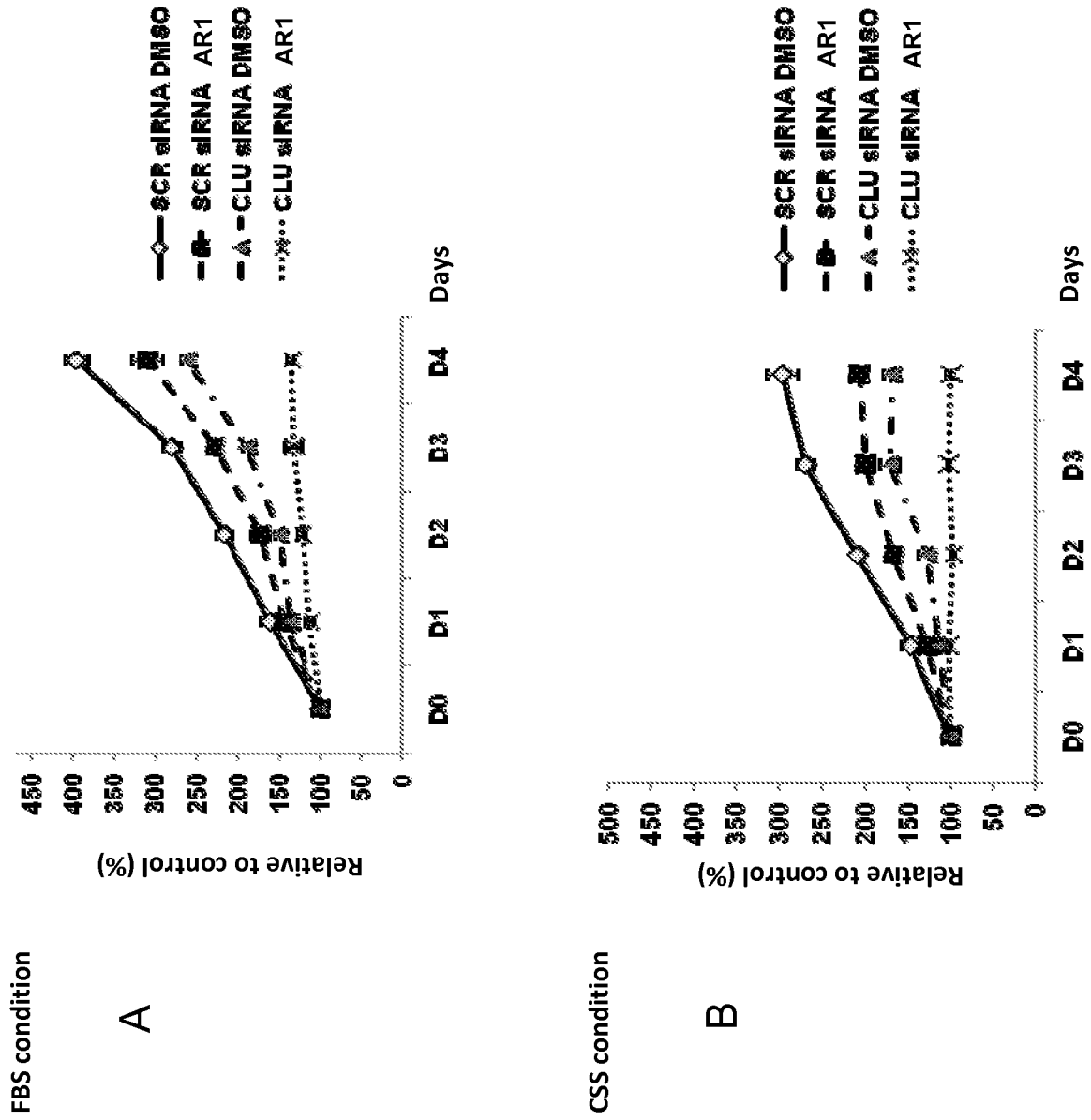
Figure 1


FBS condition
A
Relative to control (%)
450
400
350
300
250
200
150
100
50
0
D0
D1
D2
D3
D4
Days
SCR siRNA DMSO
SCR siRNA AR1
CLU siRNA DMSO
CLU siRNA AR1
CSS condition
B
Relative to control (%)
500
450
400
350
300
250
200
150
100
50
0
D0
D1
D2
D3
D4
Days
SCR siRNA DMSO
SCR siRNA AR1
CLU siRNA DMSO
CLU siRNA AR1

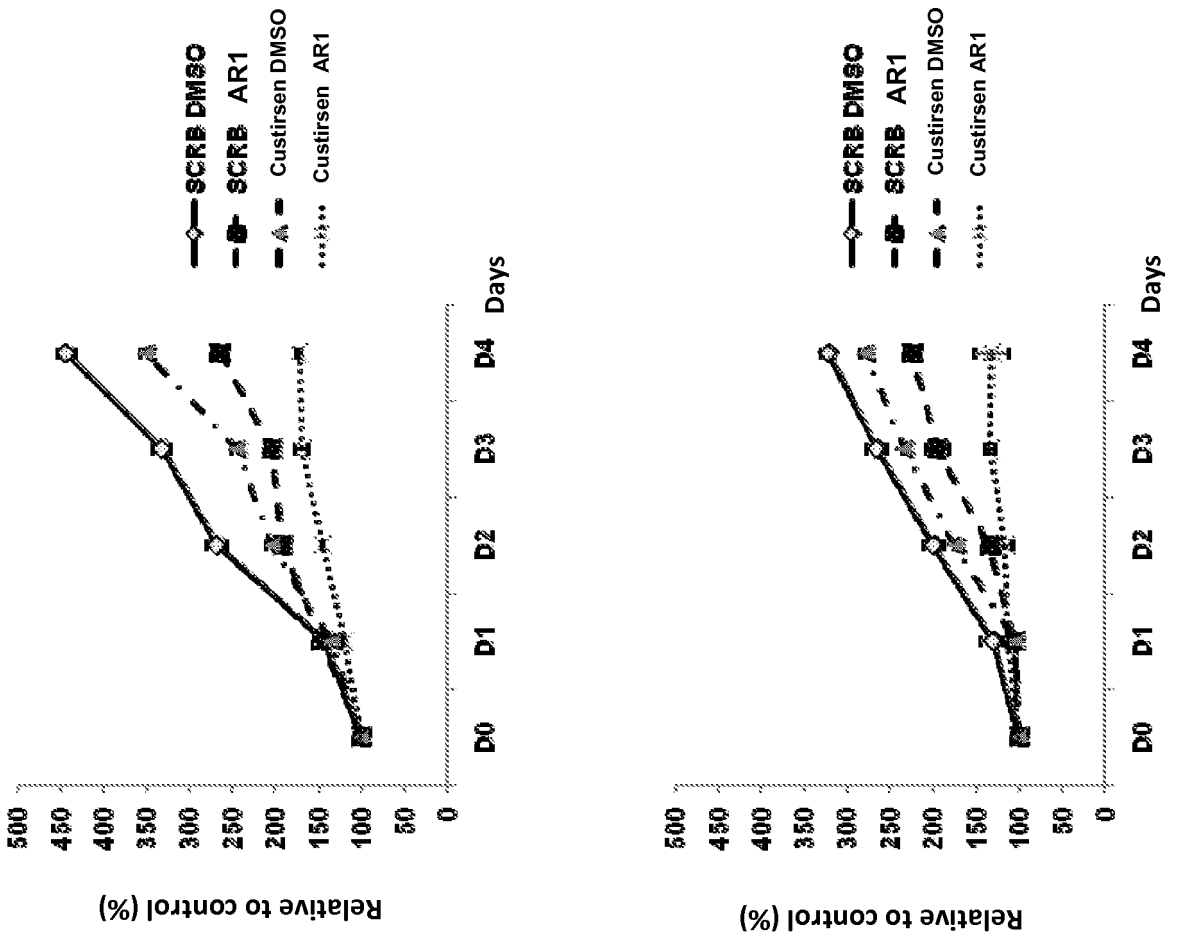

FBS condition
A
Relative to control (%)
500
450
400
350
300
250
200
150
100
50
0
D0
D1
D2
D3
D4
Days
SCRB DMSO
SCRB AR1
Custirsen DMSO
Custirsen AR1
CSS condition
B
Relative to control (%)
500
450
400
350
300
250
200
150
100
50
0
D0
D1
D2
D3
D4
Days
SCRB DMSO
SCRB AR1
Custirsen DMSO
Custirsen AR1

Figure 2

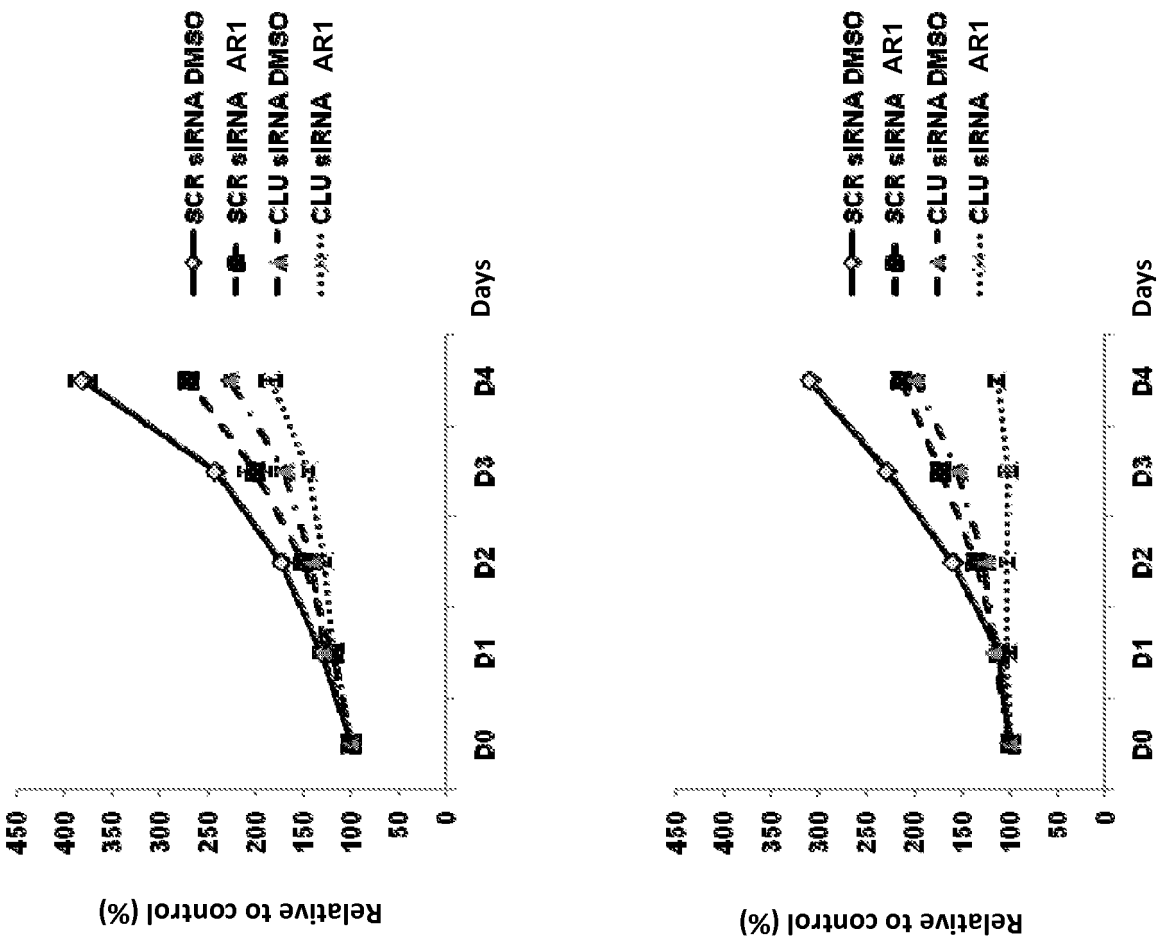

FBS condition
A
Relative to control (%)
450
400
350
300
250
200
150
100
50
0
D0
D1
D2
D3
D4
Days
SCR siRNA DMSO
SCR siRNA AR1
CLU siRNA DMSO
CLU siRNA AR1
CSS condition
B
Relative to control (%)
450
400
350
300
250
200
150
100
50
0
D0
D1
D2
D3
D4
Days
SCR siRNA DMSO
SCR siRNA AR1
CLU siRNA DMSO
CLU siRNA AR1

Figure 3

Figure 4

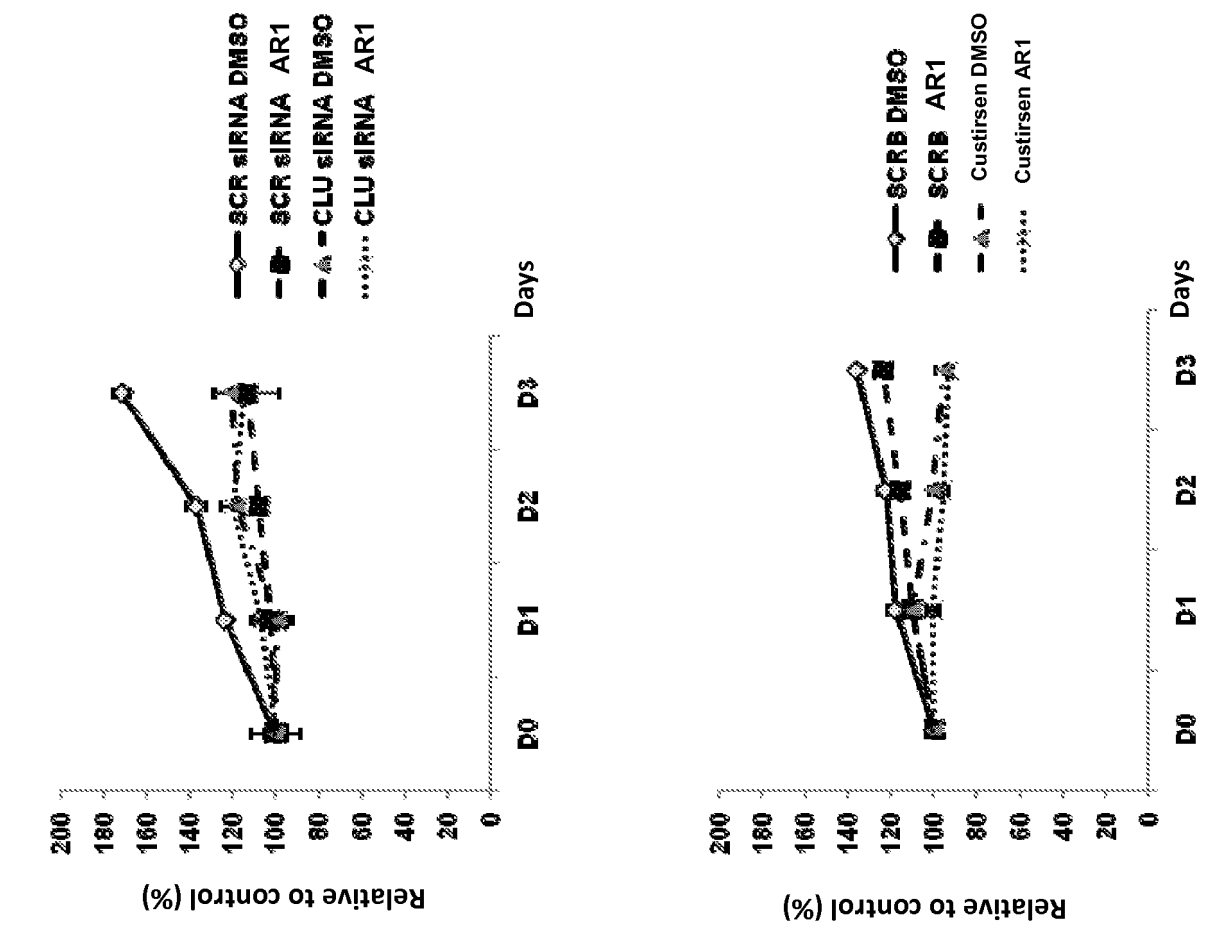
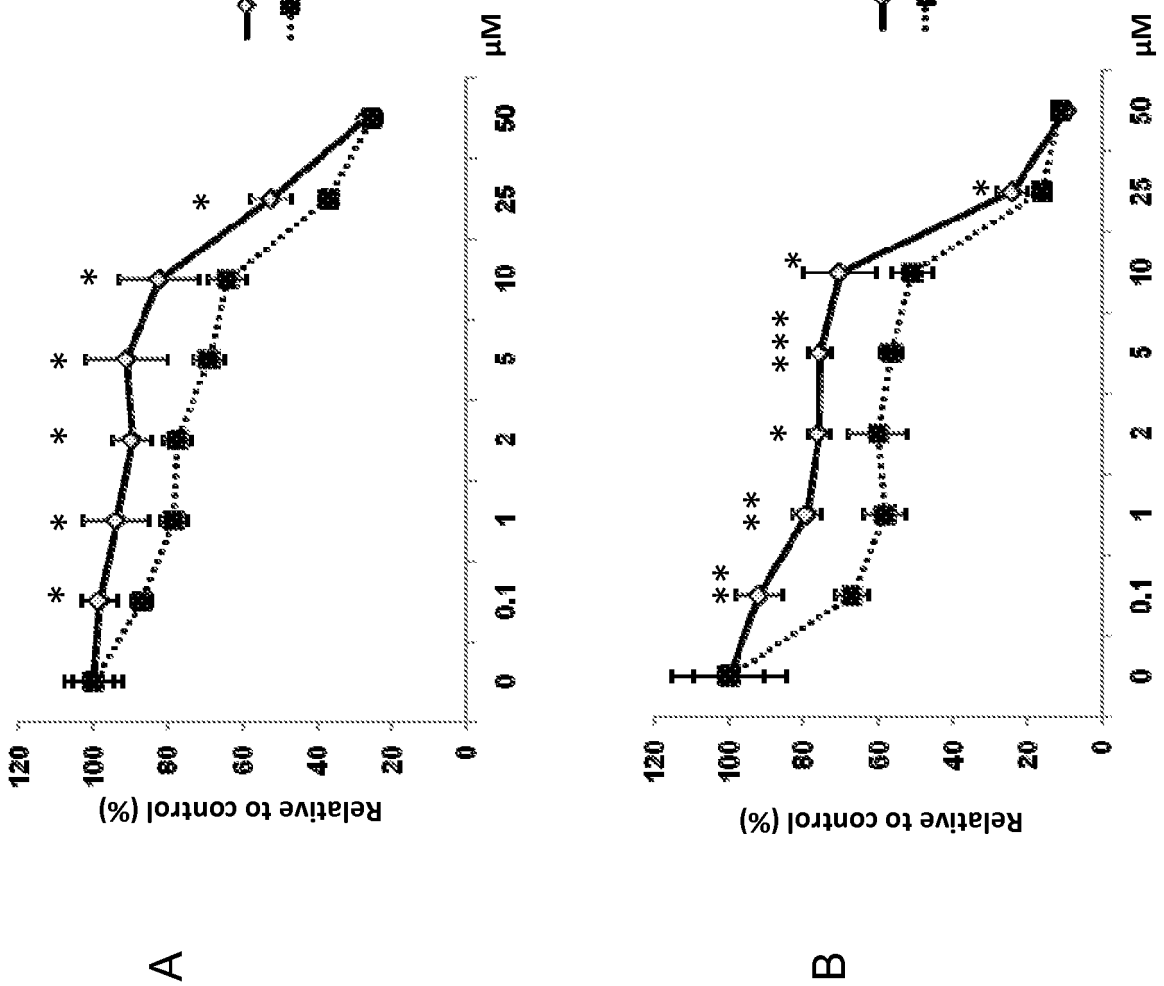

FBS condition
A
Relative to control (%)
200
180
160
140
120
100
80
60
40
20
0
D0
D1
D2
D3
Days
SCR siRNA DMSO
SCR siRNA AR1
CLU siRNA DMSO
CLU siRNA AR1
B
Relative to control (%)
SCRB DMSO
SCRB AR1
Custirsen DMSO
Custirsen AR1

Figure 5



*, $p<0.05$; **, $p<0.01$; ***, $p<0.001$

Figure 6

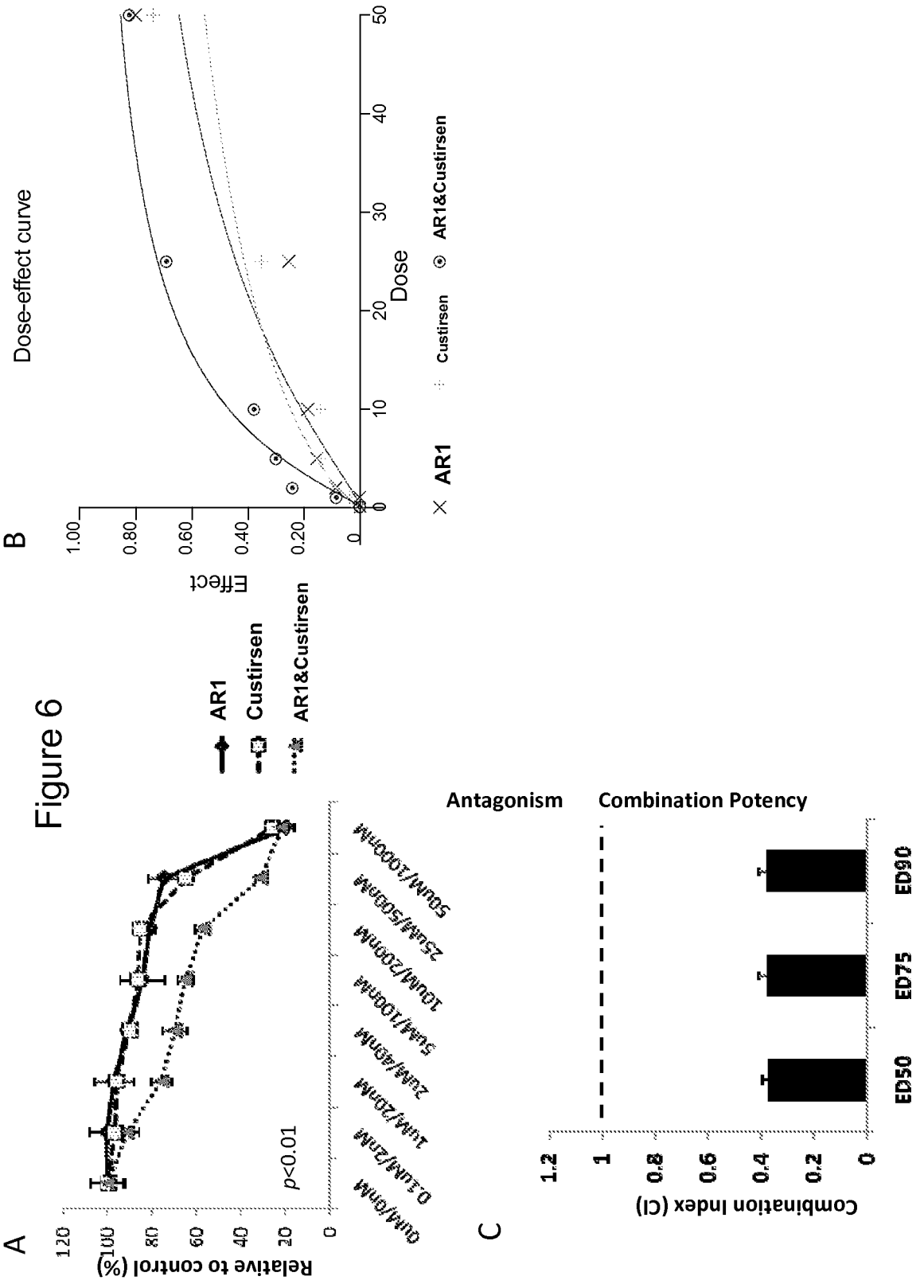
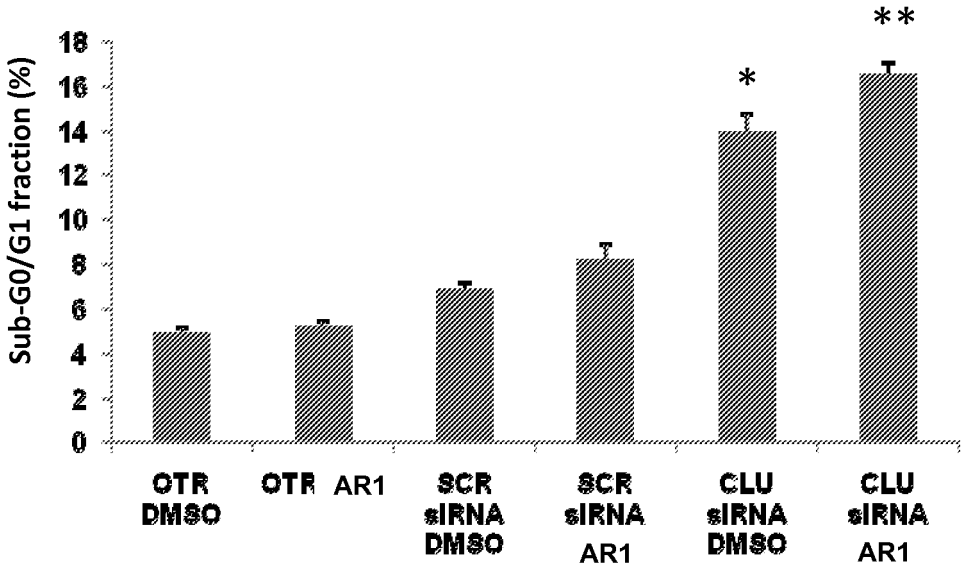

A
Relative to control (%)
120
100
80
60
40
20
0
p<0.01
AR1
Custirsen
AR1&Custirsen
0uM/0nM
0.1uM/2nM
1uM/20nM
2uM/40nM
5uM/100nM
10uM/200nM
25uM/500nM
50uM/1000nM
B
Dose-effect curve
Effect
1.00
0.80
0.60
0.40
0.20
0
0
10
20
30
40
50
Dose
AR1
Custirsen
AR1&Custirsen
C
Antagonism
Combination Potency
Combination Index (CI)
1.2
1
0.8
0.6
0.4
0.2
0
ED50
ED75
ED90

Figure 7

	Cell cycle distribution (%)			
treatment	Sub-G0/G1	G1	S	G2/M
OTR DMSO	5.0±0.2	78.4±0.6	4.2±0.3	11.7±0.3
OTR AR1	5.3±0.2	79.9±0.1	3.2±0.1	11.1±0.2
SCR siRNA DMSO	6.9±0.3	80.2±1.1	3.3±0.1	9.2±0.8
SCR siRNA AR1	8.2±0.7	80.3±0.6	2.4±0.1	8.7±0.1
CLU siRNA DMSO	14.0±0.8	77.9±0.1	1.8±0.4	6.3±0.5
CLU siRNA AR1	16.6±0.5	74.6±0.4	2.1±0.1	6.5±0.3



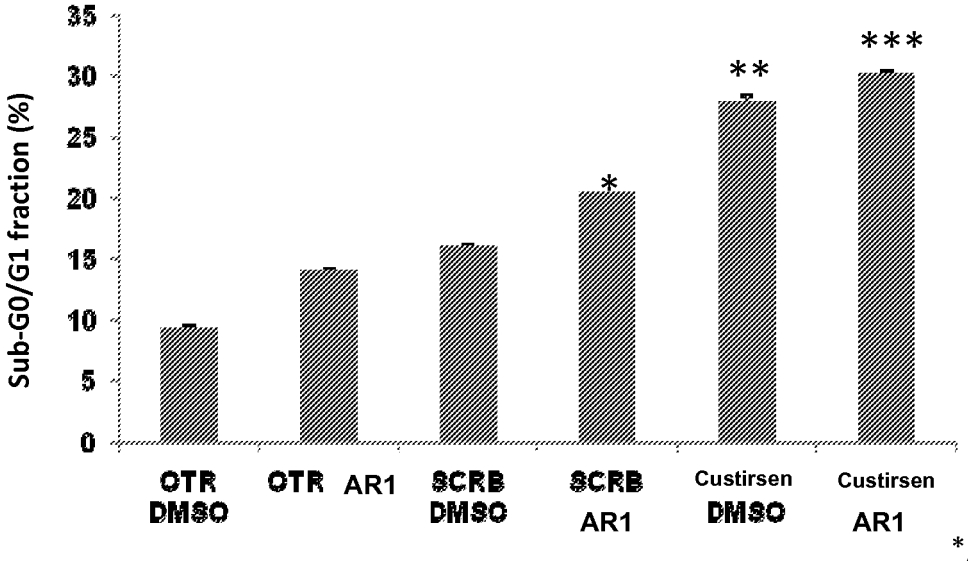
*, $p<0.05$; **, $p<0.01$; ***, $p<0.001$

Figure 8

FACS analysis for AR1 & Custirsen

Cell cycle distribution (%)

treatment	subG0/G1	G1	S	G2/M
OTR DMSO	9.3±0.3	73.8±0.1	4.7±0.1	11.6±0.1
OTR AR1	14.1±0.1	73.9±0.6	2.7±0.6	9.1±0.1
SCRB DMSO	16.1±0.1	68.6±0.3	4.2±0.4	10.8±0.1
SCRB AR1	20.4±0.1	70.1±0.2	2.2±0.1	6.9±0.3
Custirsen DMSO	28.0±0.5	60.8±1.1	2.9±0.1	7.8±0.6
Custirsen AR1	30.2±0.2	62.1±0.4	2.0±0.5	6.6±0.1



*, $p<0.05$; **, $p<0.01$; ***, $p<0.001$

Figure 9

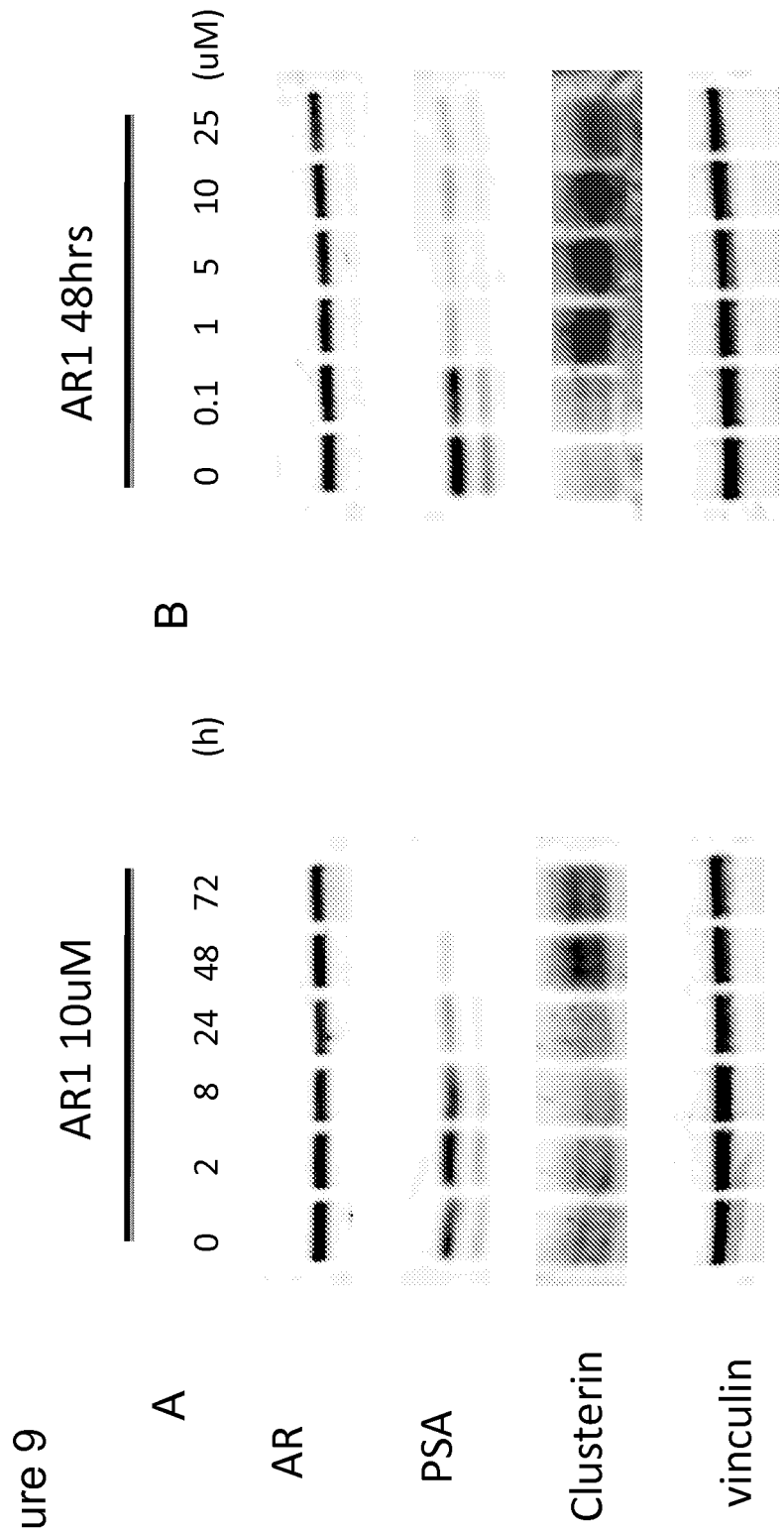

A
AR1 10uM
0 2 8 24 48 72
(h)
AR
PSA
Clusterin
vinculin
B
AR1 48hrs
0 0.1 1 5 10 25 (uM)

Figure 10

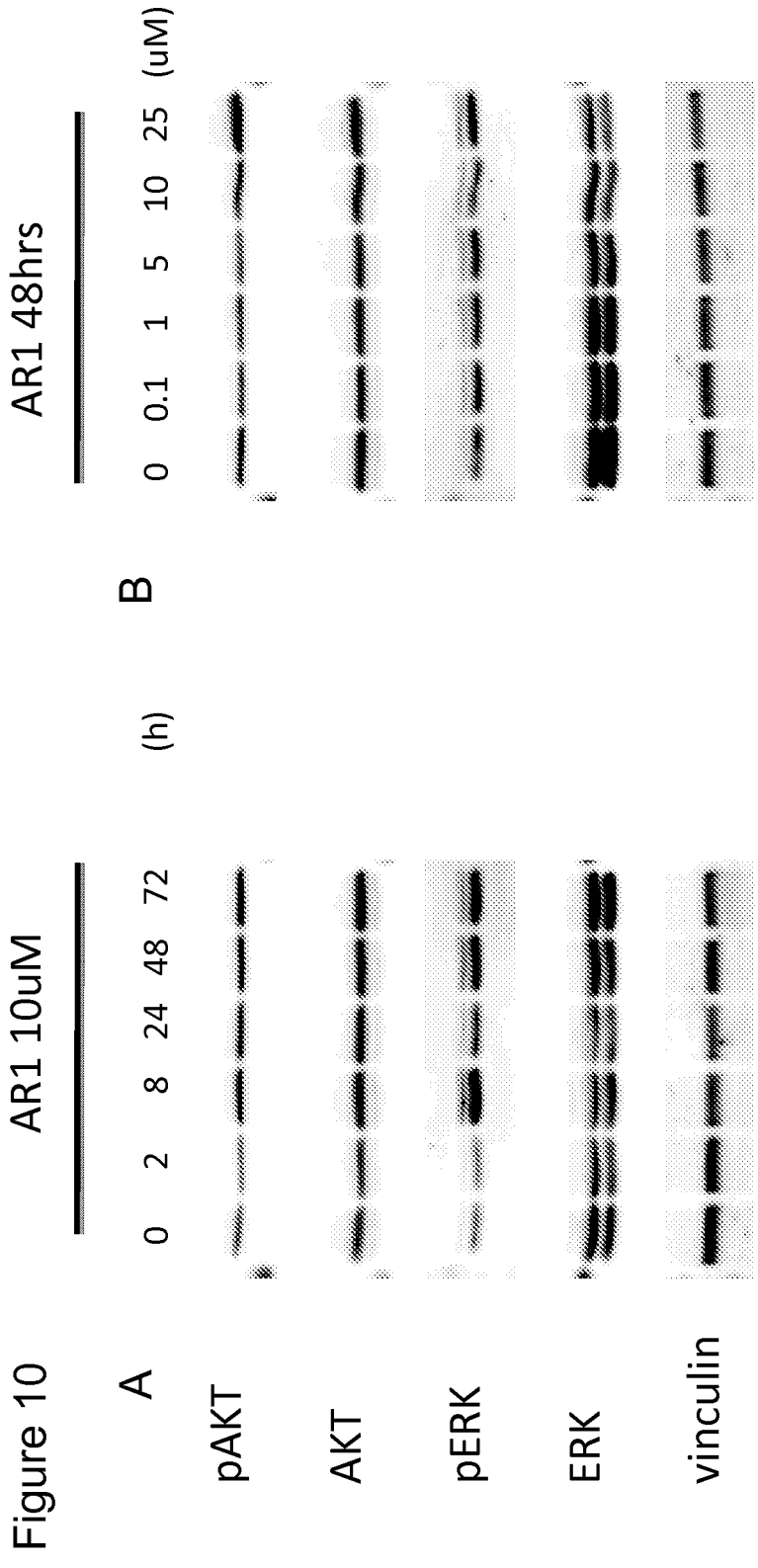

A
AR1 10uM
0 2 8 24 48 72
(h)
pAKT
AKT
pERK
ERK
vinculin
B
AR1 48hrs
0 0.1 1 5 10 25 (uM)

Figure 10 (continued)

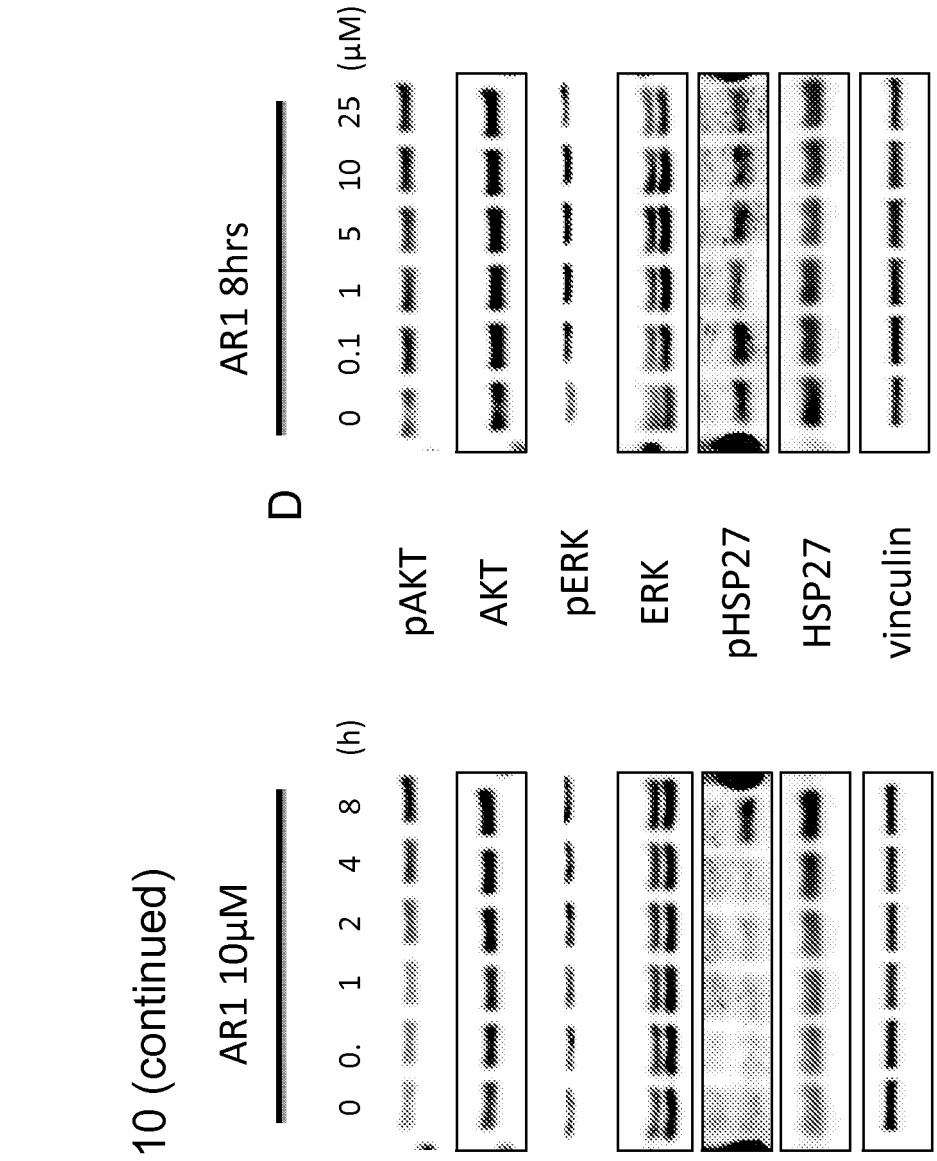
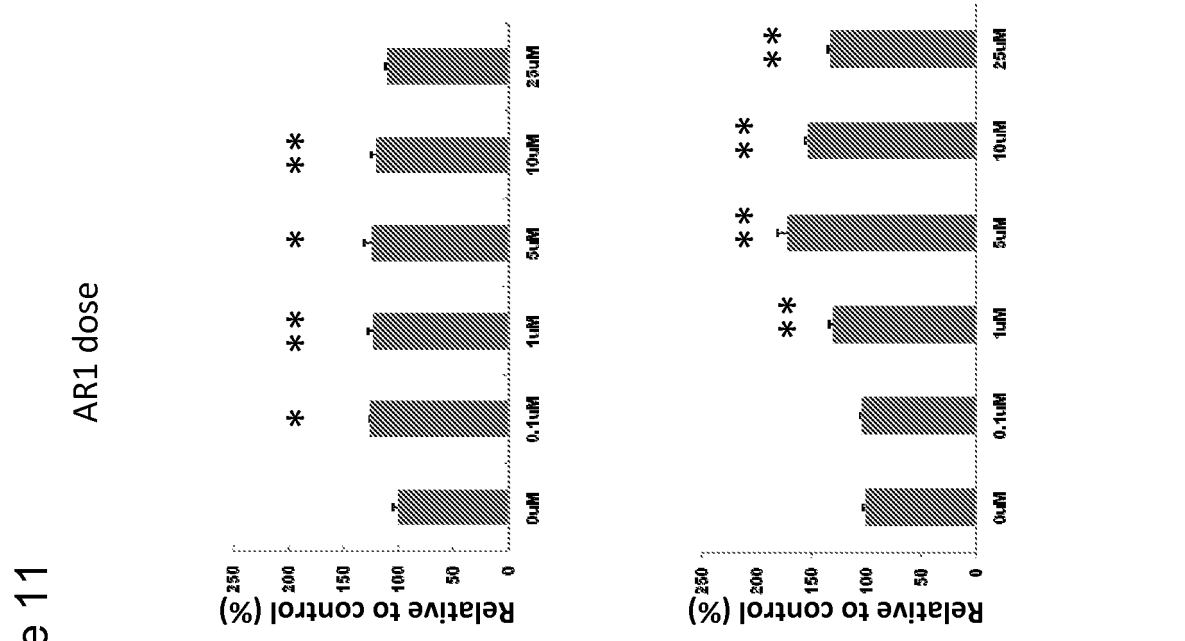


Figure 11



*, $p<0.05$; **, $p<0.01$; ***, $p<0.001$

Figure 12

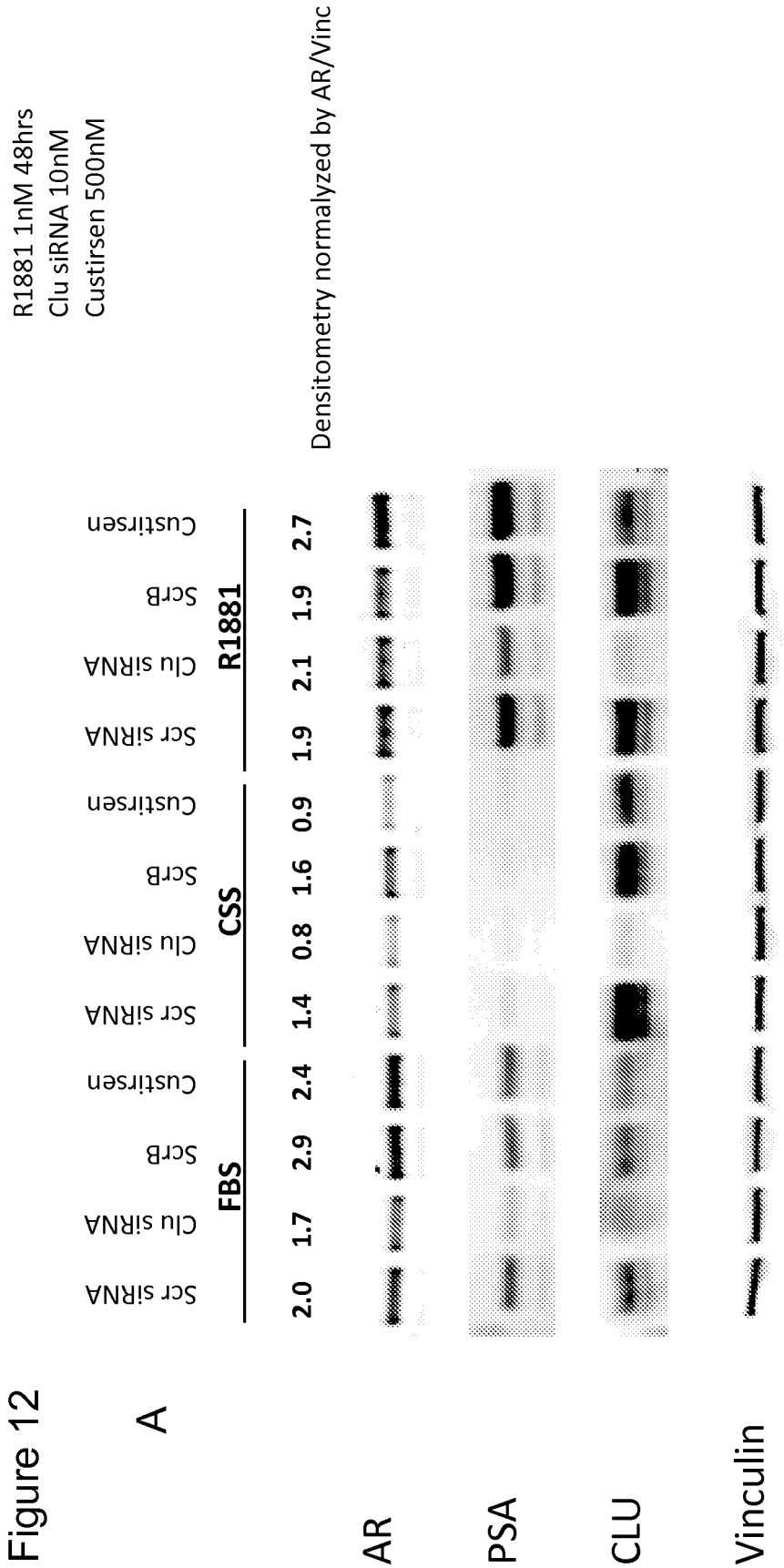

A
R1881 1nM 48hrs
Clu siRNA 10nM
Custirsen 500nM
Densitometry normalyzed by AR/Vinc
FBS
CSS
R1881
Scr siRNA
Clu siRNA
ScrB
Custirsen
2.0 1.7 2.9 2.4 1.4 0.8 1.6 0.9 1.9 2.1 1.9 2.7
AR
PSA
CLU
Vinculin

Figure 12 (continued)

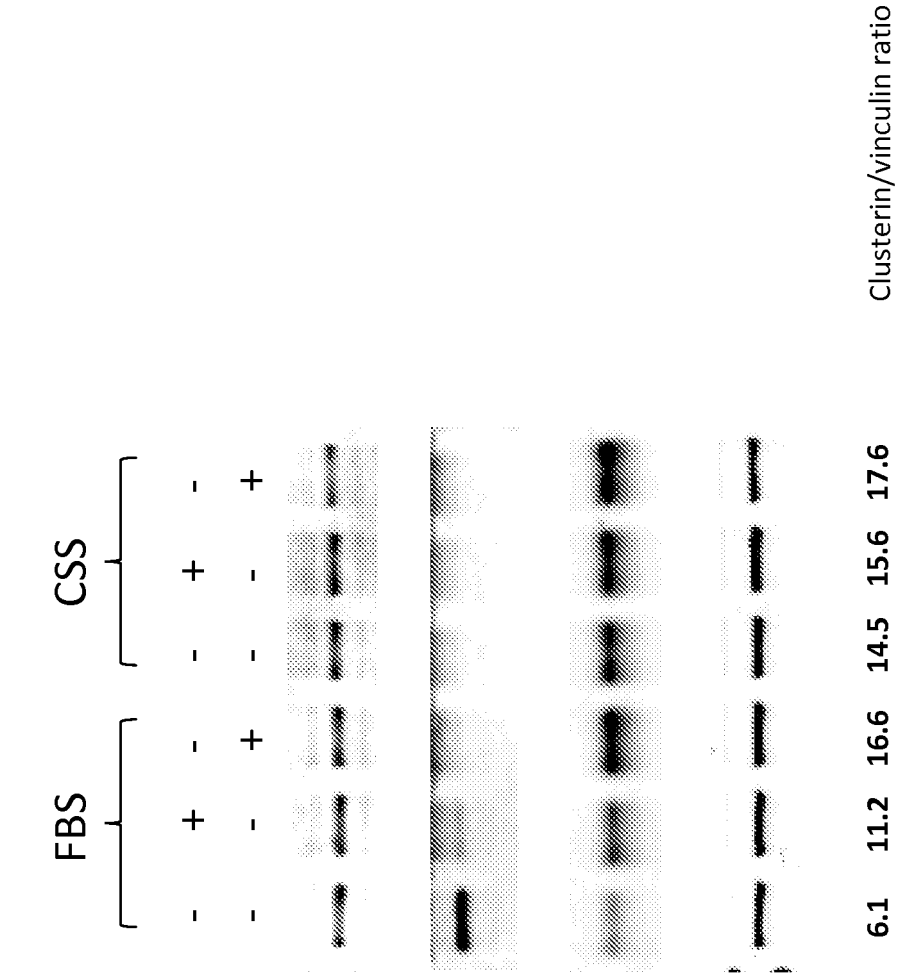


Figure 12 (continued)

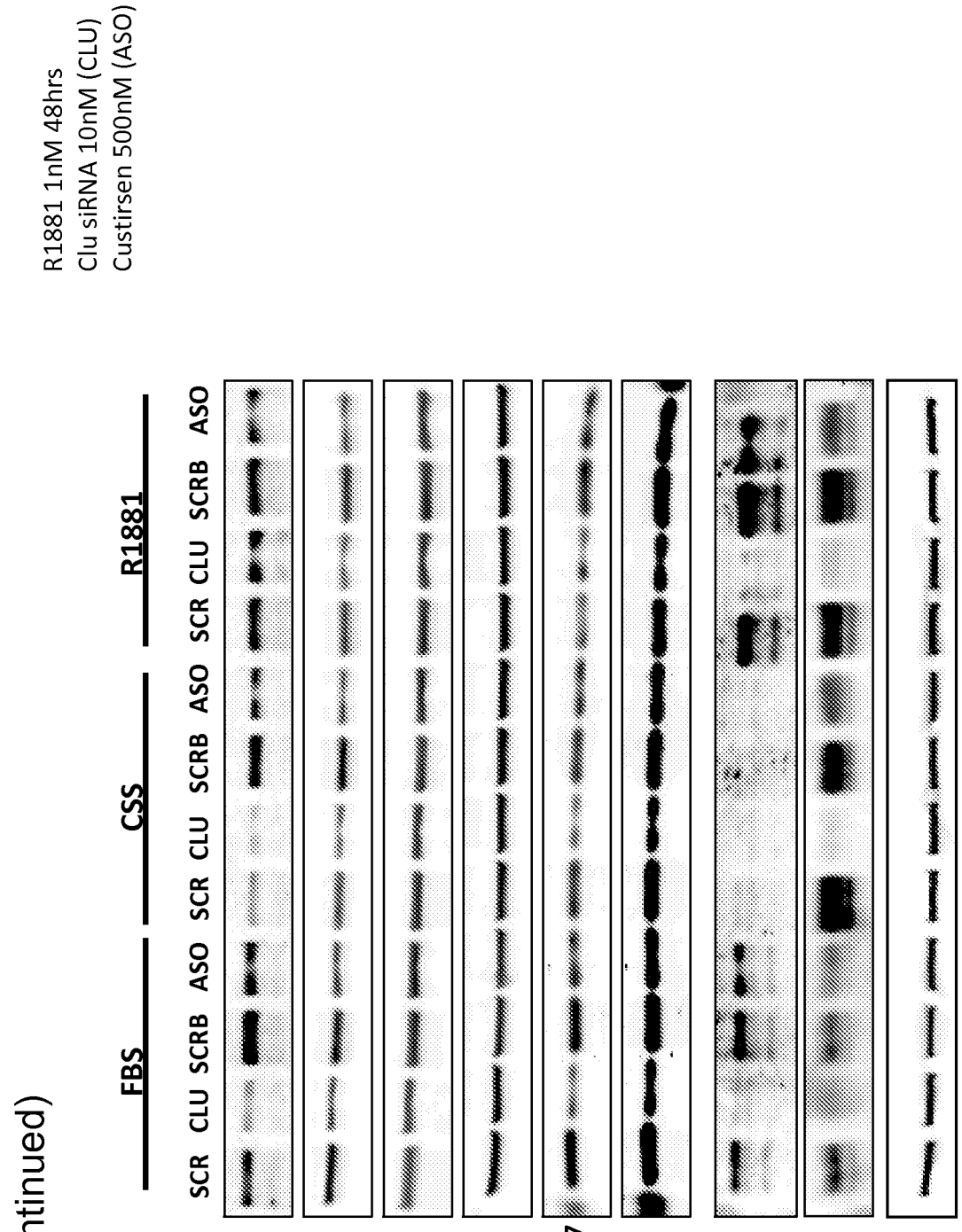


Figure 12 (continued)

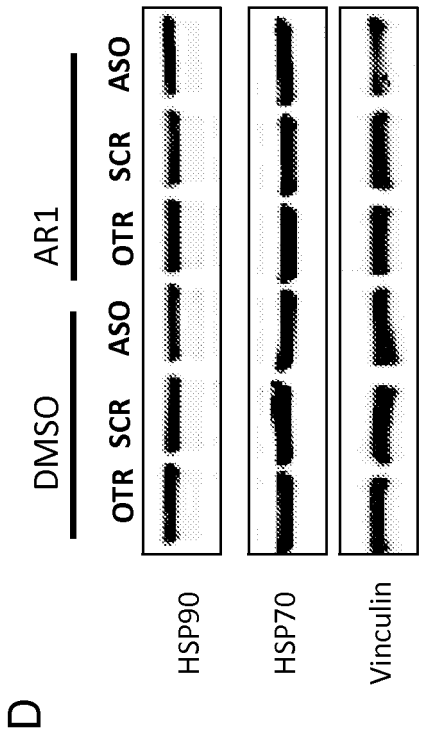
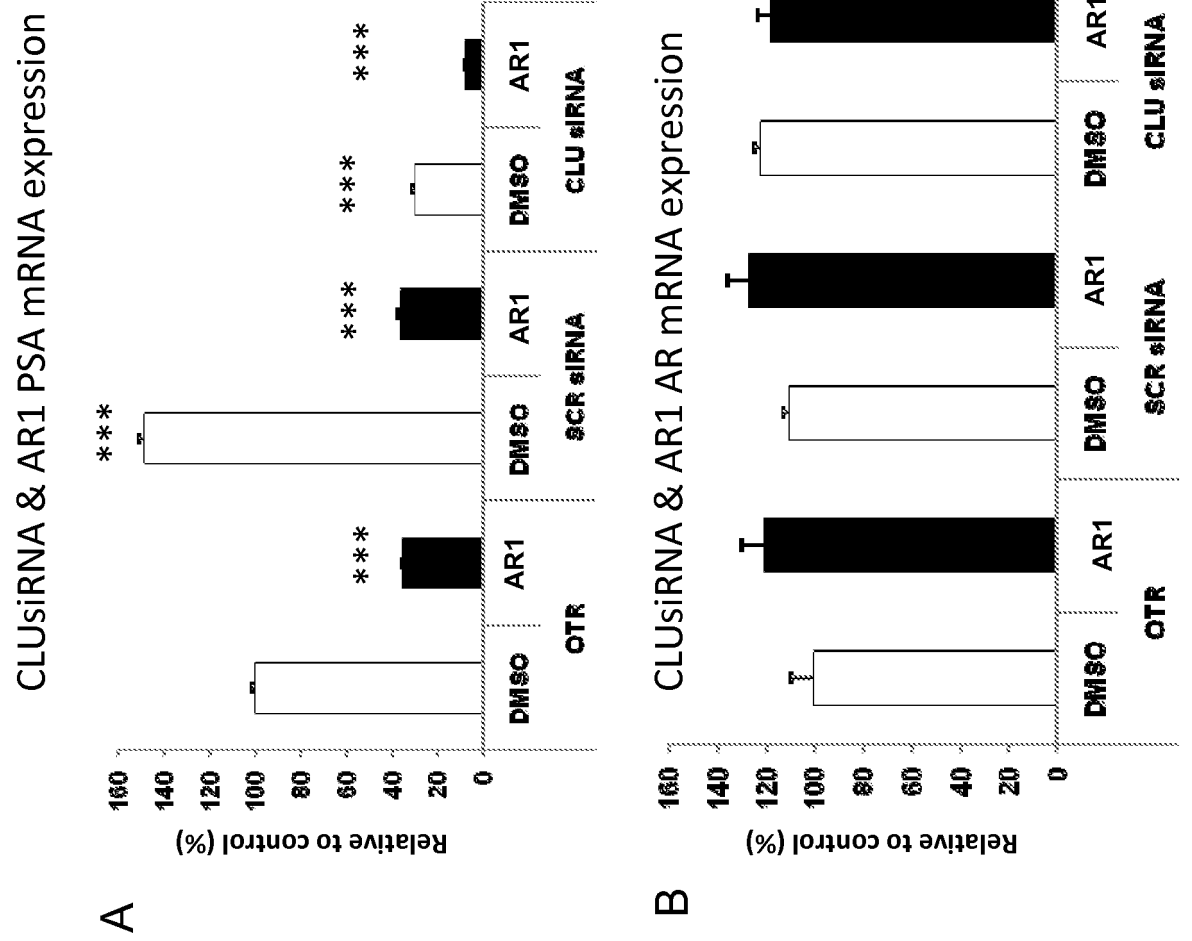
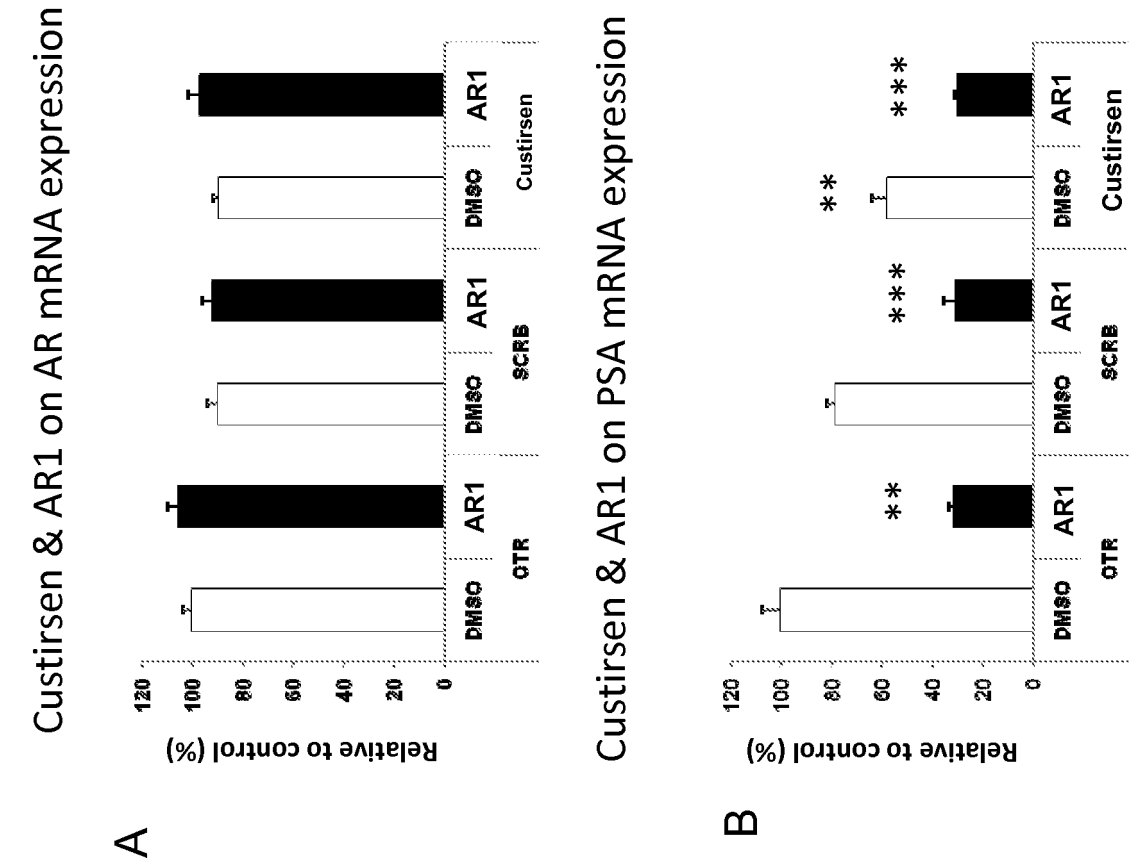


Figure 13



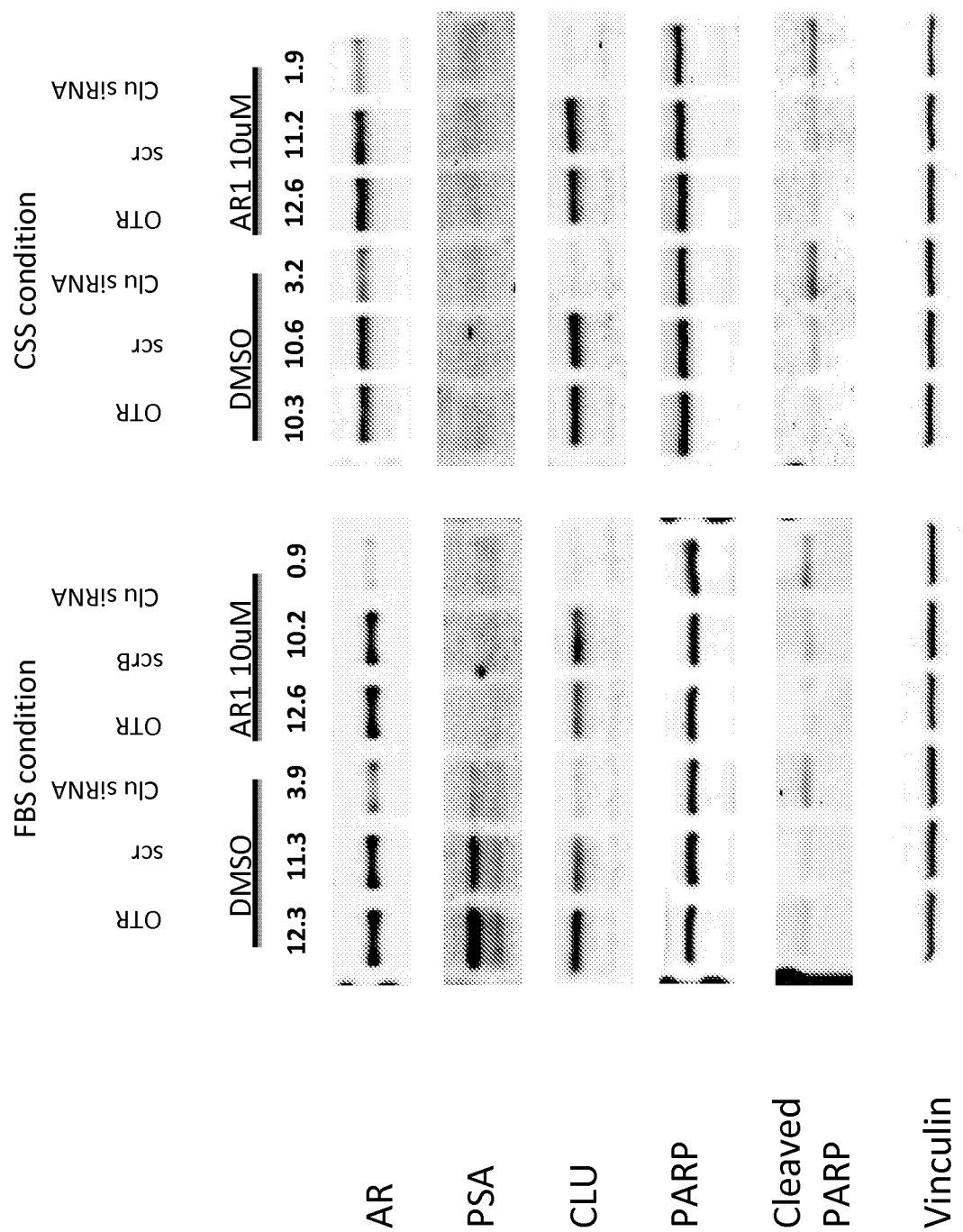
*, $p<0.05$; **, $p<0.01$; ***, $p<0.001$

Figure 14



*, $p<0.05$; **, $p<0.01$; ***,

Figure 15


FBS condition
CSS condition
DMSO
AR1 10uM
OTR
scr
Clu siRNA
scrB
12.3
11.3
3.9
12.6
10.2
0.9
10.3
10.6
3.2
12.6
11.2
1.9
AR
PSA
CLU
PARP
Cleaved PARP
Vinculin

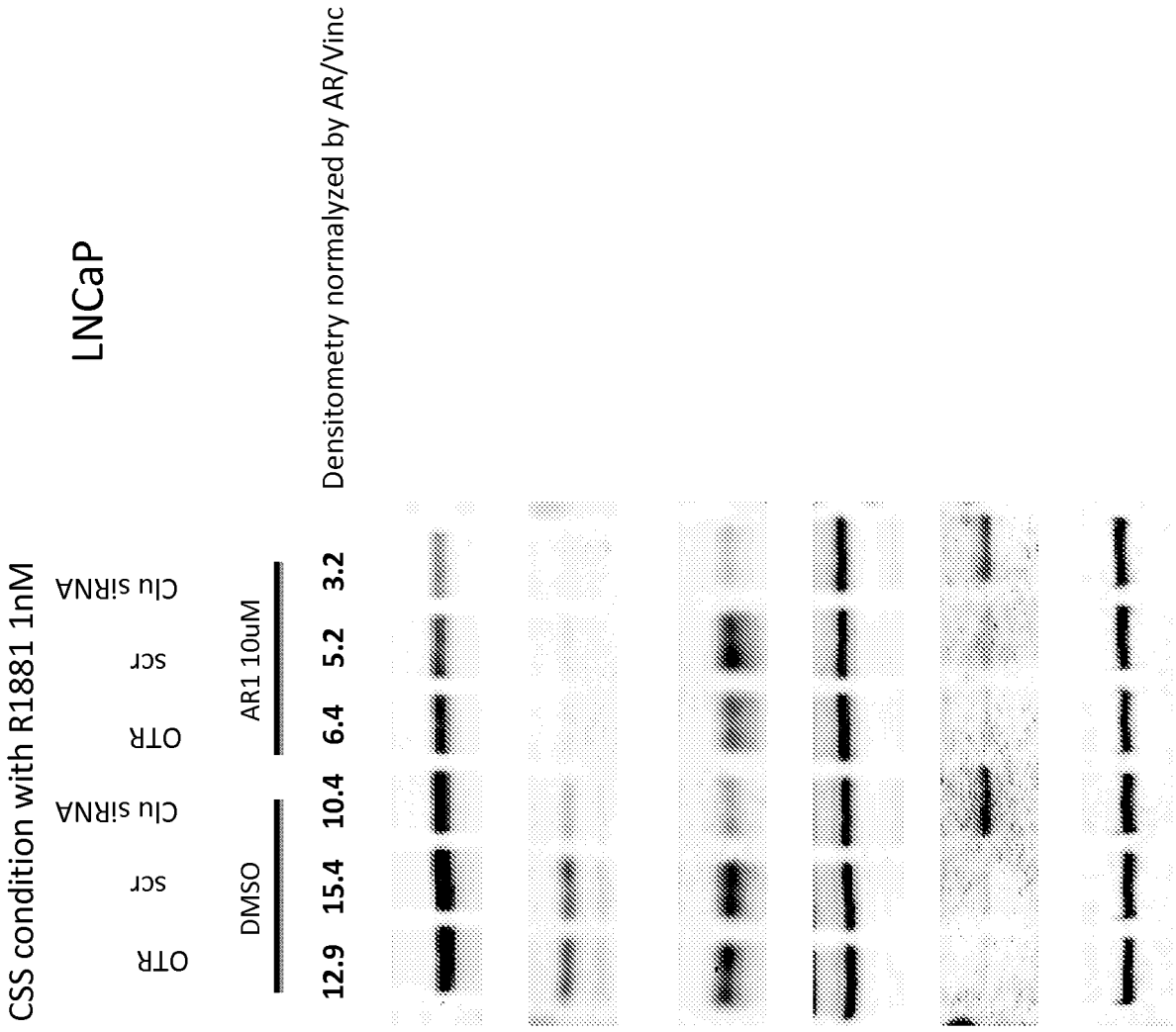

LNCaP
CSS condition with R1881 1nM
DMSO
AR1 10uM
OTR
scr
Clu siRNA
OTR
scr
Clu siRNA
Densitometry normalyzed by AR/Vinc
12.9 15.4 10.4 6.4 5.2 3.2
AR
PSA
CLU
PARP
Cleaved PARP
Vinculin

Figure 16

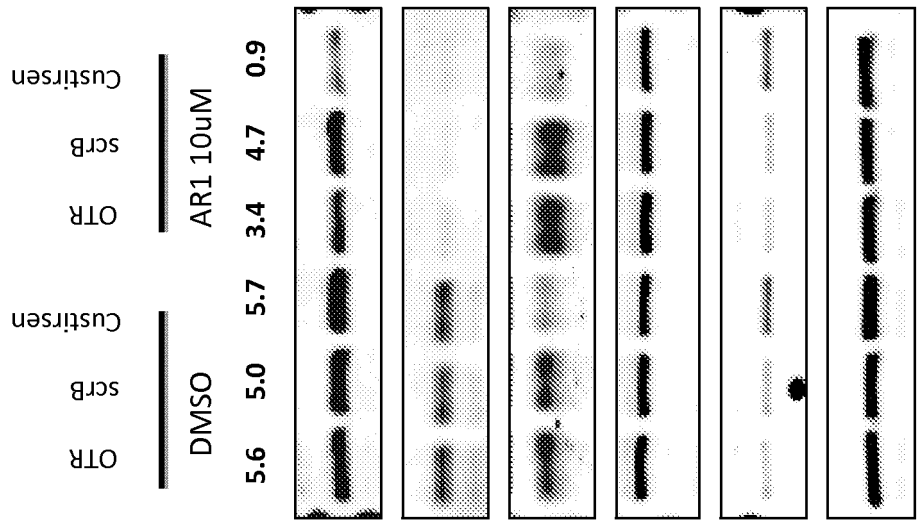

OTR
scrB
Custirsen
DMSO
5.6
5.0
5.7
OTR
scrB
Custirsen
AR1 10uM
3.4
4.7
0.9
AR
PSA
CLU
PARP
Cleaved PARP
Vinculin

Figure 17

Figure 18

Protein expression for AR1 and clusiRNA combination therapy by WB (LNCaP cells)

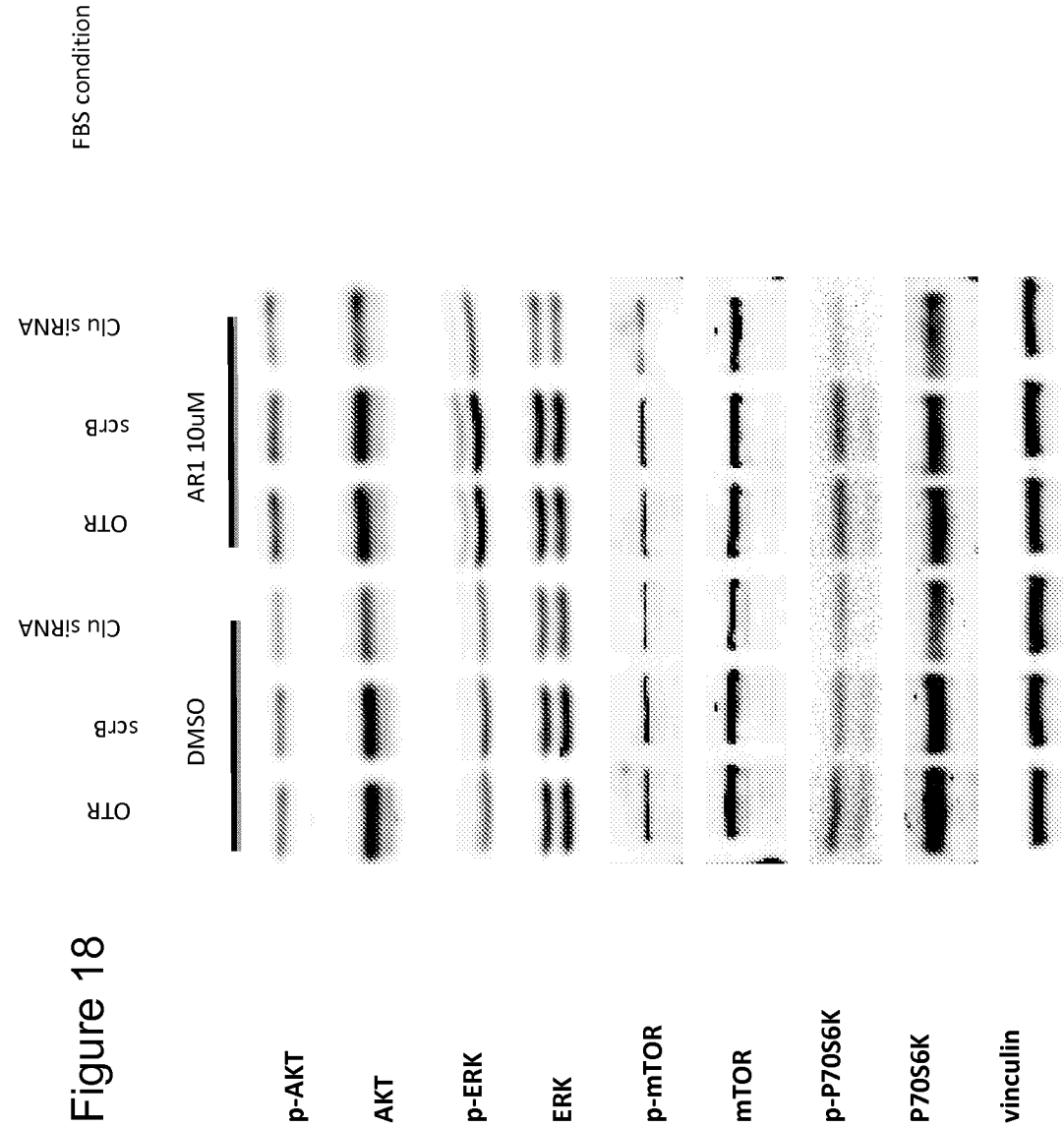


Figure 19

LNCaP FBS condition

AR degradation - by proteasome inhibition and protein synthesis inhibition

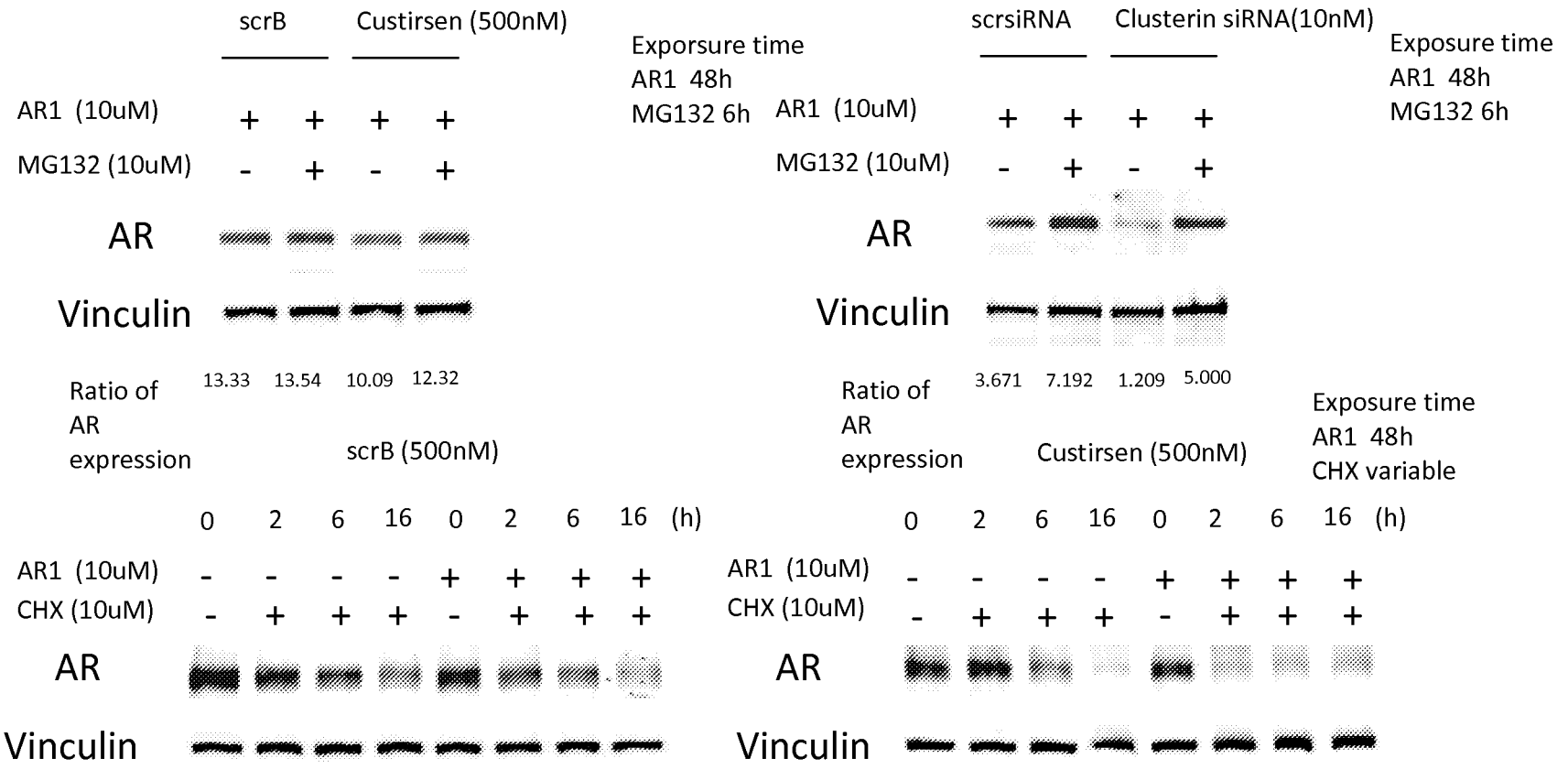


Figure 20

Effect of combination therapy for AR transcriptional activity

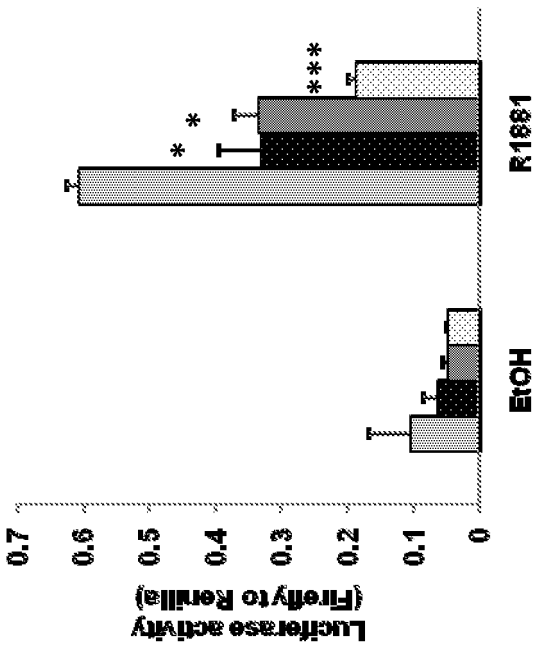


Figure 21

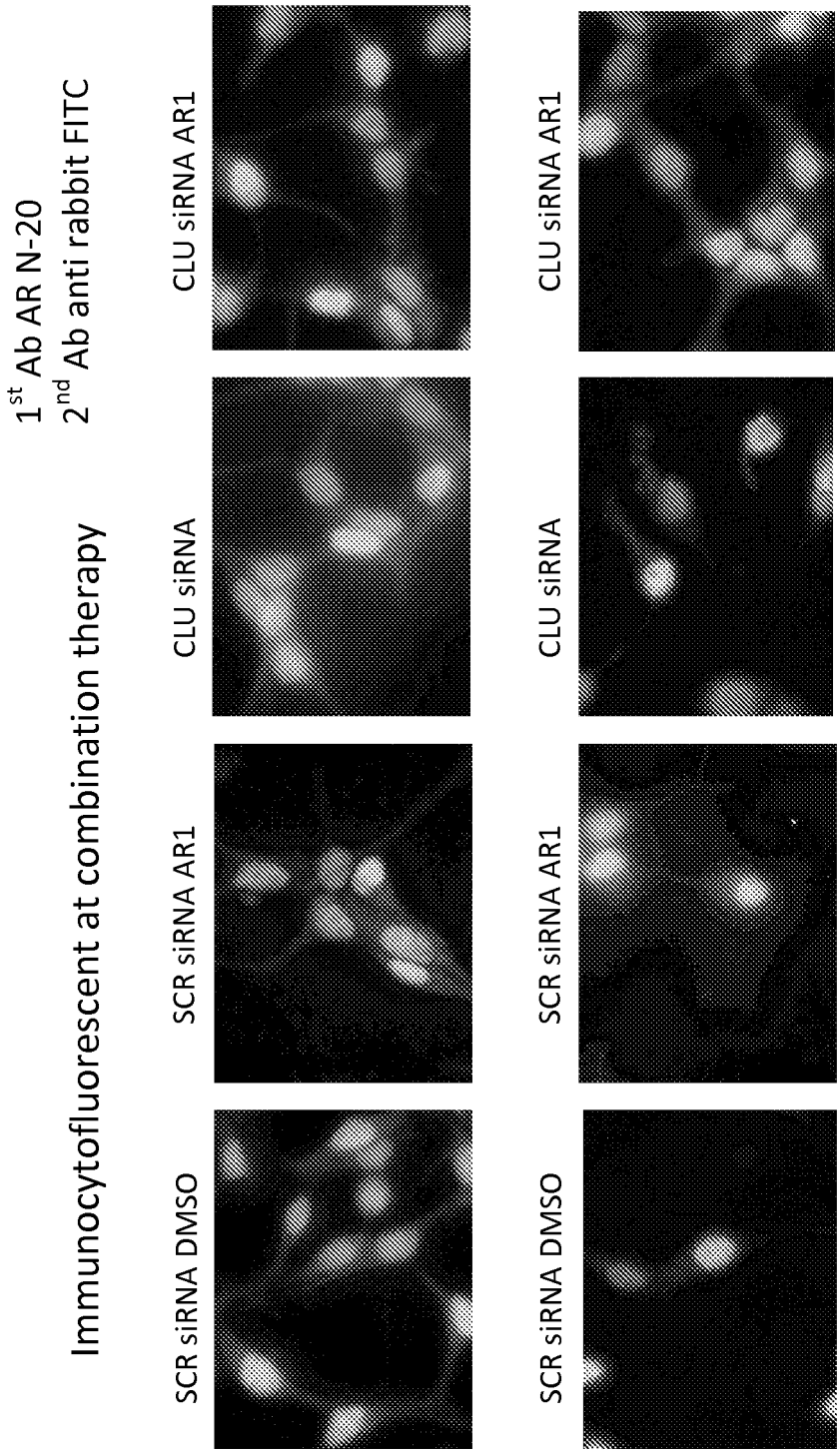

Immunocytofluorescent at combination therapy
1st Ab AR N-20
2nd Ab anti rabbit FITC
SCR siRNA DMSO
SCR siRNA AR1
CLU siRNA
CLU siRNA AR1
CSS only
R1881 1nM

Figure 22

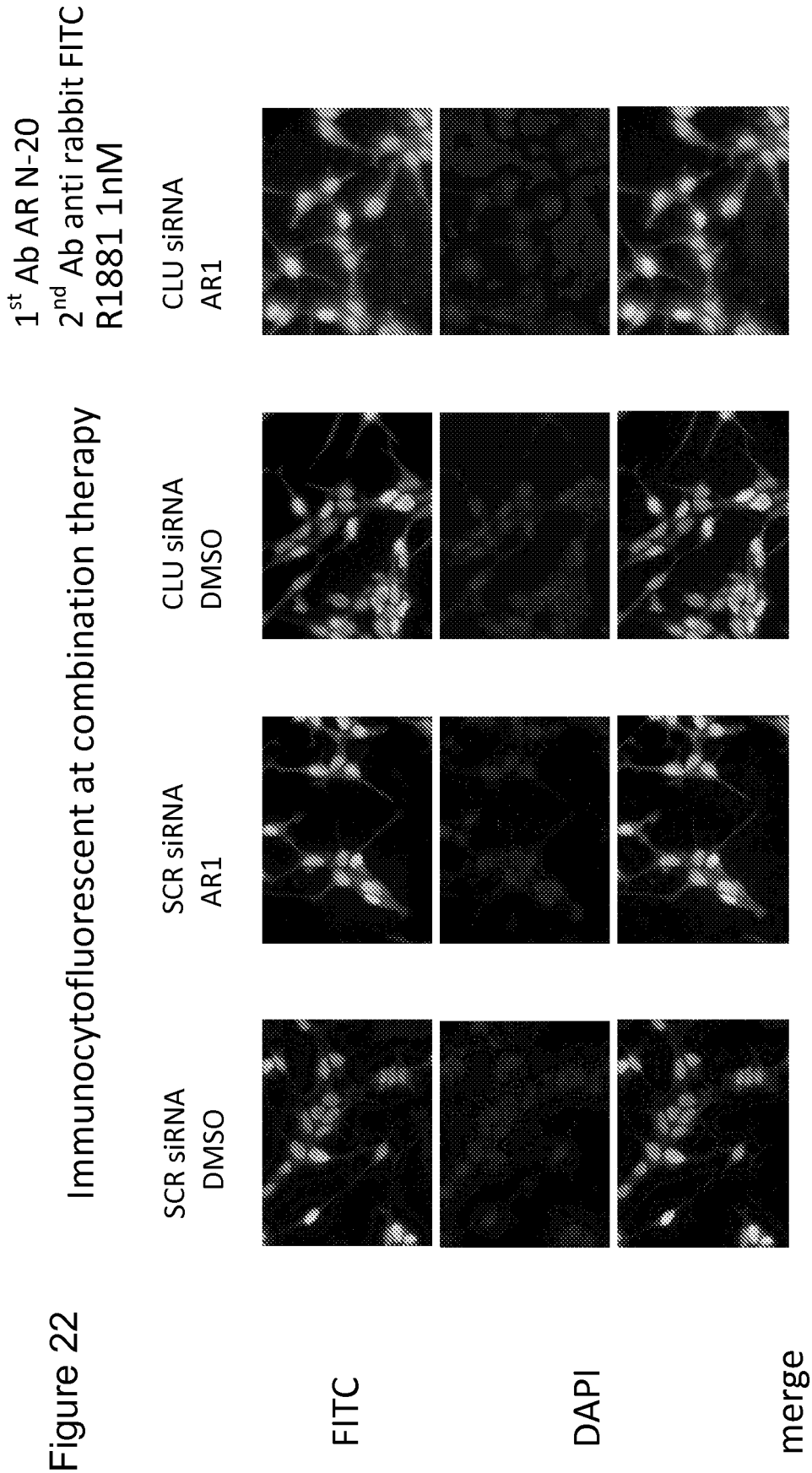

Immunocytofluorescent at combination therapy
1st Ab AR N-20
2nd Ab anti rabbit FITC
R1881 1nM
SCR siRNA DMSO
SCR siRNA AR1
CLU siRNA DMSO
CLU siRNA AR1
FITC
DAPI
merge

Figure 23
Effect of CLU knock down and AR1 combination treatment on AR/ubiquitin association

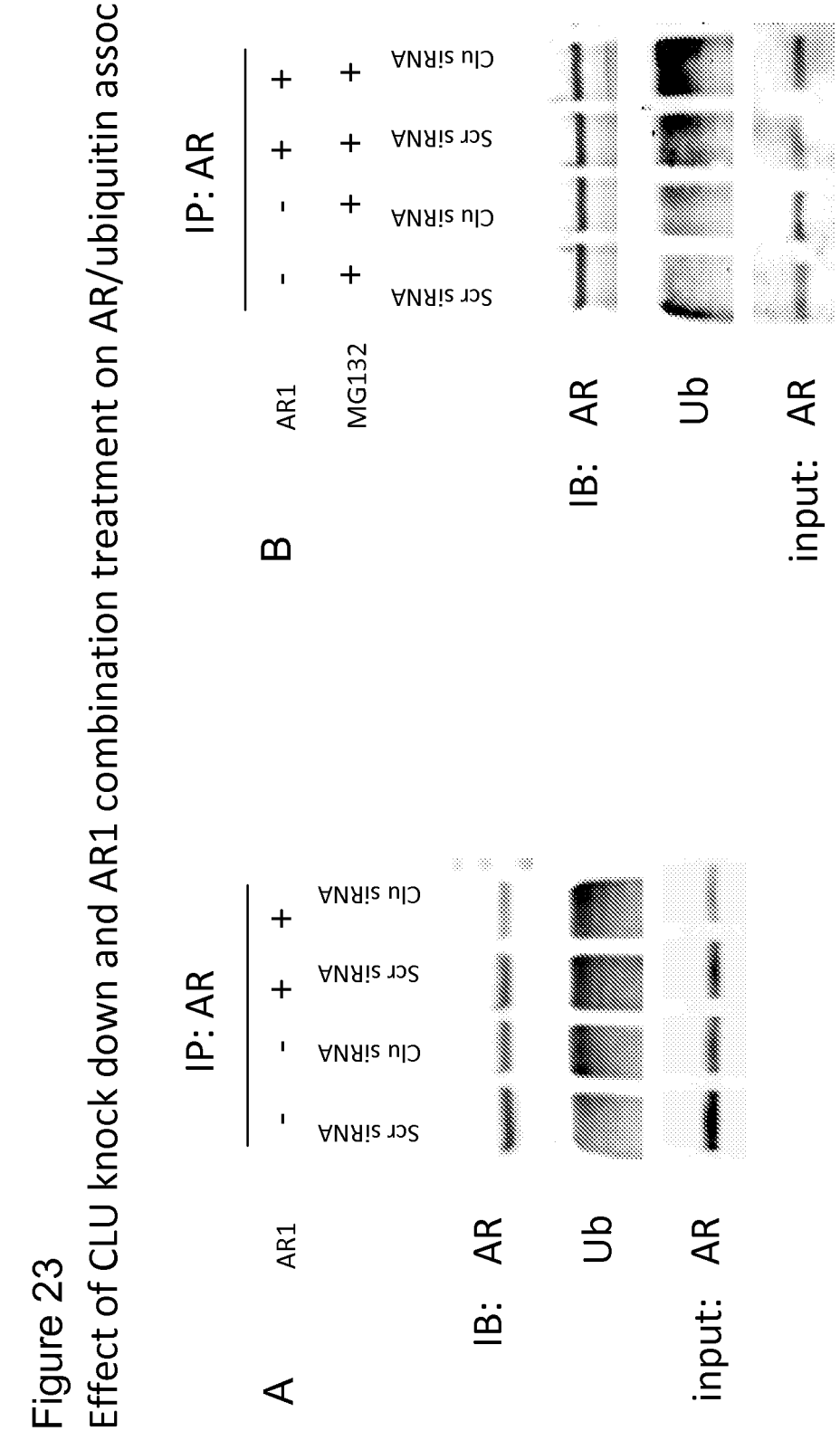


Figure 24

CLU knock down effect combined with bicalutamide or AR1

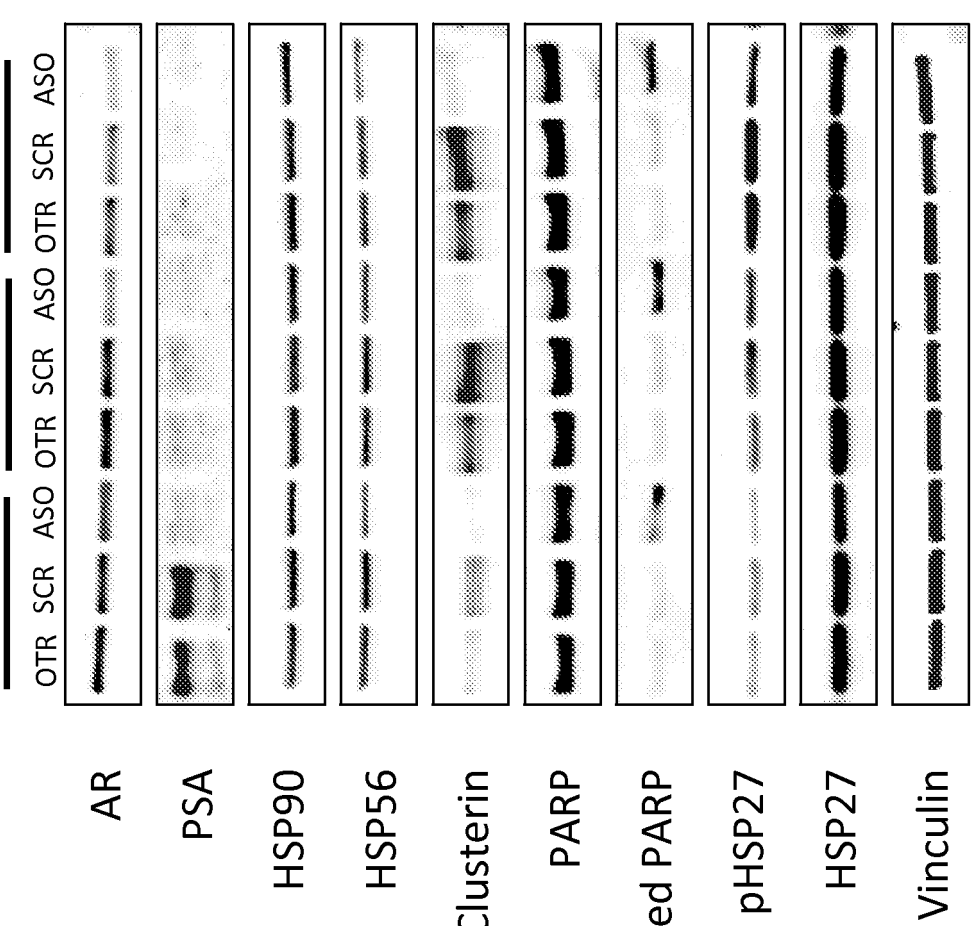


Figure 25

FKBP52 overexpression might rescue the degradation of AR from CLU knock down

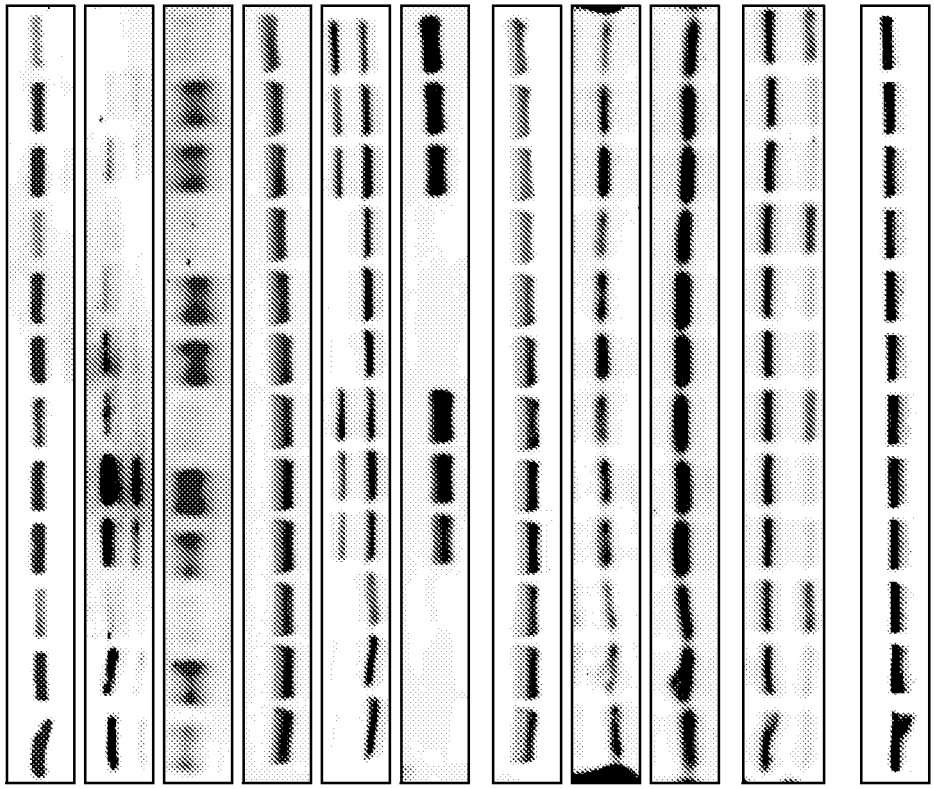
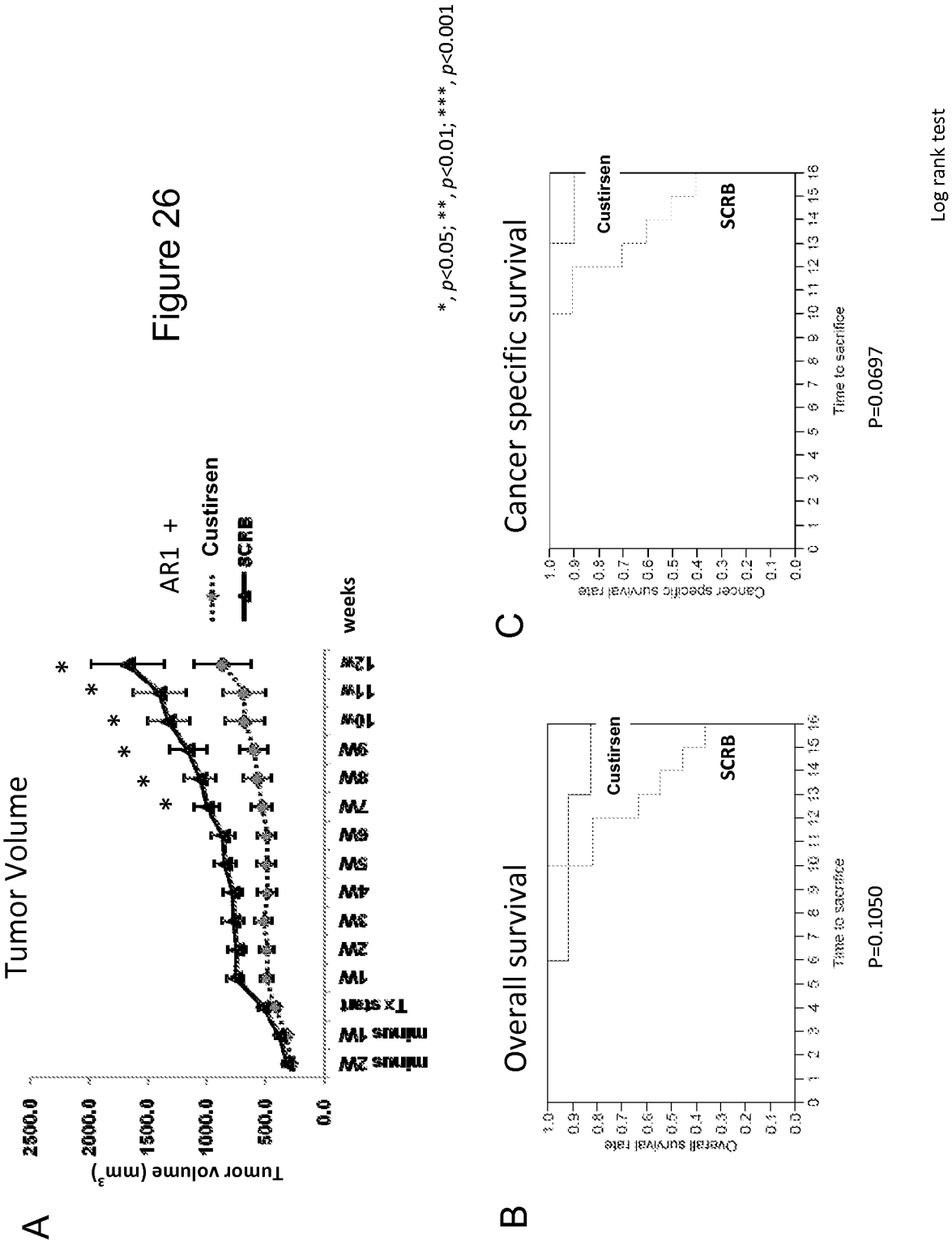


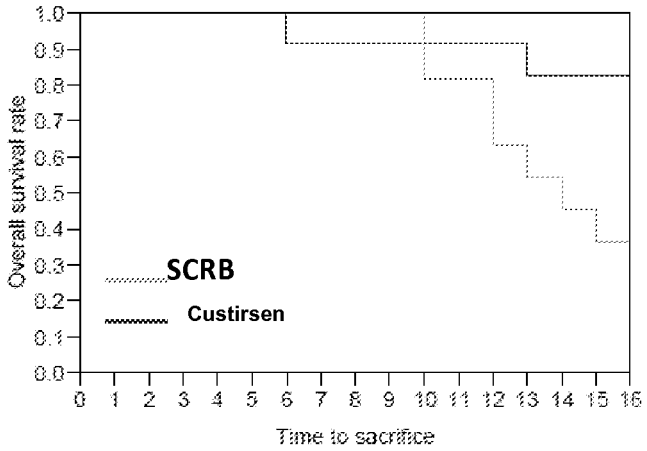
Figure 26


A
Tumor Volume
AR1 +
Custirsen
SCRB
Tumor volume (mm³)
2500.0
2000.0
1500.0
1000.0
500.0
0.0
weeks
minus 2W
minus 1W
Tx start
1W
2W
3W
4W
5W
6W
7W
8W
9W
10W
11W
12W
*, p<0.05; **, p<0.01; ***, p<0.001
B
Overall survival
Overall survival rate
Time to sacrifice
Custirsen
SCRB
P=0.1050
C
Cancer specific survival
Cancer specific survival rate
Time to sacrifice
Custirsen
SCRB
P=0.0697
Log rank test

In vivo mouse model survival curve

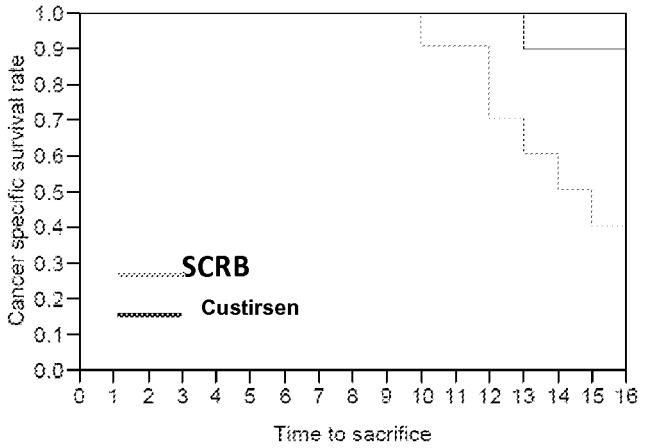
Figure 27

A Overall survival



P=0.1050

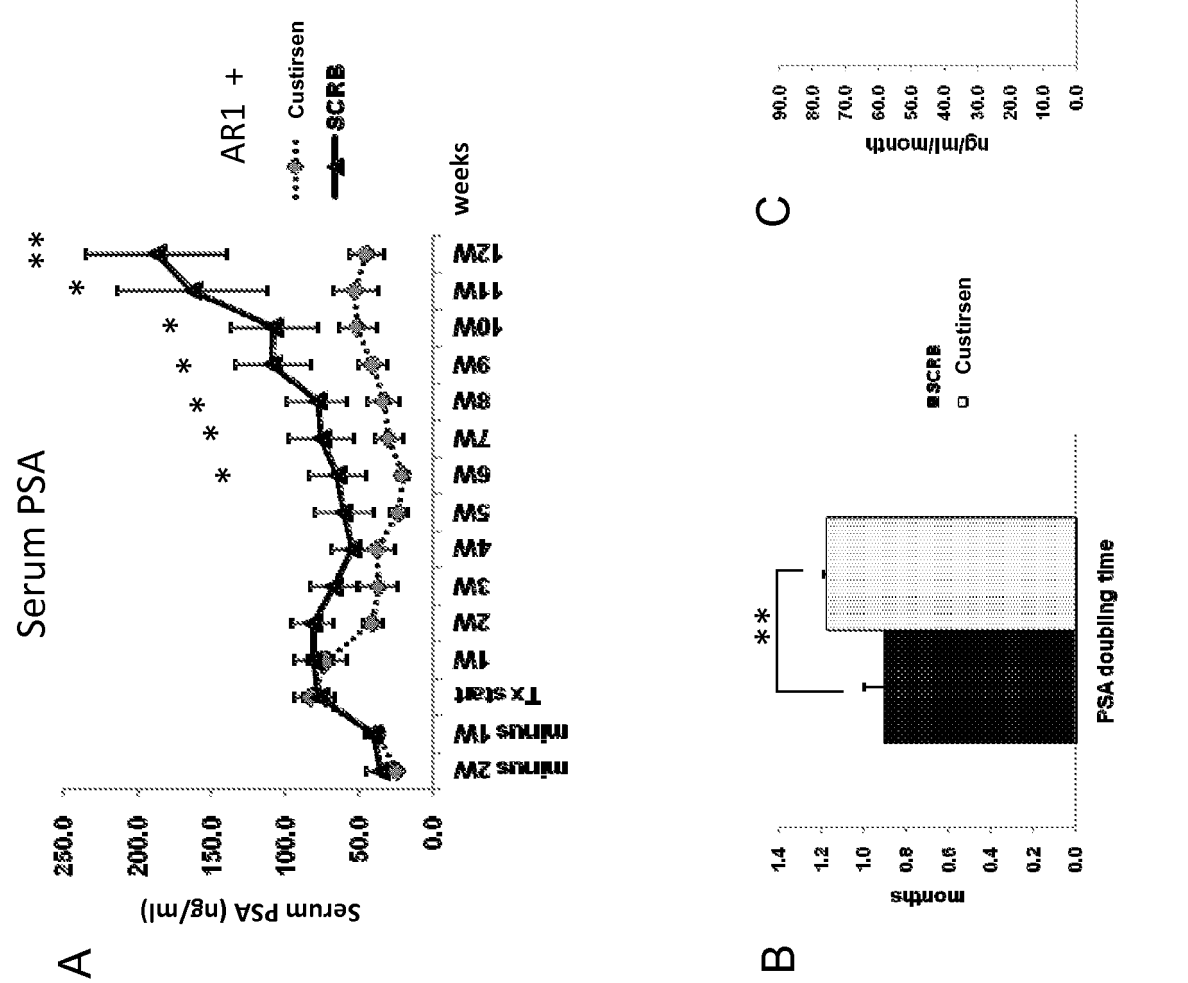
B Cancer specific survival



P=0.0697

Log rank test

Figure 28



*, $p<0.05$; **, $p<0.01$; ***, $p<0.001$

Figure 29

Protein expression in mice tissue after 7 days of treatment

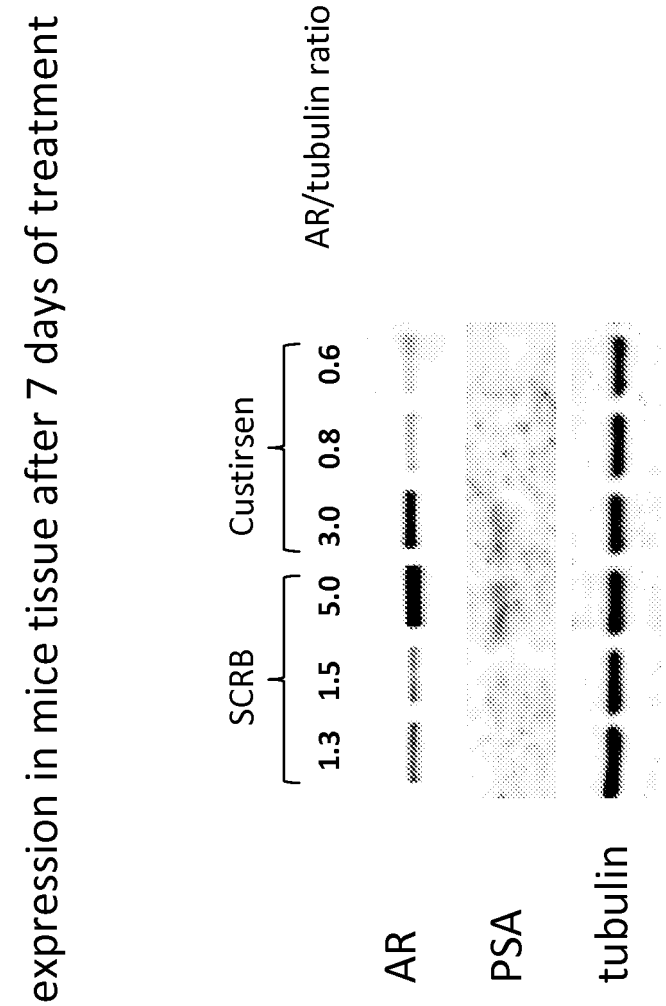
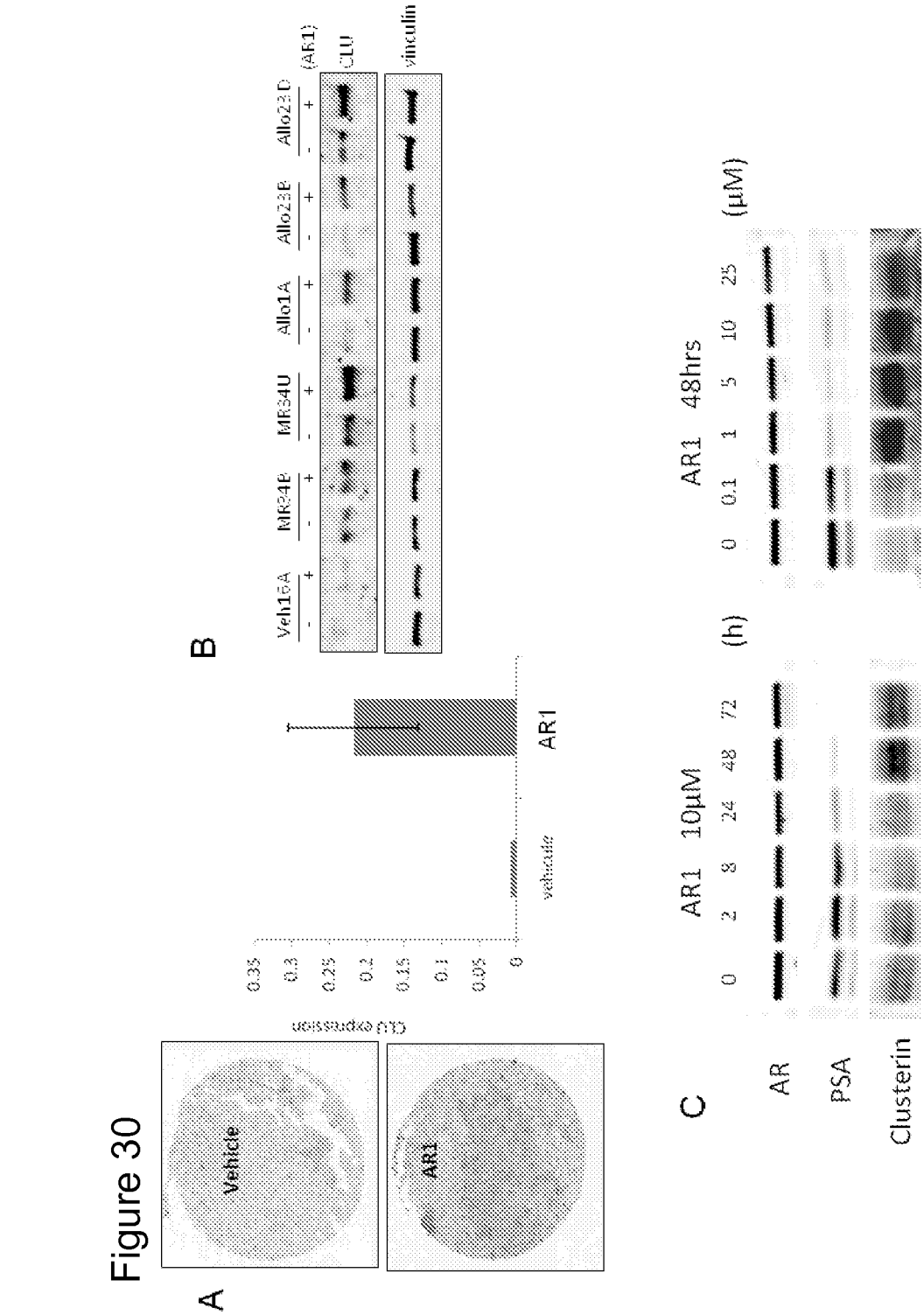


Figure 30


A
Vehicle
AR1
CLU expression
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
vehicle
AR1
B
Veh16A
MF34E
MF34U
Allo1A
Allo23E
Allo23D
(AR1)
CLU
vinculin
C
AR1 10μM
0 2 8 24 48 72 (h)
AR1 48hrs
0 0.1 1 5 10 25 (μM)
AR
PSA
Clusterin

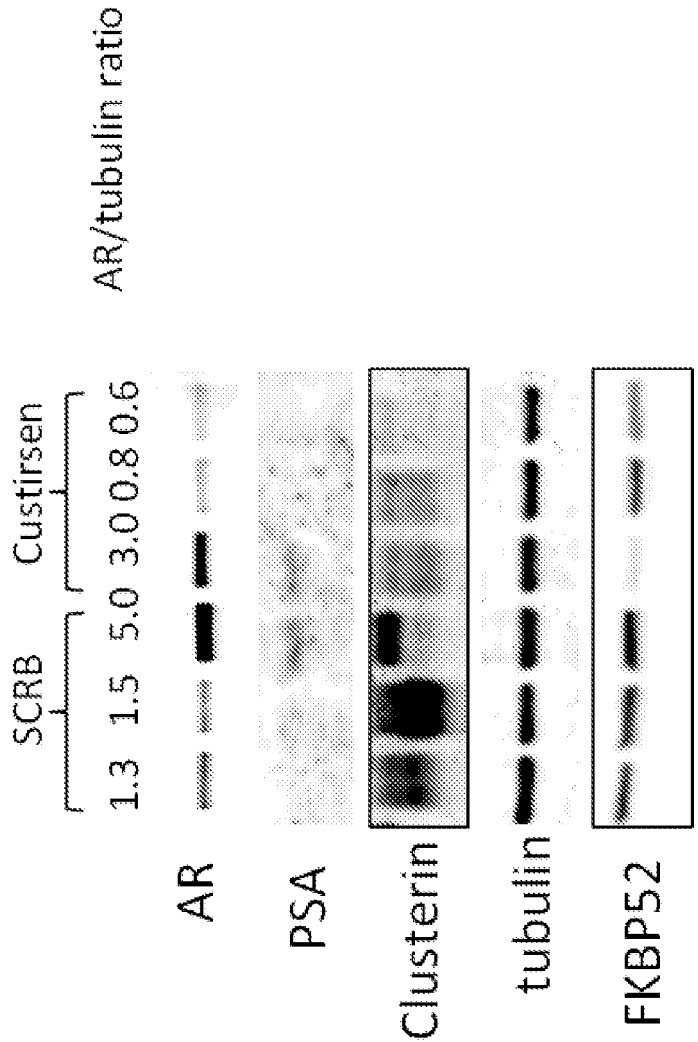

SCRB
Custirsen
1.3 1.5 5.0 3.0 0.8 0.6
AR/tubulin ratio
AR
PSA
Clusterin
tubulin
FKBP52

Figure 31

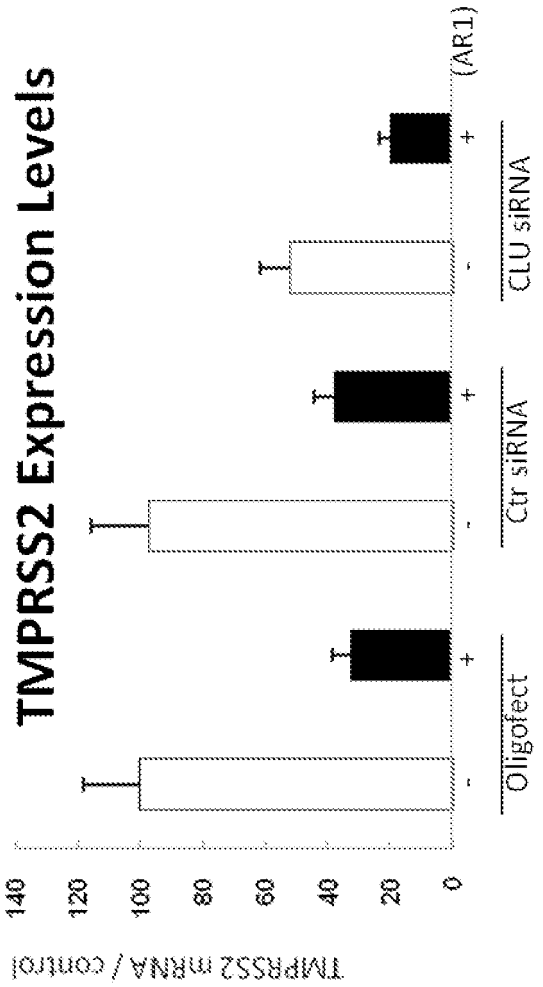

TMPRSS2 Expression Levels
TMPRSS2 mRNA / control
140
120
100
80
60
40
20
0
Oligofect
Ctr siRNA
CLU siRNA
(AR1)
-
+
-
+
-
+

Figure 32

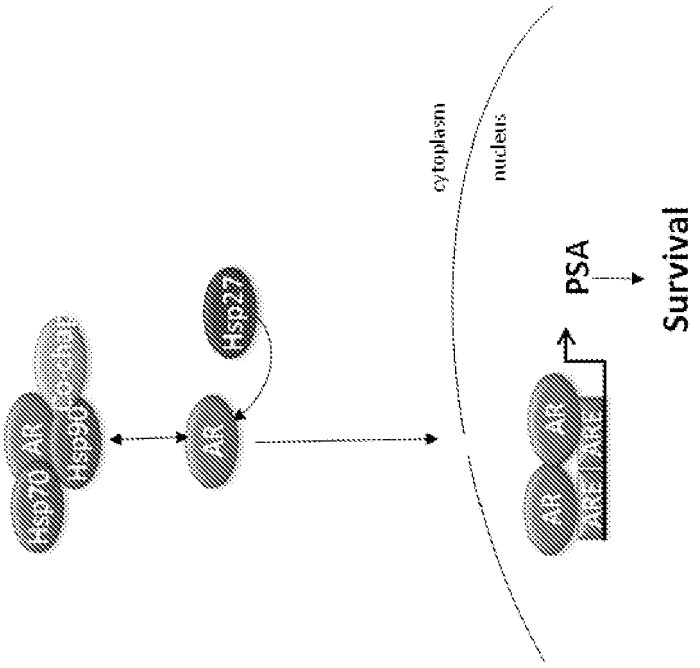

cytoplasm
nucleus
PSA
Survival
AR
AR
ARE
AR
Hsp27
Hsp70
Hsp90

Figure 33

Figure 34

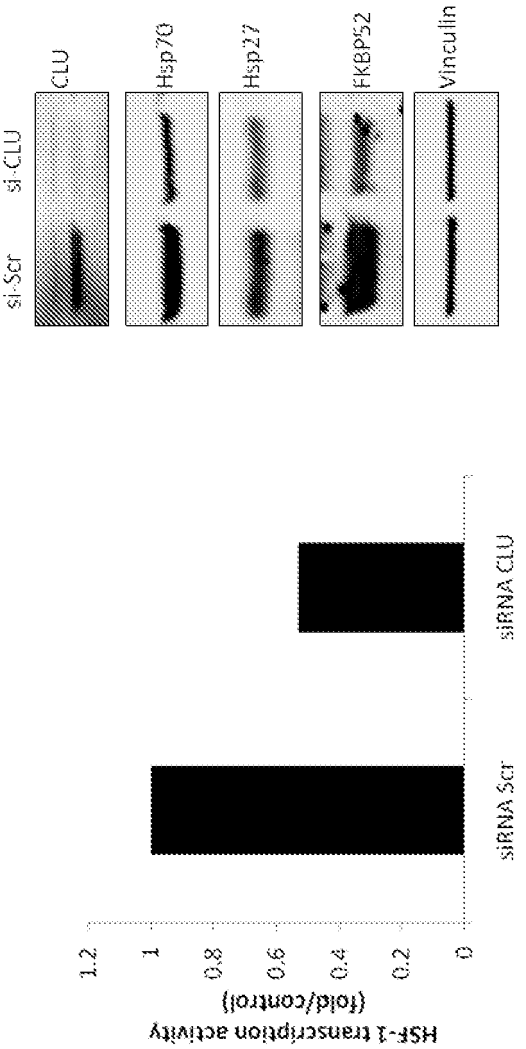

si-Scr
si-CLU
CLU
Hsp70
Hsp27
FKBP52
Vinculin
HSF-1 transcription activity
(fold/control)
1.2
1
0.8
0.6
0.4
0.2
0
siRNA Scr
siRNA CLU

Figure 35

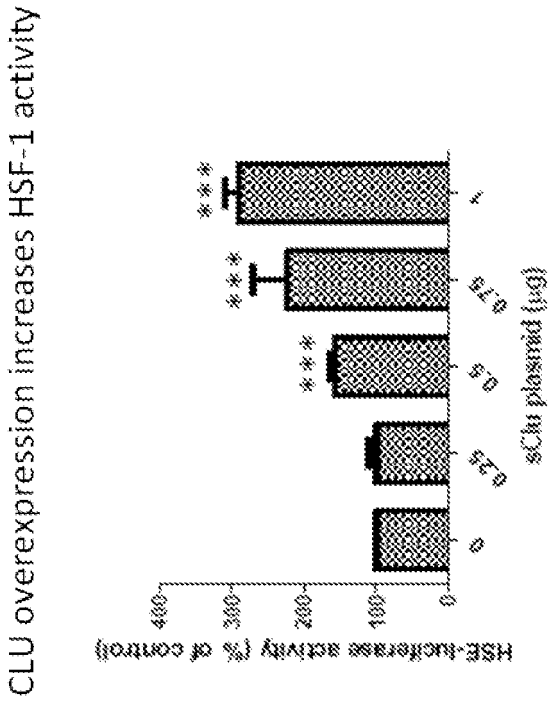

CLU overexpression increases HSF-1 activity
HSE-luciferase activity (% of control)
400
300
200
100
0
***
***
***
***
0
50
100
200
sClu plasmid (µg)

Figure 36

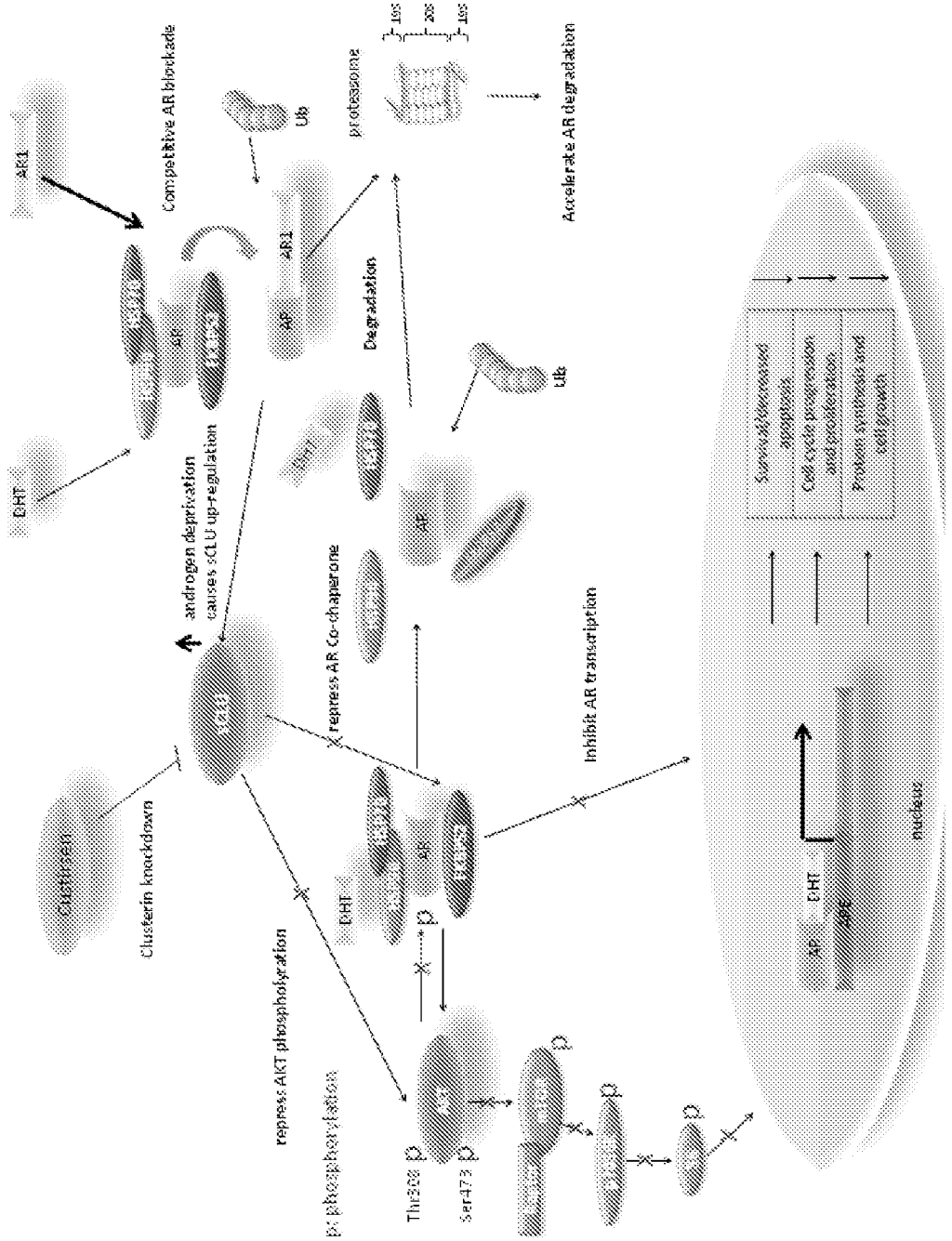

AR1
Competitive AR blockade
Ub
proteasome
DHT
androgen deprivation
causes sCLU up-regulation
Degradation
Accelerate AR degradation
Ub
repress AR Co-chaperone
Inhibit AR transcription
Survival/decreased apoptosis
Cell cycle progression and proliferation
Protein synthesis and cell growth
nucleus
Clusterin
Clusterin knockdown
repress AKT phosphorylation
P= phosphorylation
Thr308
Ser473
DHT
AR

Figure 37

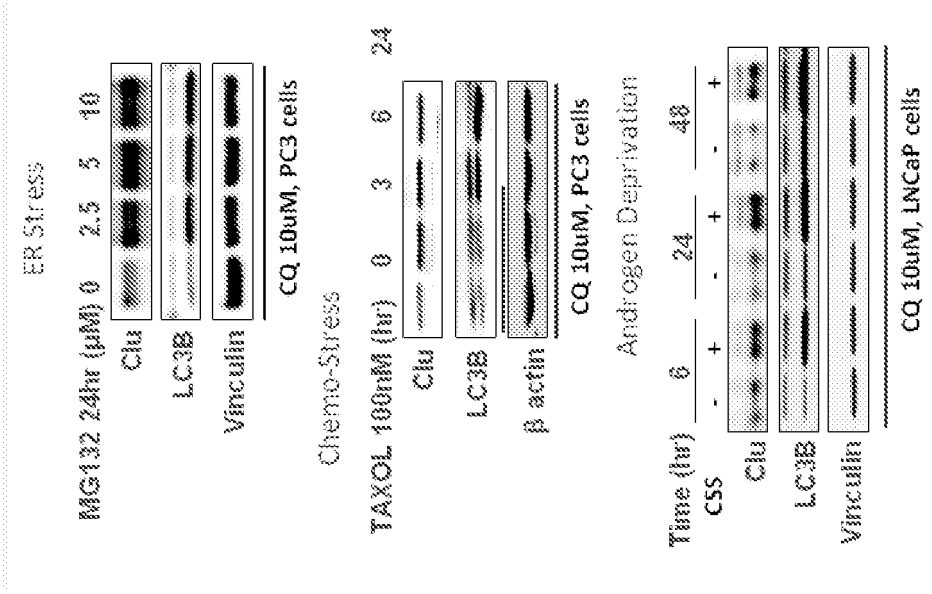

ER Stress
MG132 24hr (μM) 0 2.5 5 10
Clu
LC3B
Vinculin
CQ 10uM, PC3 cells
Chemo-Stress
TAXOL 100nM (hr) 0 3 6 24
Clu
LC3B
β actin
CQ 10uM, PC3 cells
Androgen Deprivation
Time (hr) 6 24 48
CSS - + - + - +
Clu
LC3B
Vinculin
CQ 10uM, LNCaP cells

Figure 38

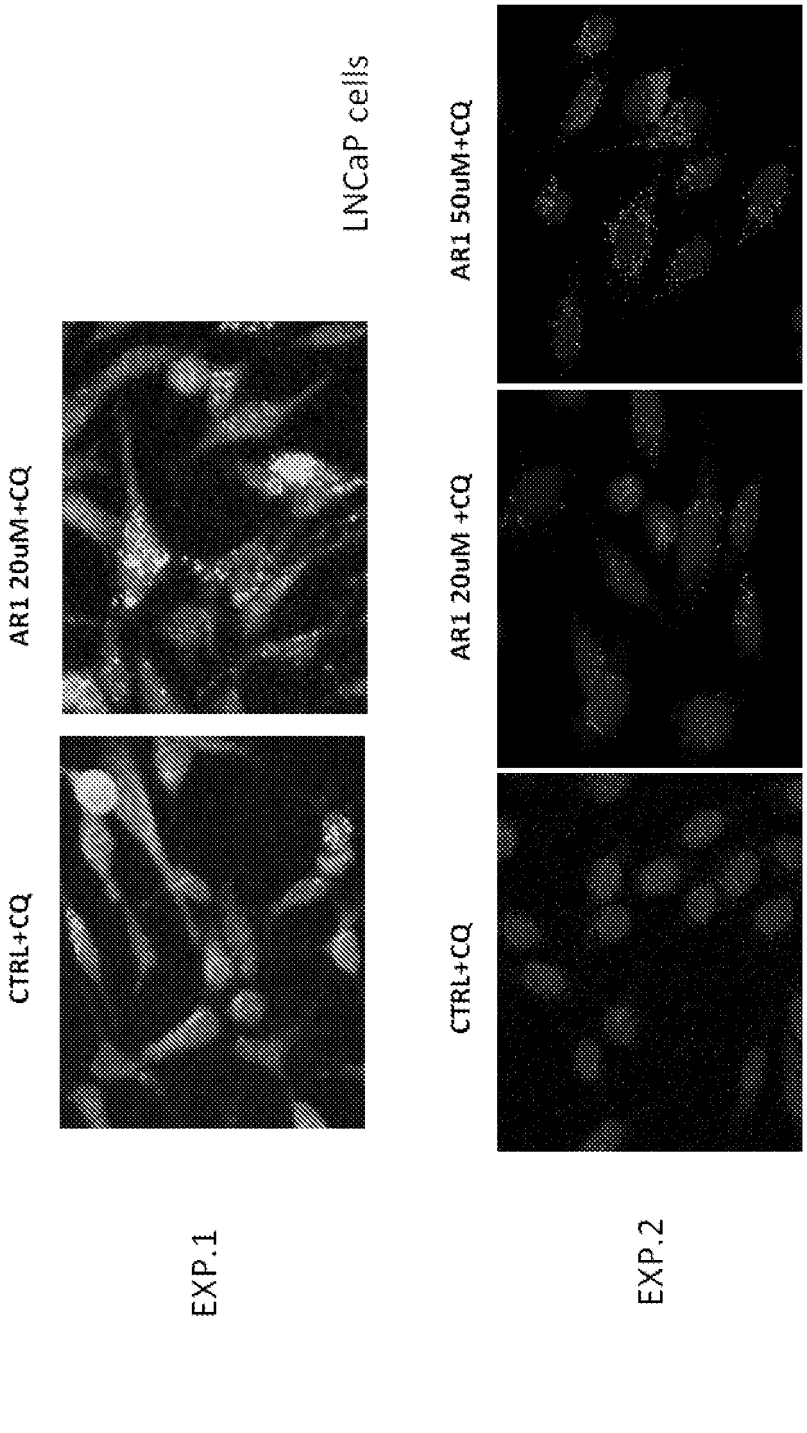

LNCaP cells
CTRL+CQ
AR1 20uM+CQ
EXP.1
CTRL+CQ
AR1 20uM +CQ
AR1 50uM+CQ
EXP.2
CQ: chloroquine, 10uM

Figure 39

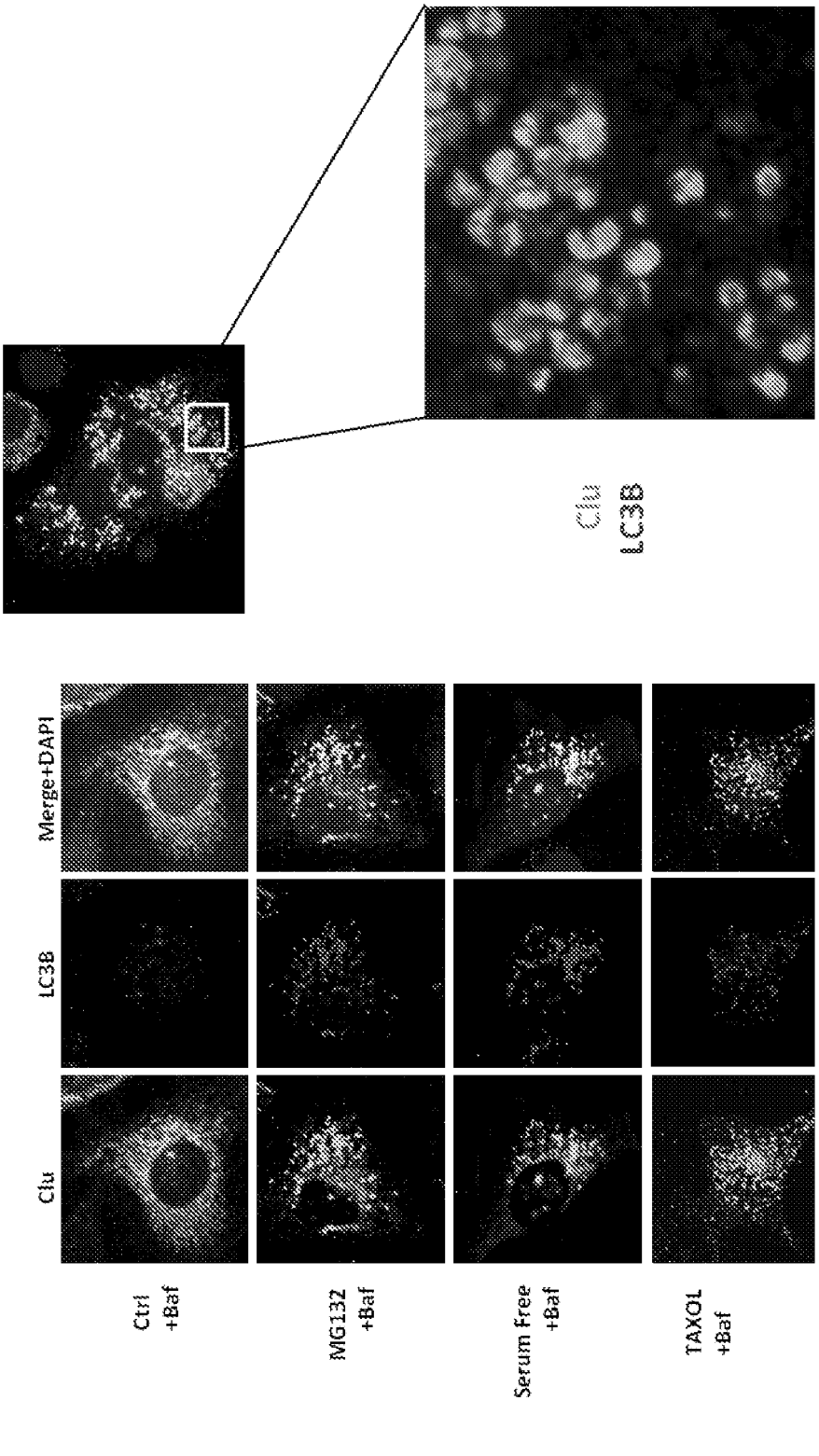

Clu
LC3B
Merge+DAPI
Ctrl +Baf
MG132 +Baf
Serum Free +Baf
TAXOL +Baf

Figure 40

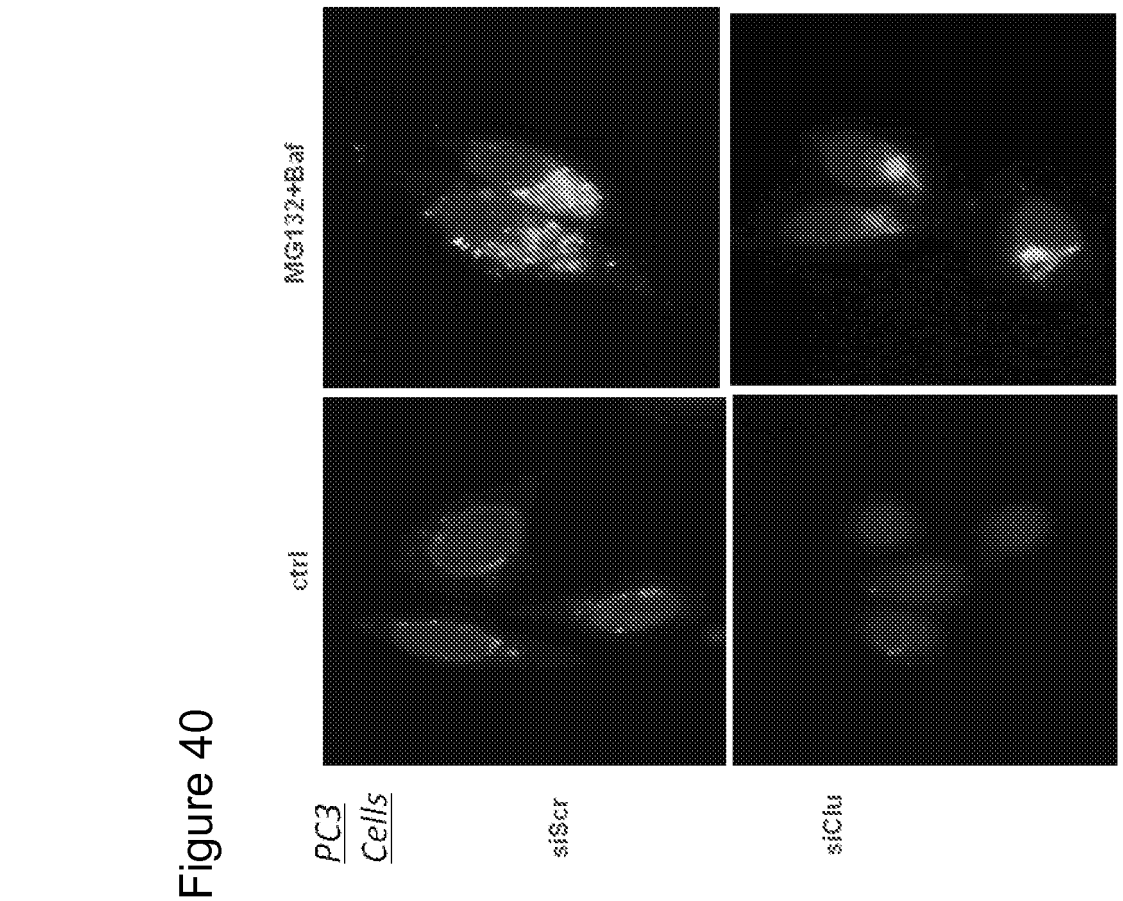

PC3
Cells
ctrl
MG132+Baf
siScr
siClu
siScr (10nM)
siClu (10nM)
Chloroquine
MG132
Clu
LC3B
Vinculin

Figure 41

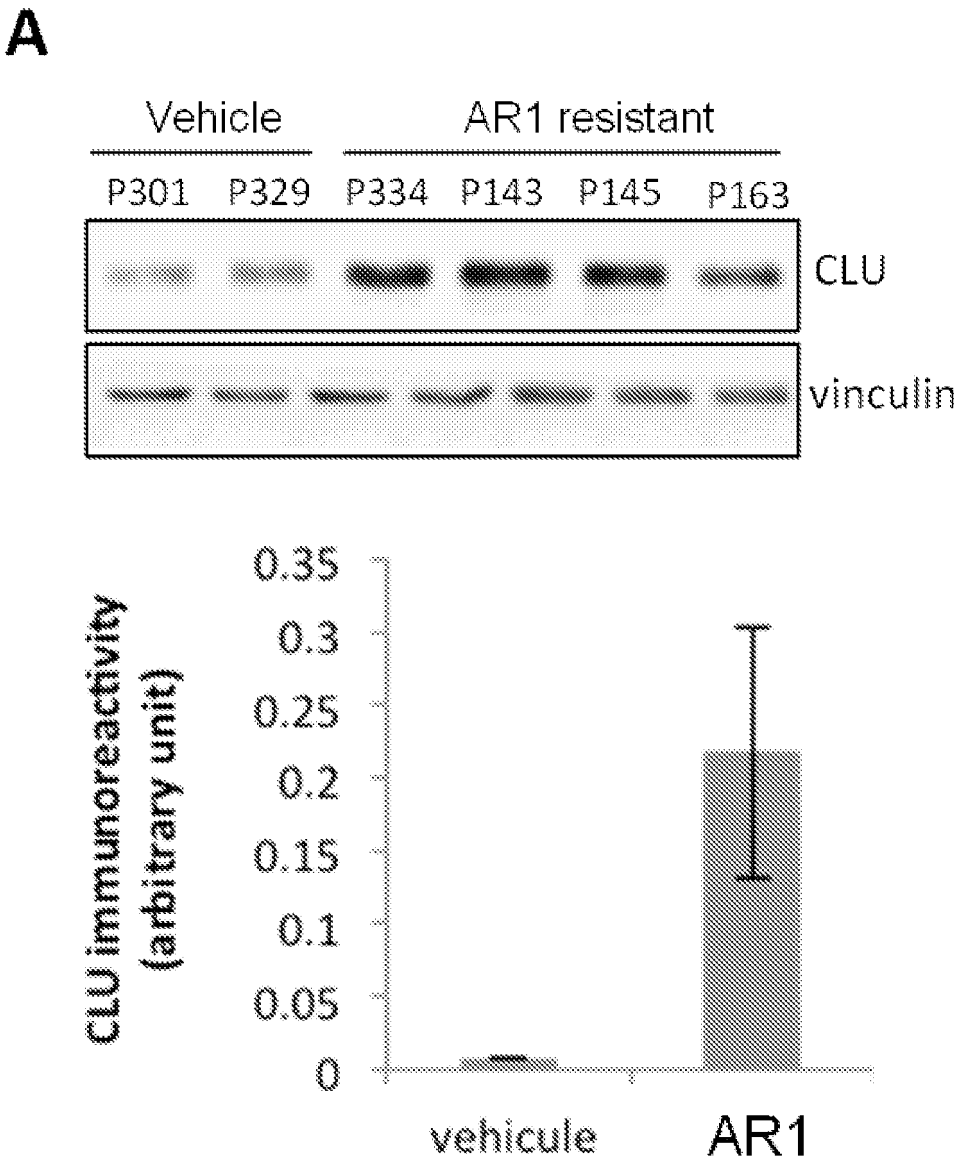

A
Vehicle
AR1 resistant
P301
P329
P334
P143
P145
P163
CLU
vinculin
CLU immunoreactivity (arbitrary unit)
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
vehicule
AR1

Figure 41 (Continued)

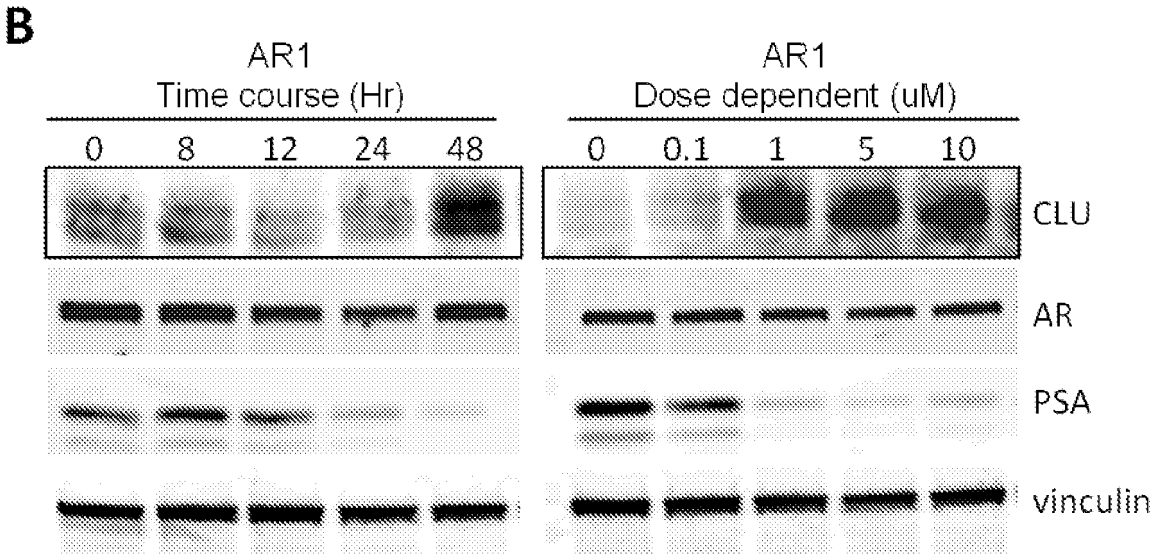


Figure 41 (Continued)

C

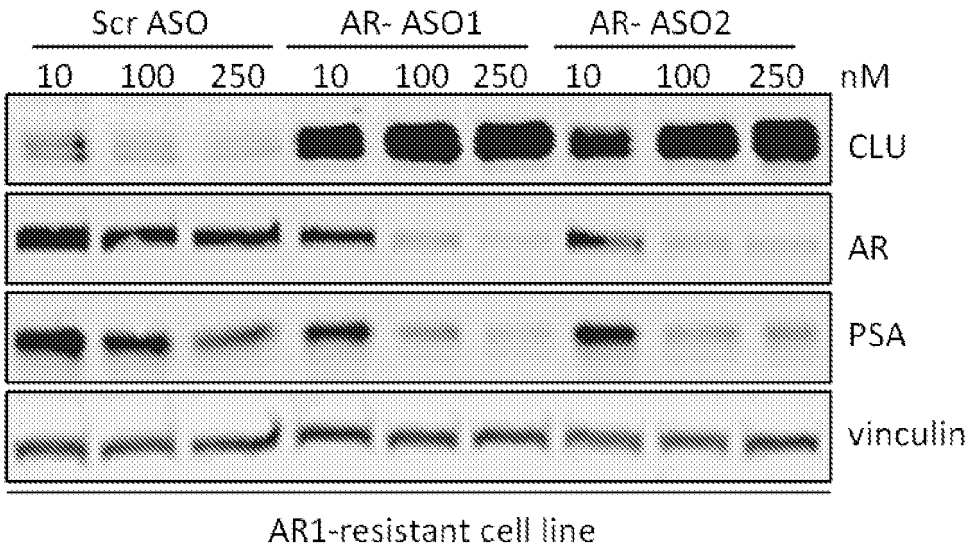


Figure 42

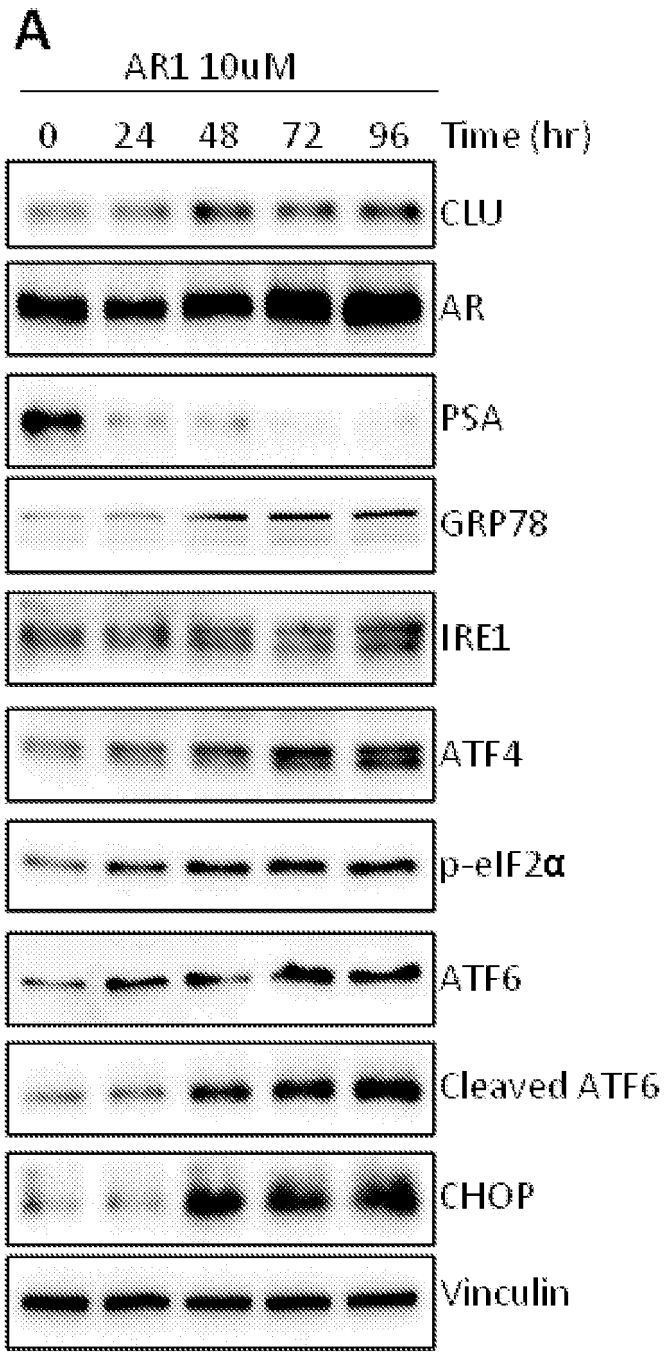

A
AR1 10uM
0
24
48
72
96
Time (hr)
CLU
AR
PSA
GRP78
IRE1
ATF4
p-eIF2α
ATF6
Cleaved ATF6
CHOP
Vinculin

Figure 42 (Continued)

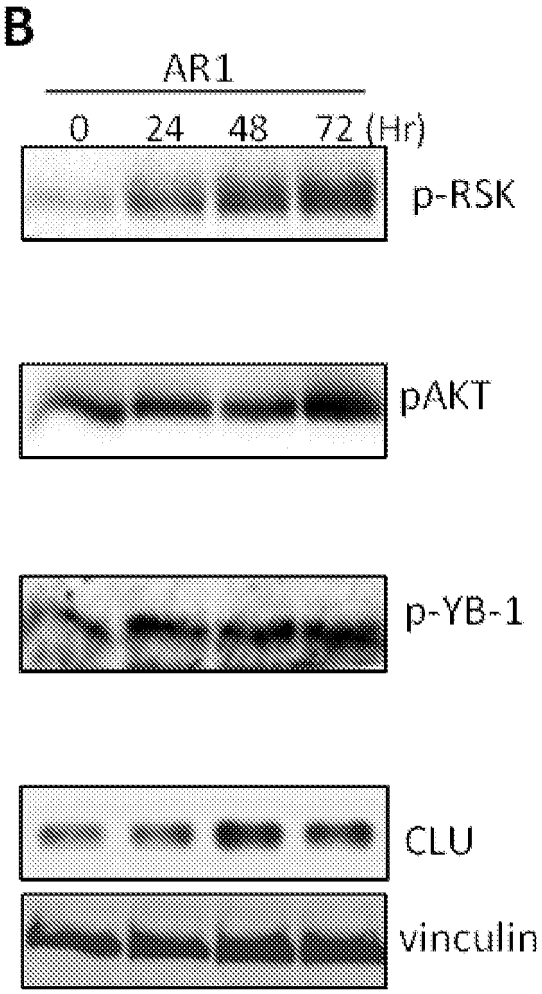


Figure 42 (Continued)

C

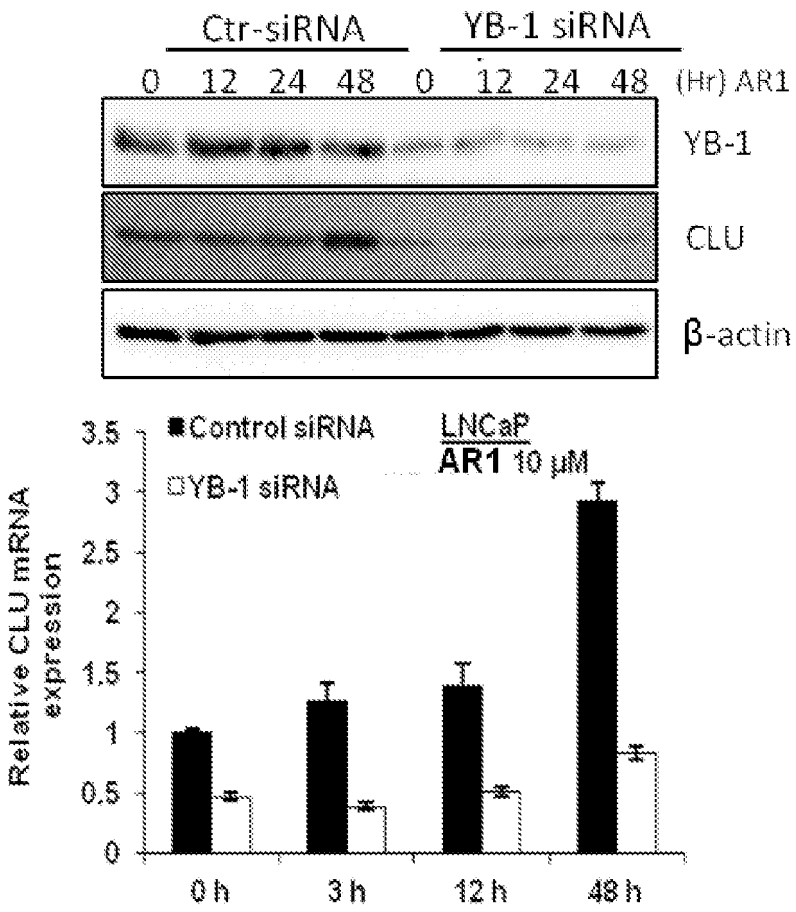


Figure 42 (Continued)

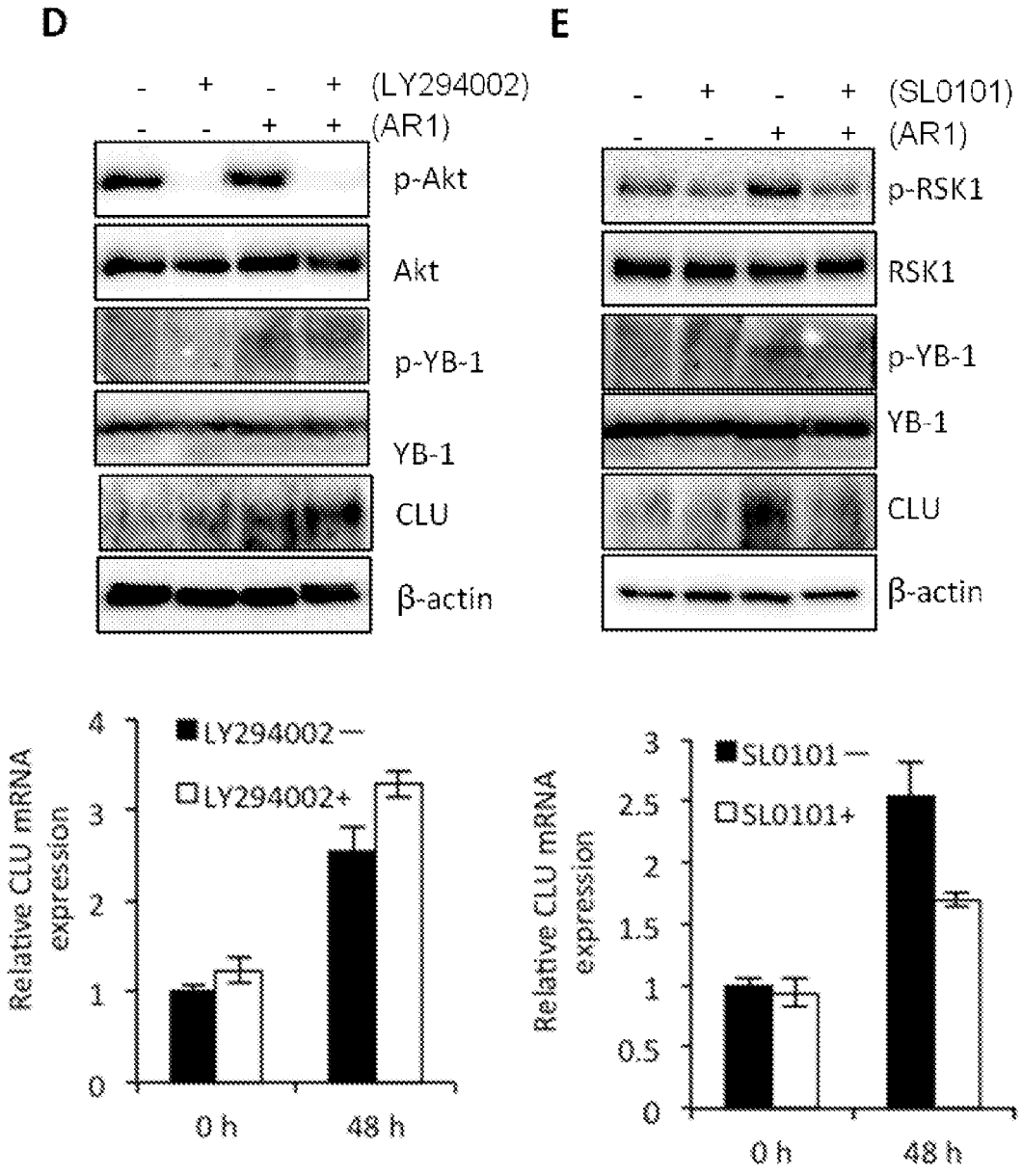


Figure 43

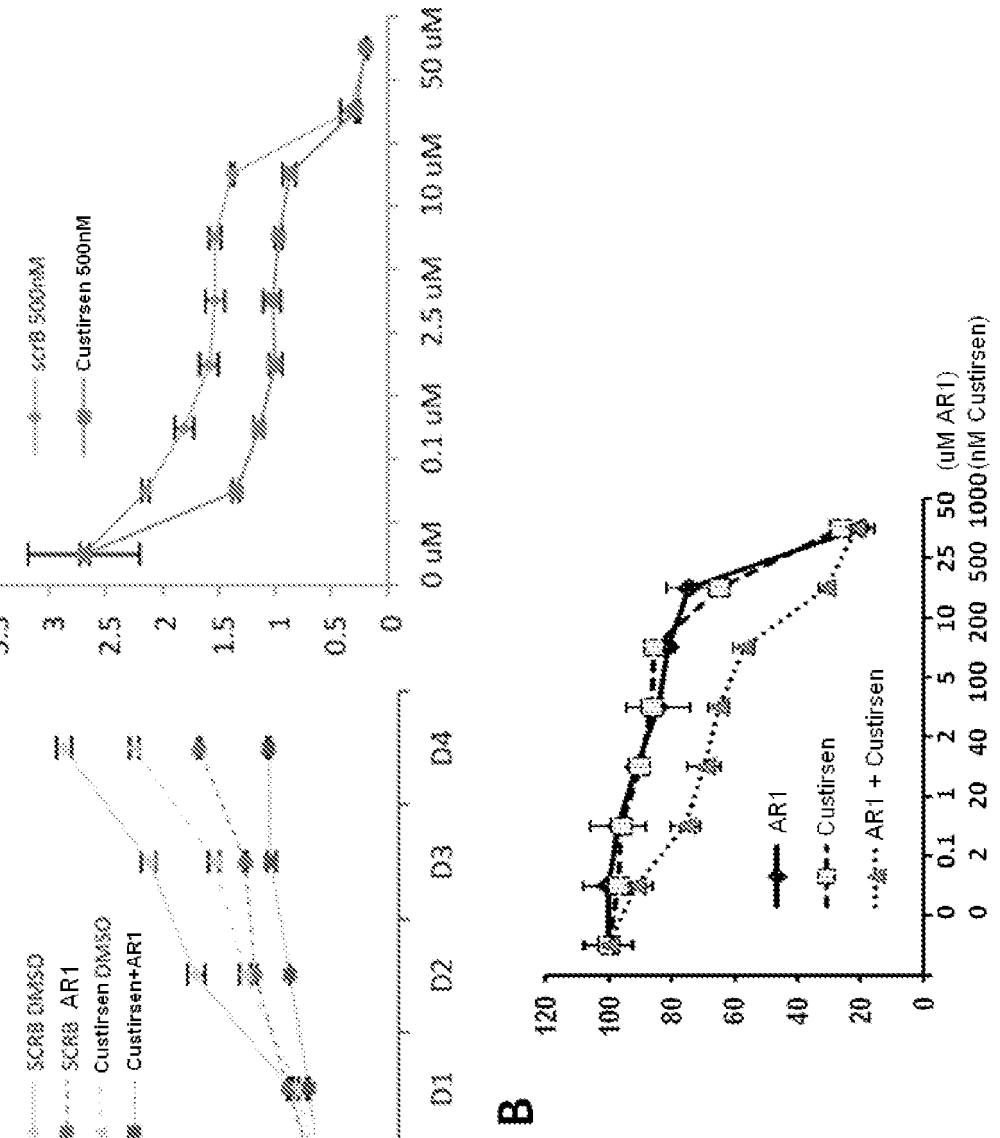

A
% of control
SCRB DMSO
SCRB AR1
Custirsen DMSO
Custirsen+AR1
SCRB 500nM
Custirsen 500nM
D0
D1
D2
D3
D4
0 uM
0.1 uM
2.5 uM
10 uM
50 uM
B
AR1
Custirsen
AR1 + Custirsen
(uM AR1)
(nM Custirsen)

Figure 43 (Continued)

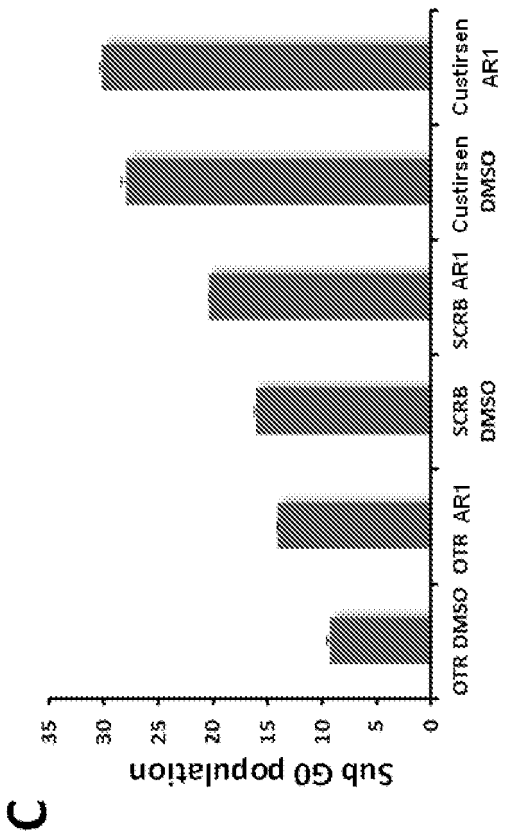


Figure 44

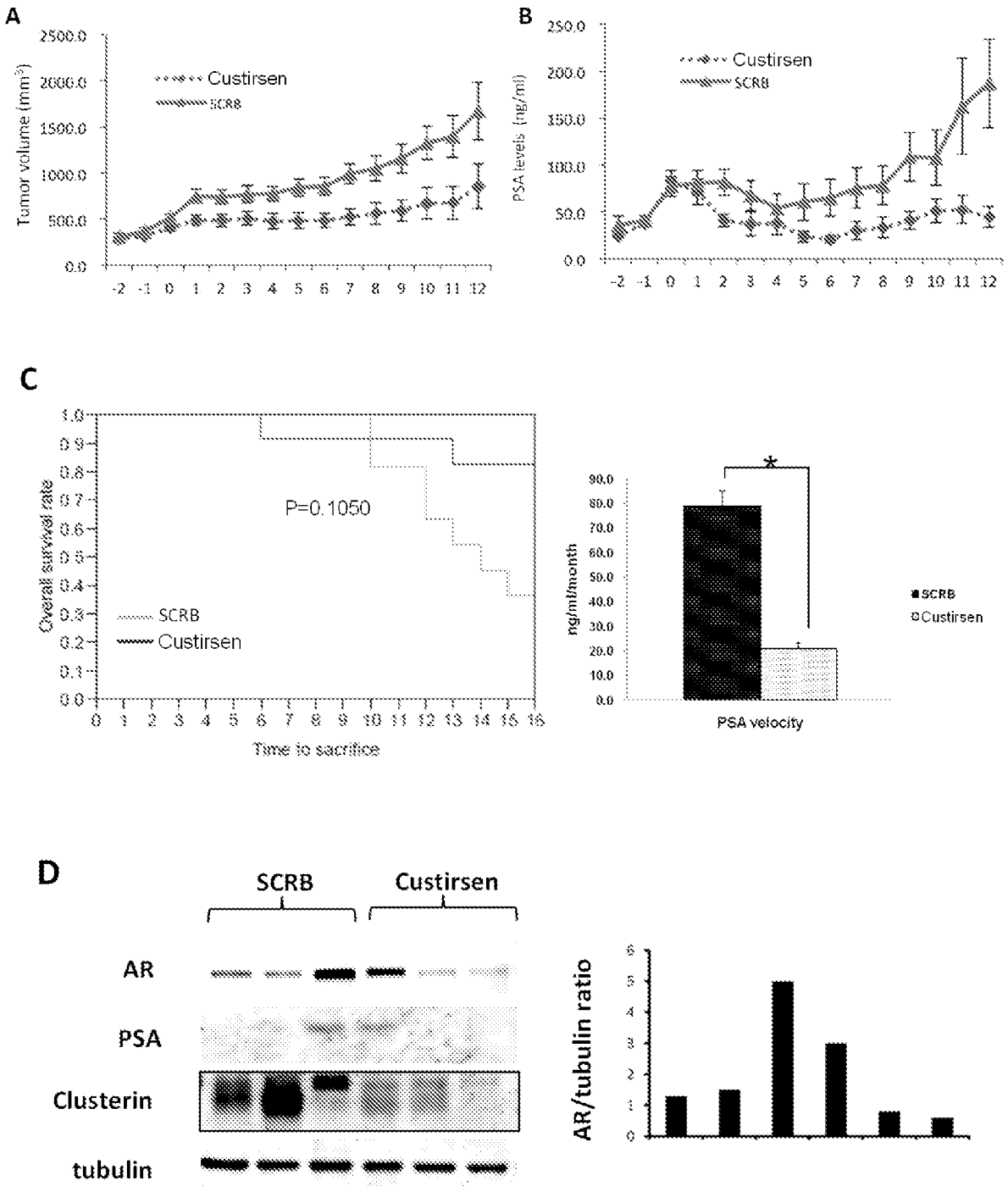

A
Tumor volume (mm³)
Custirsen
SCRB
B
PSA levels (ng/ml)
Custirsen
SCRB
C
Overall survival rate
P=0.1050
SCRB
Custirsen
Time to sacrifice
ng/ml/month
PSA velocity
SCRB
Custirsen
D
SCRB
Custirsen
AR
PSA
Clusterin
tubulin
AR/tubulin ratio

Figure 45

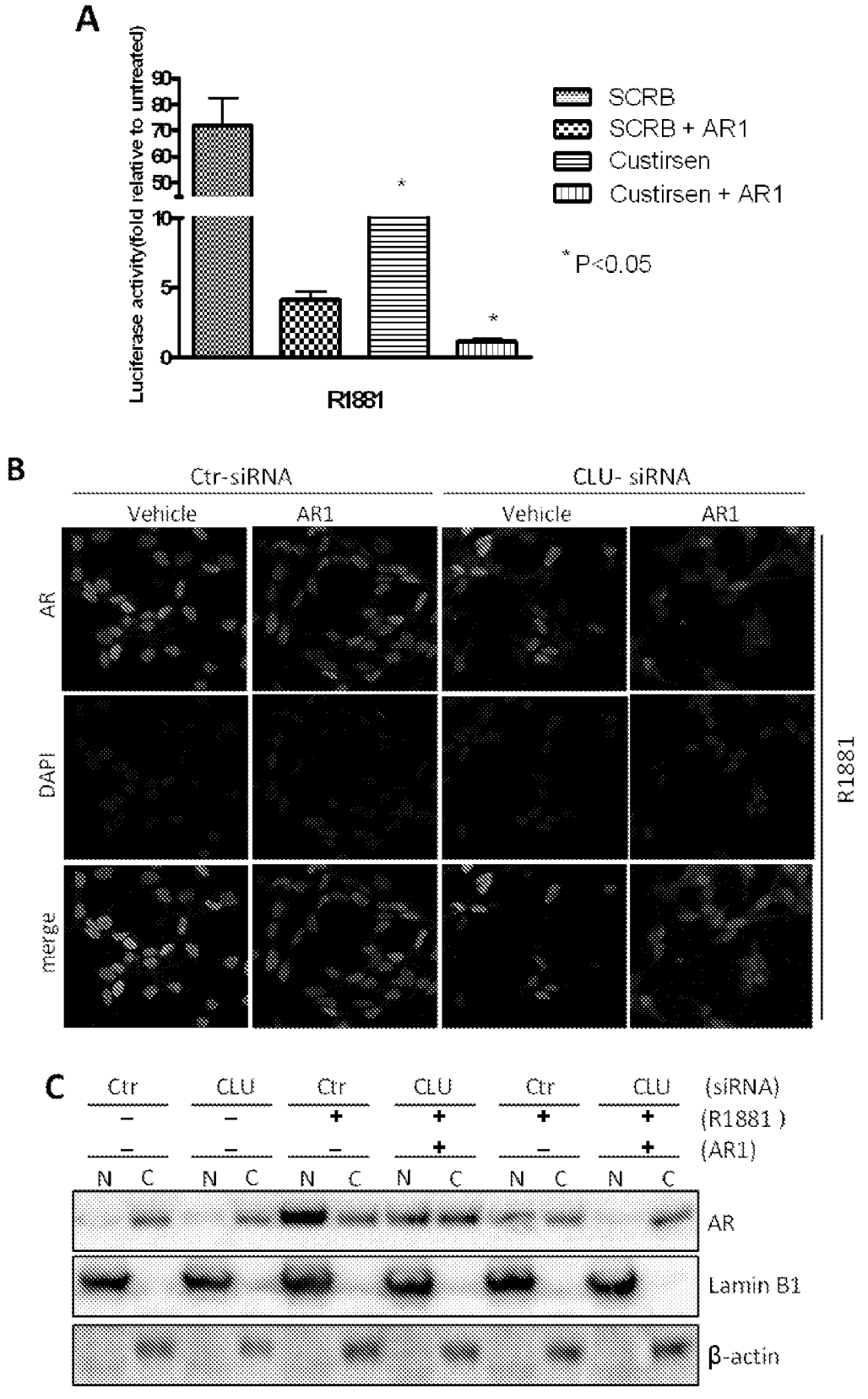

A
Luciferase activity(fold relative to untreated)
90
80
70
60
50
10
5
0
*
*
R1881
SCRB
SCRB + AR1
Custirsen
Custirsen + AR1
*P<0.05
B
Ctr-siRNA
CLU- siRNA
Vehicle
AR1
Vehicle
AR1
AR
DAPI
merge
R1881
C
Ctr
CLU
Ctr
CLU
Ctr
CLU
(siRNA)
(R1881)
(AR1)
N C N C N C N C N C N C
AR
Lamin B1
β-actin

Figure 46

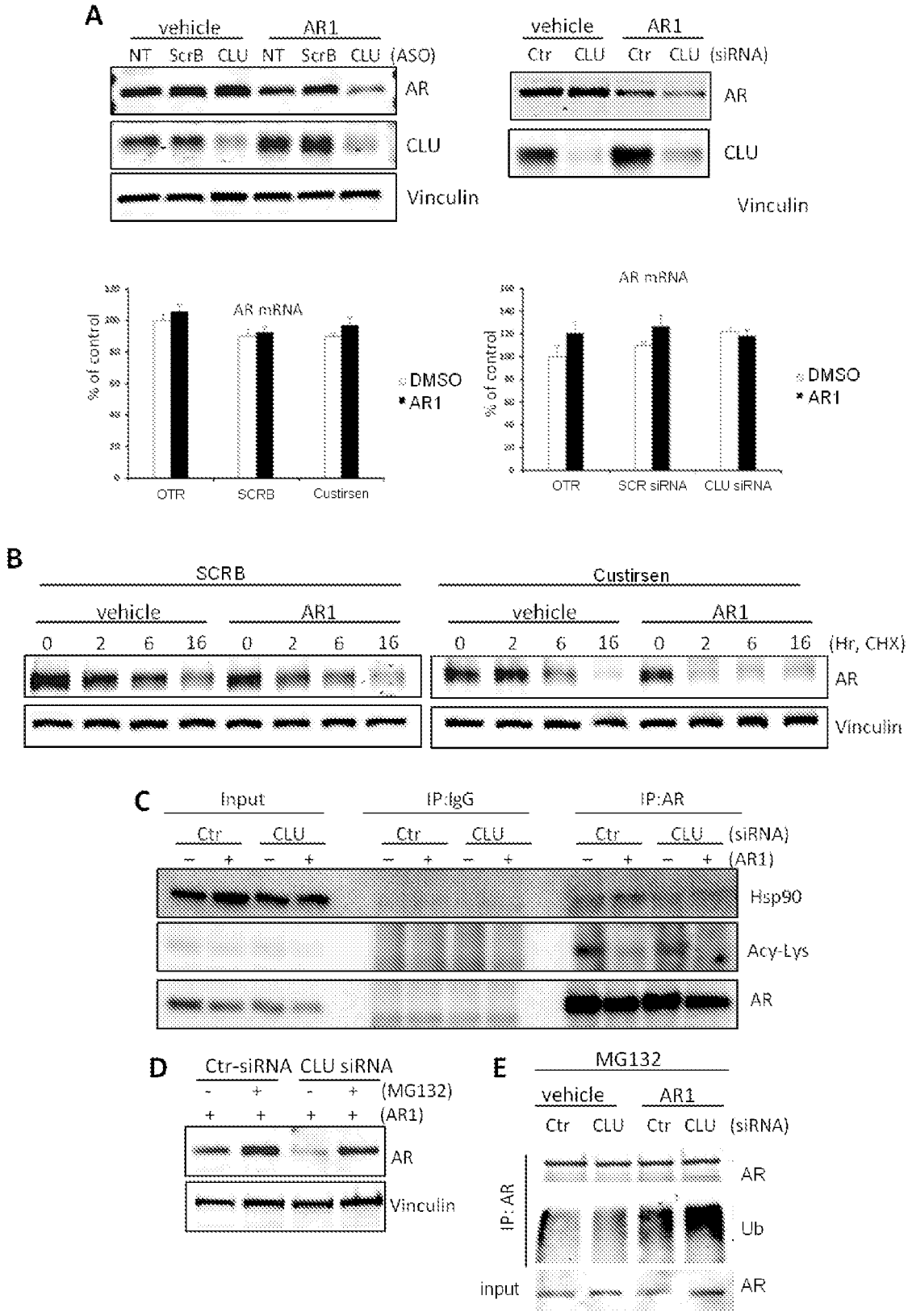
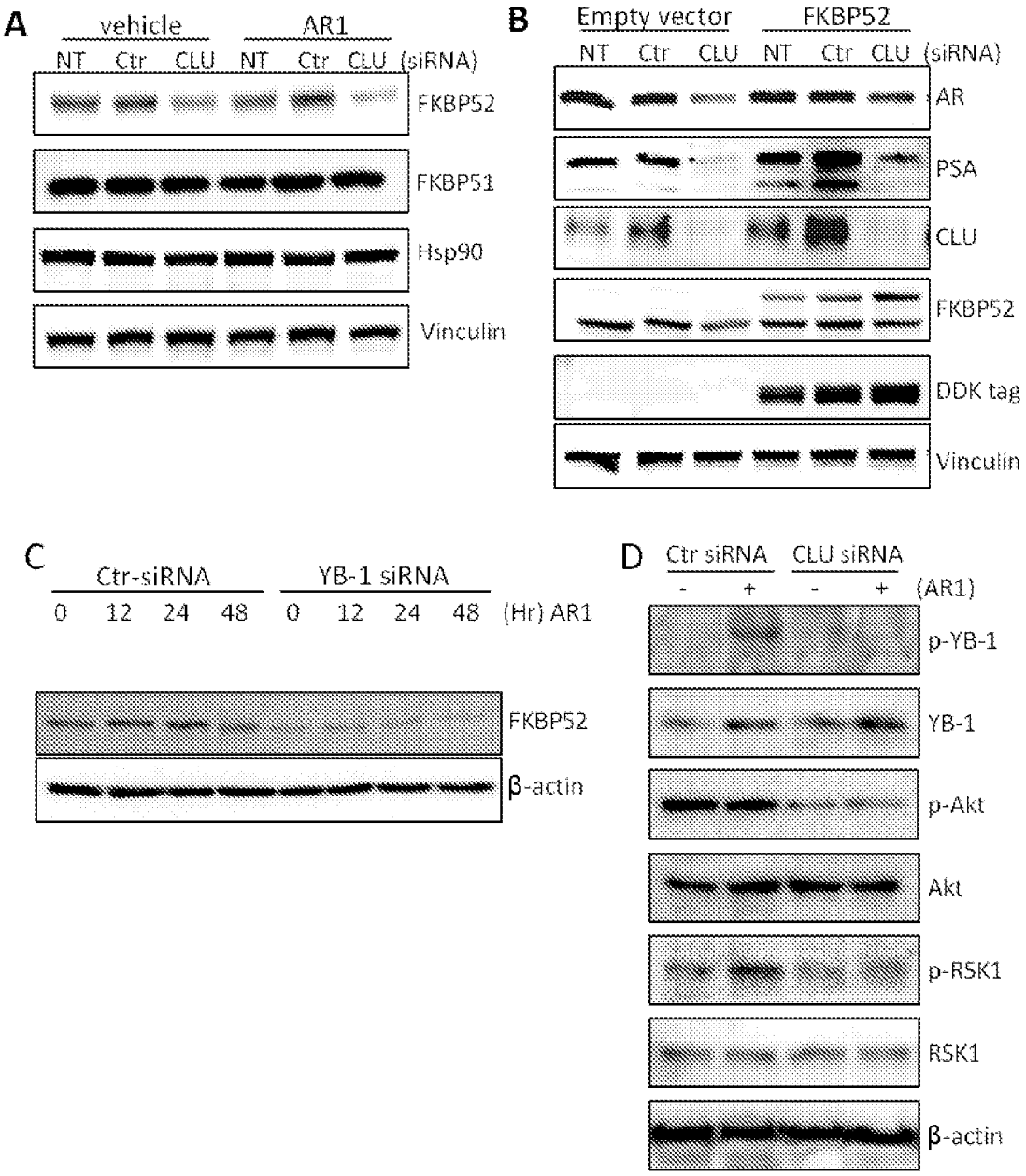

A
vehicle
AR1
NT ScrB CLU NT ScrB CLU (ASO)
AR
CLU
Vinculin
vehicle
AR1
Ctr CLU Ctr CLU (siRNA)
AR
CLU
Vinculin
AR mRNA
% of control
DMSO
AR1
OTR
SCRB
Custirsen
AR mRNA
% of control
DMSO
AR1
OTR
SCR siRNA
CLU siRNA
B
SCRB
Custirsen
vehicle
AR1
0 2 6 16 0 2 6 16 (Hr, CHX)
AR
Vinculin
C
Input
IP:IgG
IP:AR
Ctr CLU Ctr CLU Ctr CLU (siRNA)
(AR1)
Hsp90
Acy-Lys
AR
D
Ctr-siRNA CLU siRNA
(MG132)
(AR1)
AR
Vinculin
E
MG132
vehicle
AR1
Ctr CLU Ctr CLU (siRNA)
IP: AR
AR
Ub
input
AR

Figure 47


A
vehicle
AR1
NT Ctr CLU NT Ctr CLU (siRNA)
FKBP52
FKBP51
Hsp90
Vinculin
B
Empty vector
FKBP52
NT Ctr CLU NT Ctr CLU (siRNA)
AR
PSA
CLU
FKBP52
DDK tag
Vinculin
C
Ctr-siRNA
YB-1 siRNA
0 12 24 48 0 12 24 48 (Hr) AR1
FKBP52
β-actin
D
Ctr siRNA
CLU siRNA
- + - + (AR1)
p-YB-1
YB-1
p-Akt
Akt
p-RSK1
RSK1
β-actin