24 hr exposure

FIG. 18



AR	IC50 (nM)
PC3-AR$_{wt}$	445
LAPC4 (AR$_{wt}$)	463
LNCaP (T878A)	576
PC3-AR-W741C	188
PC3-AR-W741L	980

FIG. 19

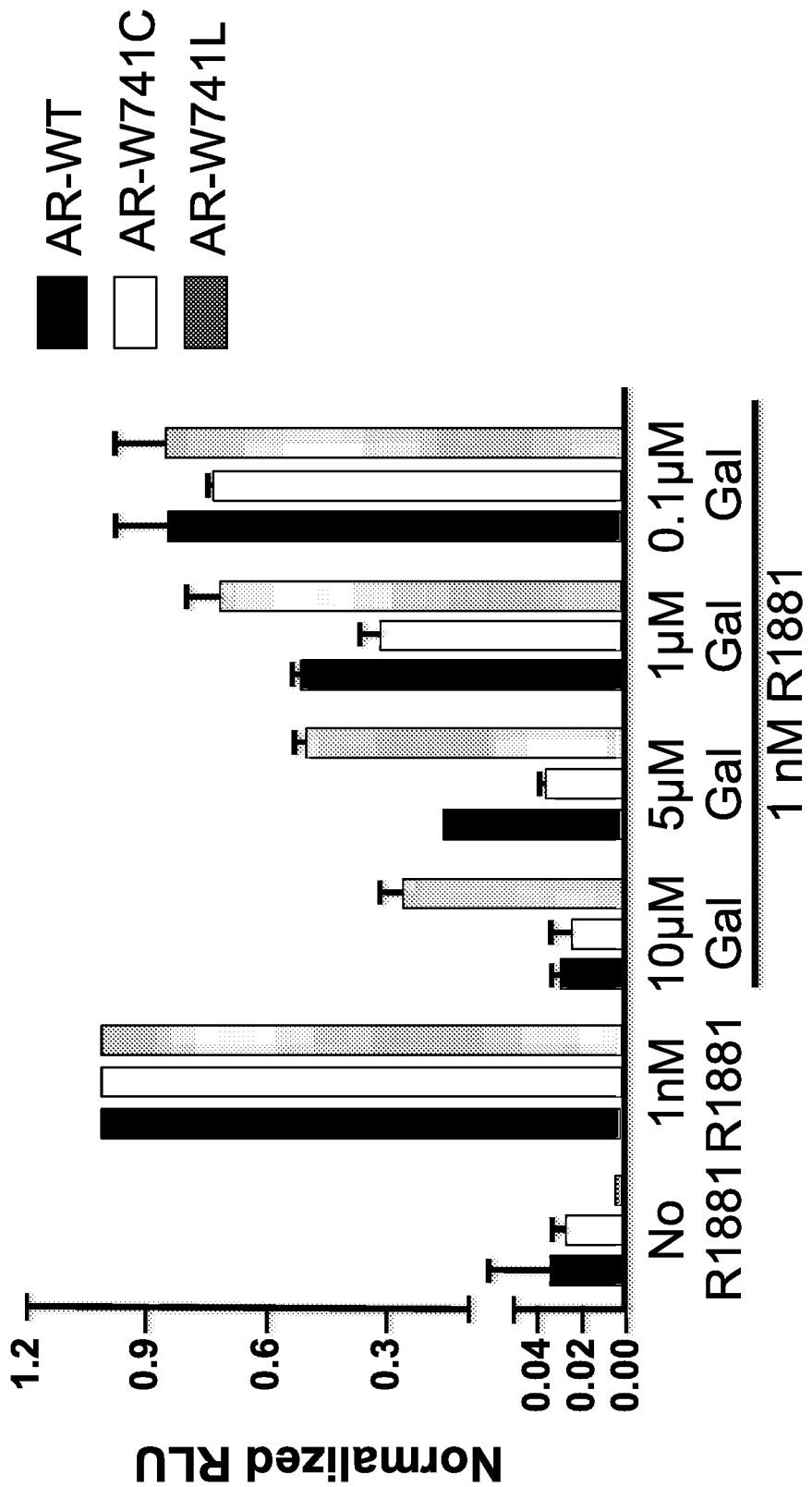

AR-WT
AR-W741C
AR-W741L
Normalized RLU
1.2
0.9
0.6
0.3
0.04
0.02
0.00
No R1881
1nM R1881
10μM Gal
5μM Gal
1μM Gal
0.1μM Gal
1 nM R1881

FIG. 20

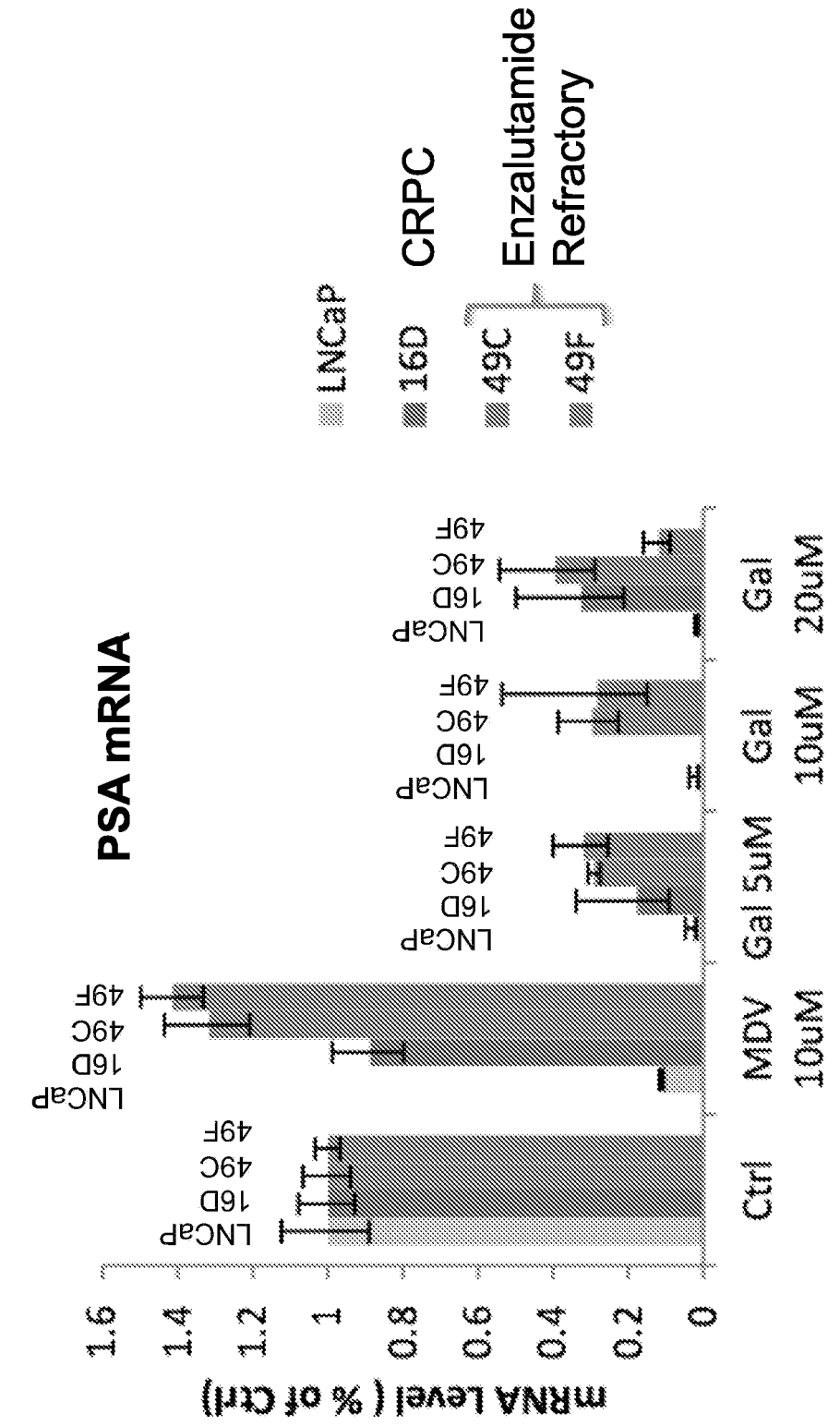

PSA mRNA
mRNA Level (% of Ctrl)
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
LNCaP
16D
49C
49F
Ctrl
MDV 10uM
Gal 5uM
Gal 10uM
Gal 20uM
LNCaP
16D
49C
49F
CRPC
Enzalutamide Refractory

FIG. 21

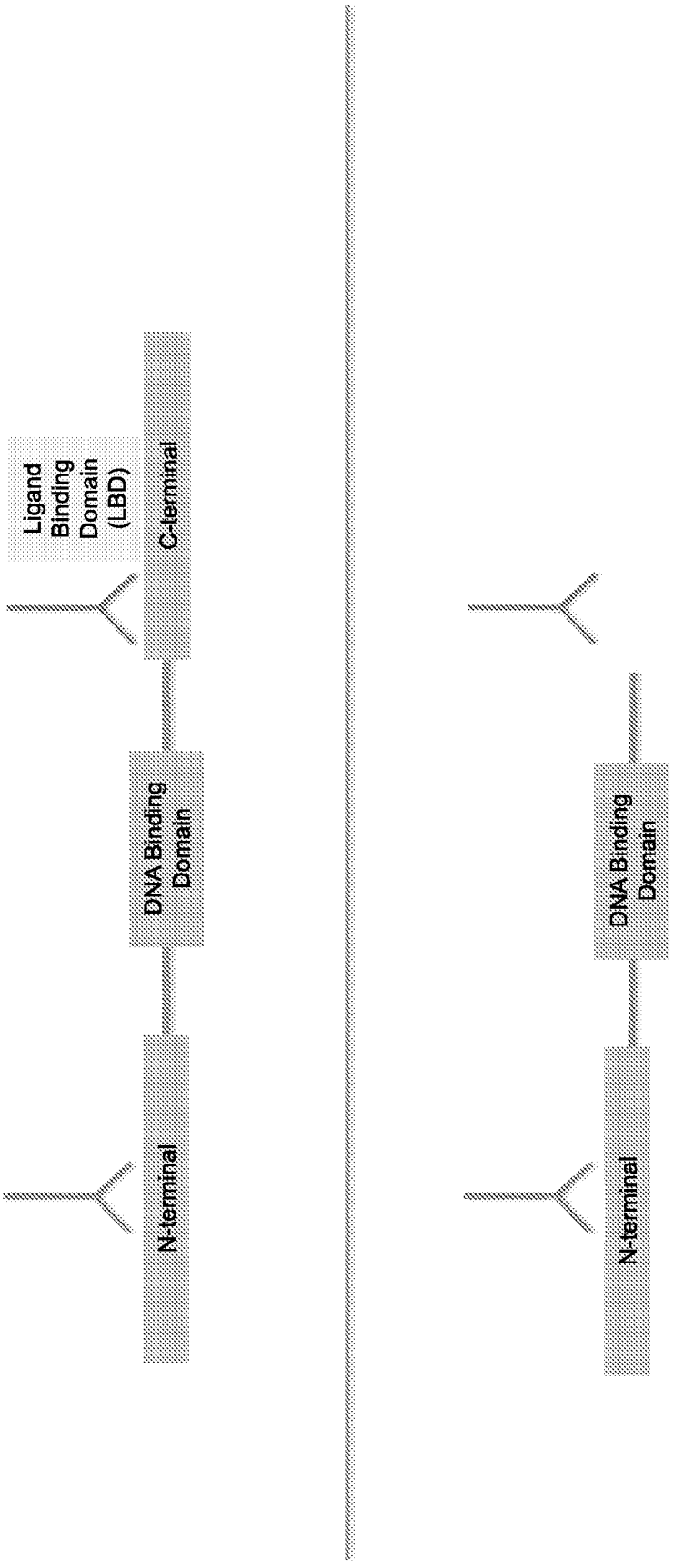
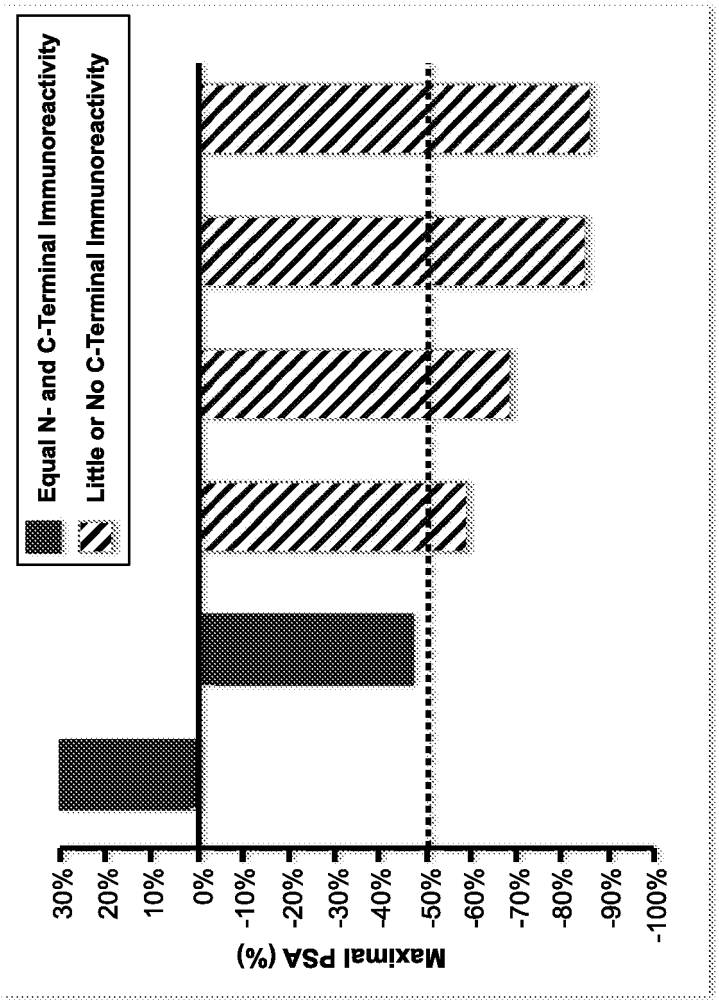

Ligand Binding Domain (LBD)
C-terminal
DNA Binding Domain
N-terminal
DNA Binding Domain
N-terminal

FIG. 22


Equal N- and C-Terminal Immunoreactivity
Little or No C-Terminal Immunoreactivity
Maximal PSA (%)
30%
20%
10%
0%
-10%
-20%
-30%
-40%
-50%
-60%
-70%
-80%
-90%
-100%