TC İSTANBUL KÜLTÜR ÜNİVERSİTESİ

TÜBİTAK 112T433

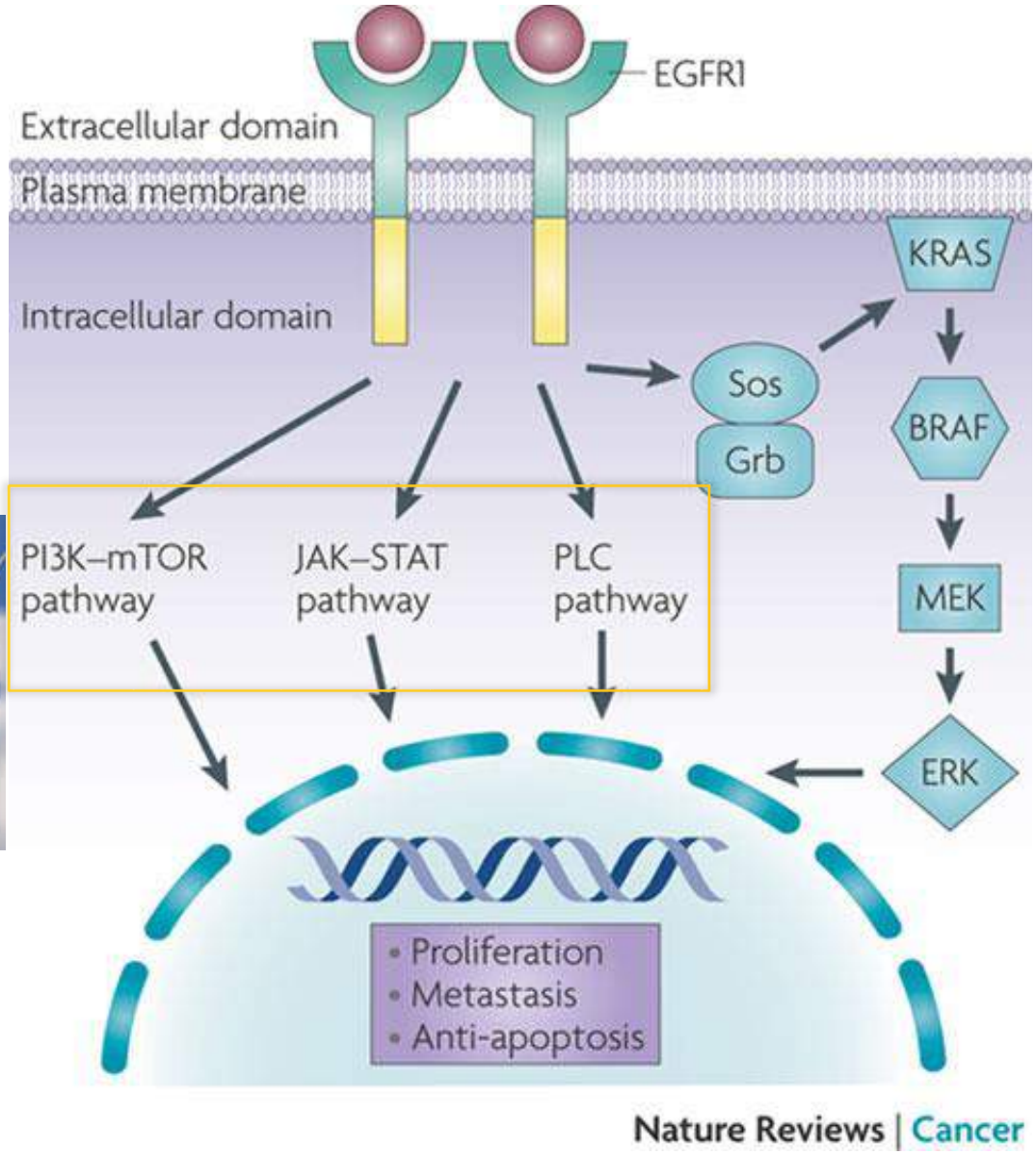
Roscovitine treatment inhibited cell proliferation and induced apoptosis via modulating activity of Stat3 and Foxo1 under control of AKT in LNCaP prostate cancer cells

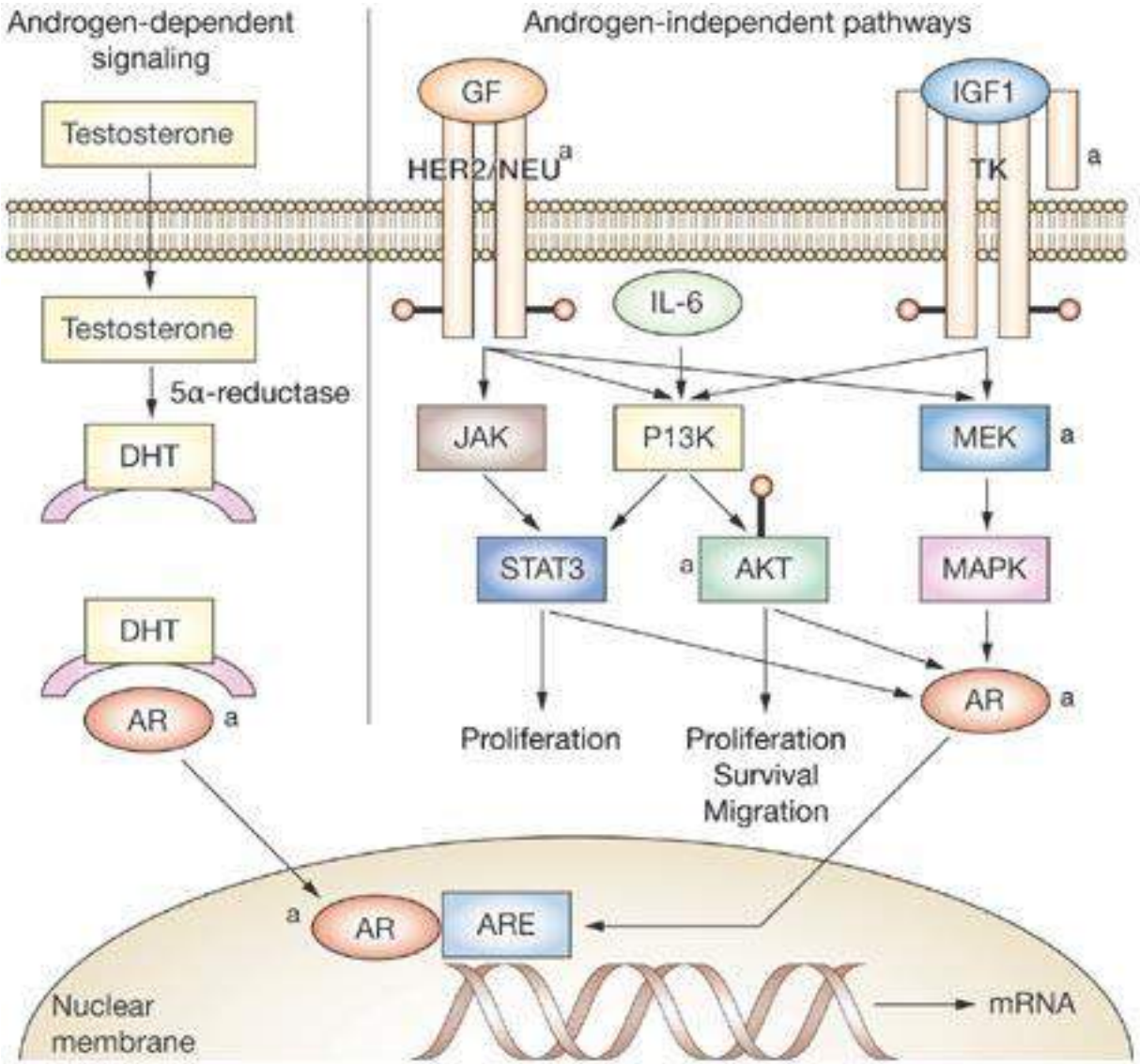
Elif Damla ARISAN, PhD

Istanbul Kultur University

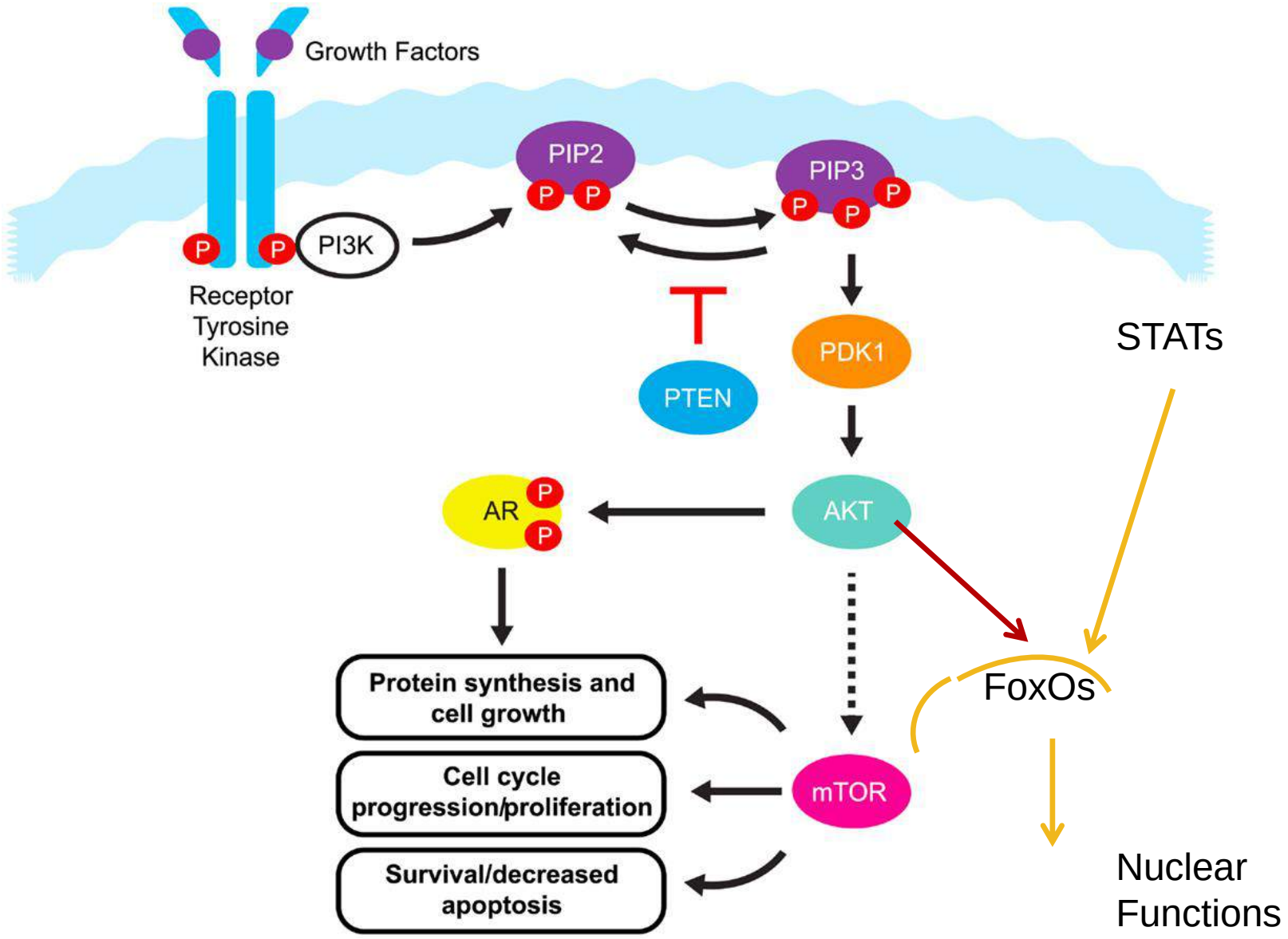
Dept of Molecular Biology and Genetics, Istanbul-Turkey



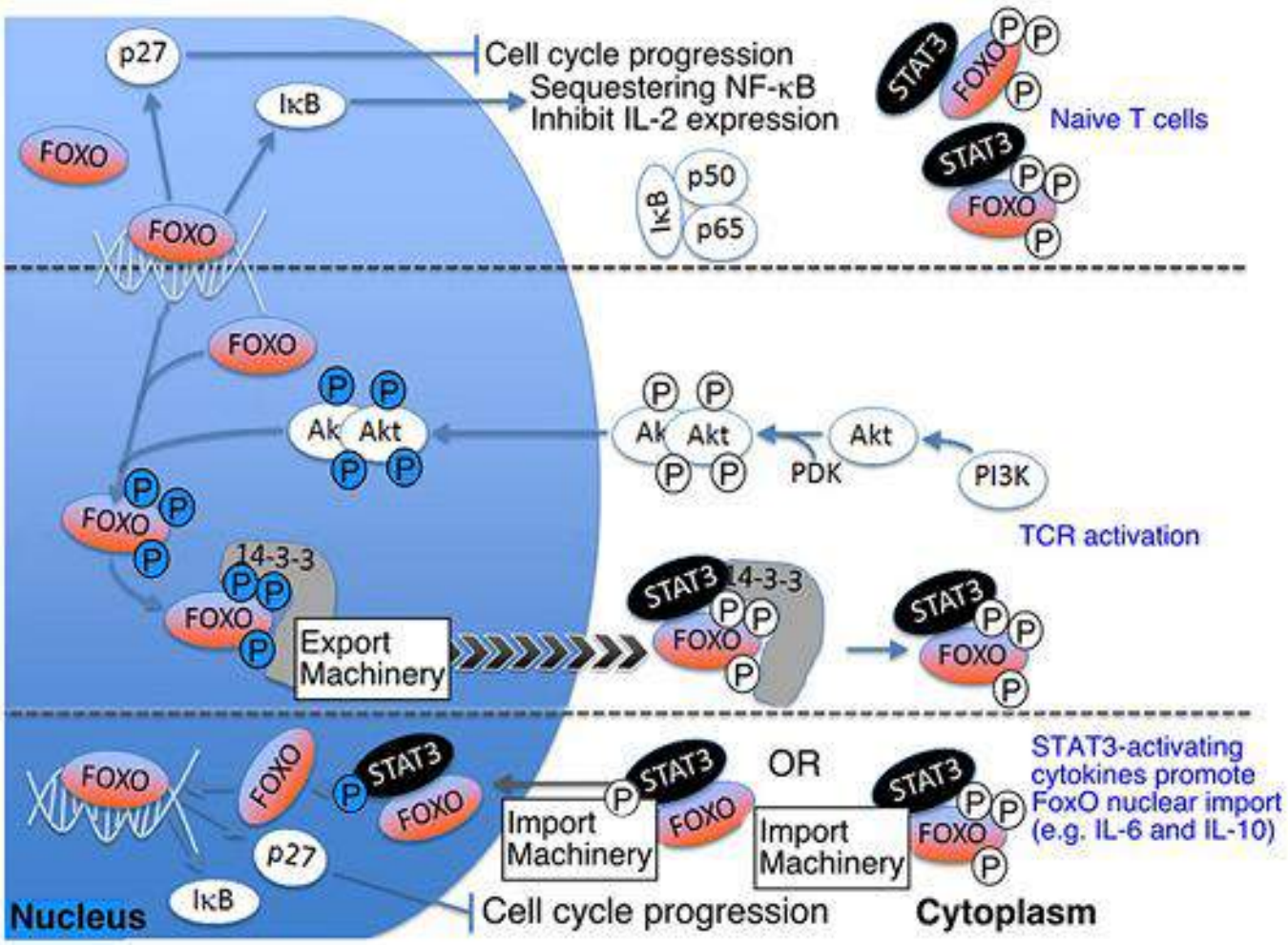

EGFR1
Extracellular domain
Plasma membrane
Intracellular domain
KRAS
Sos
Grb
BRAF
MEK
ERK
PI3K–mTOR pathway
JAK–STAT pathway
PLC pathway
• Proliferation
• Metastasis
• Anti-apoptosis
Nature Reviews | Cancer



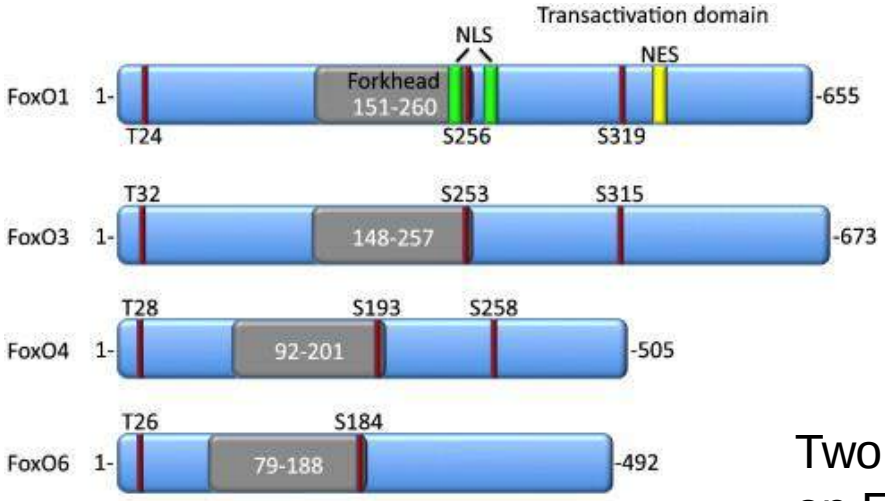
Mechanisms of Disease: the role of heat-shock protein 90 in genitourinary malignancy
Jean-Baptiste Lattouf, Ramaprasad Srinivasan, Peter A Pinto, W Marston Linehan and Leonard Neckers
Nature Clinical Practice Urology (2006) 3, 590-601



Phin et al. Front. Oncol., 2013

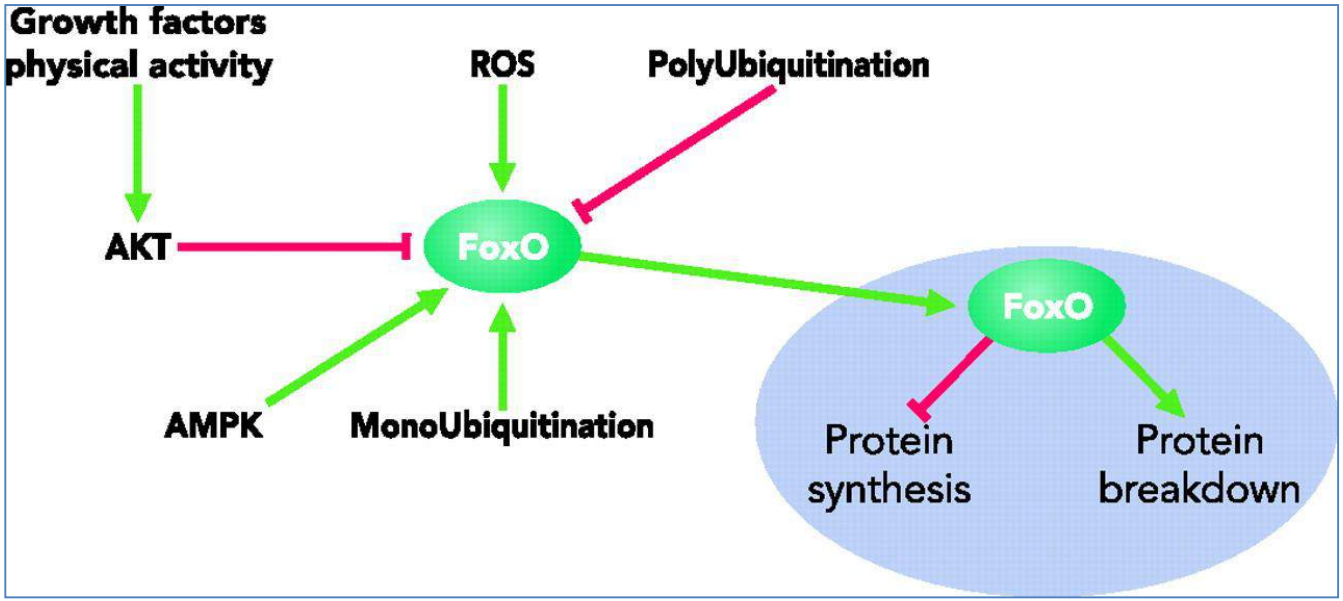


Oh et al, JBC 2012

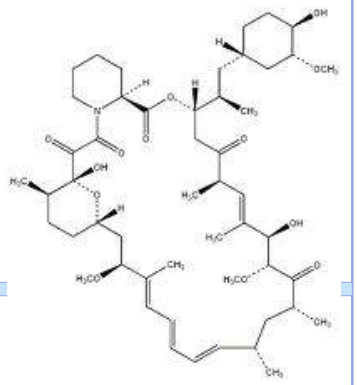


AKT phosphorylation sites are conserved

Two of the three AKT phosphorylation sites on FoxO3, T32 and S253 cooperatively mediated the binding to the 14-3-3z isoform

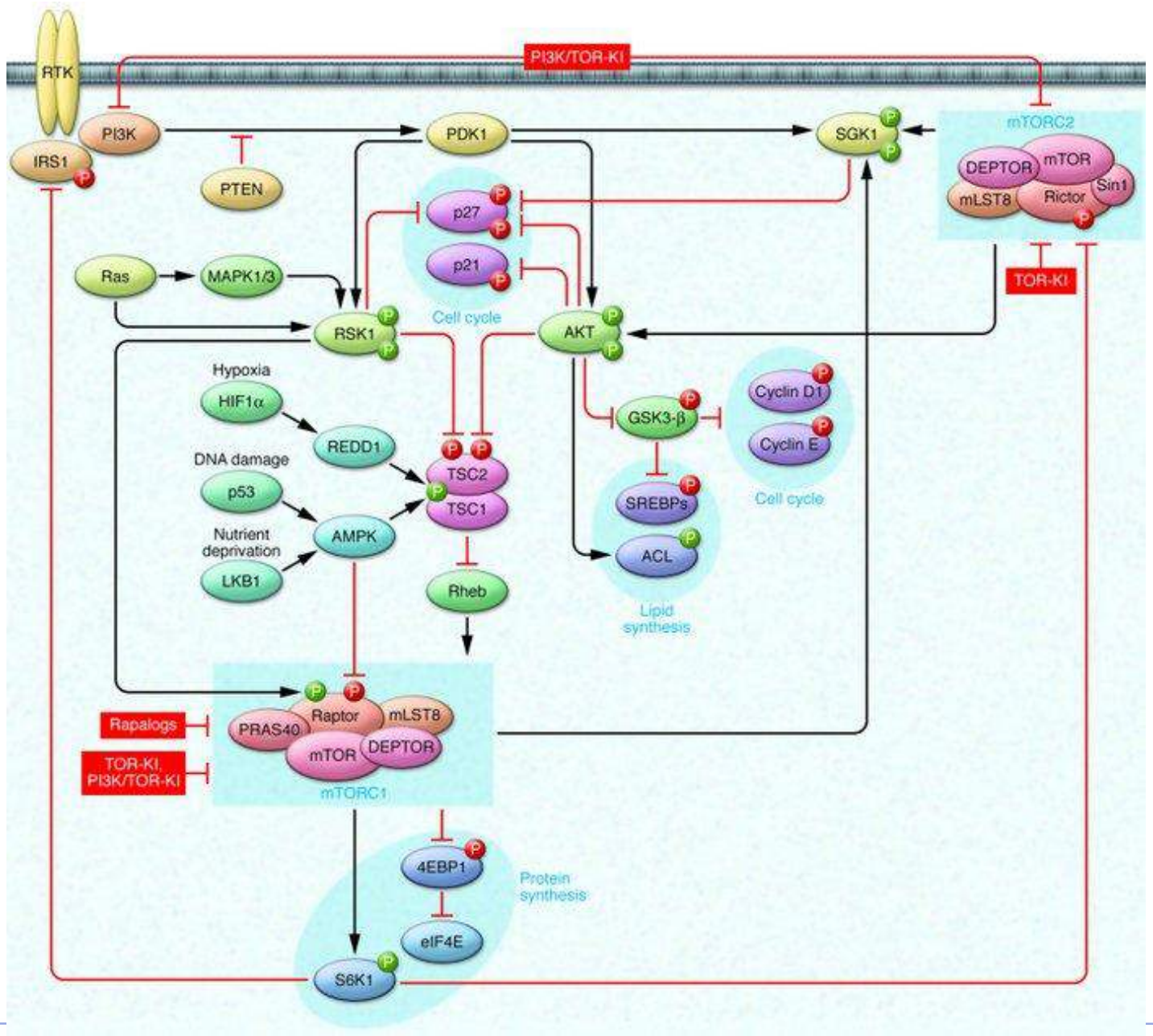


Rapamycin-mTOR inhibitor

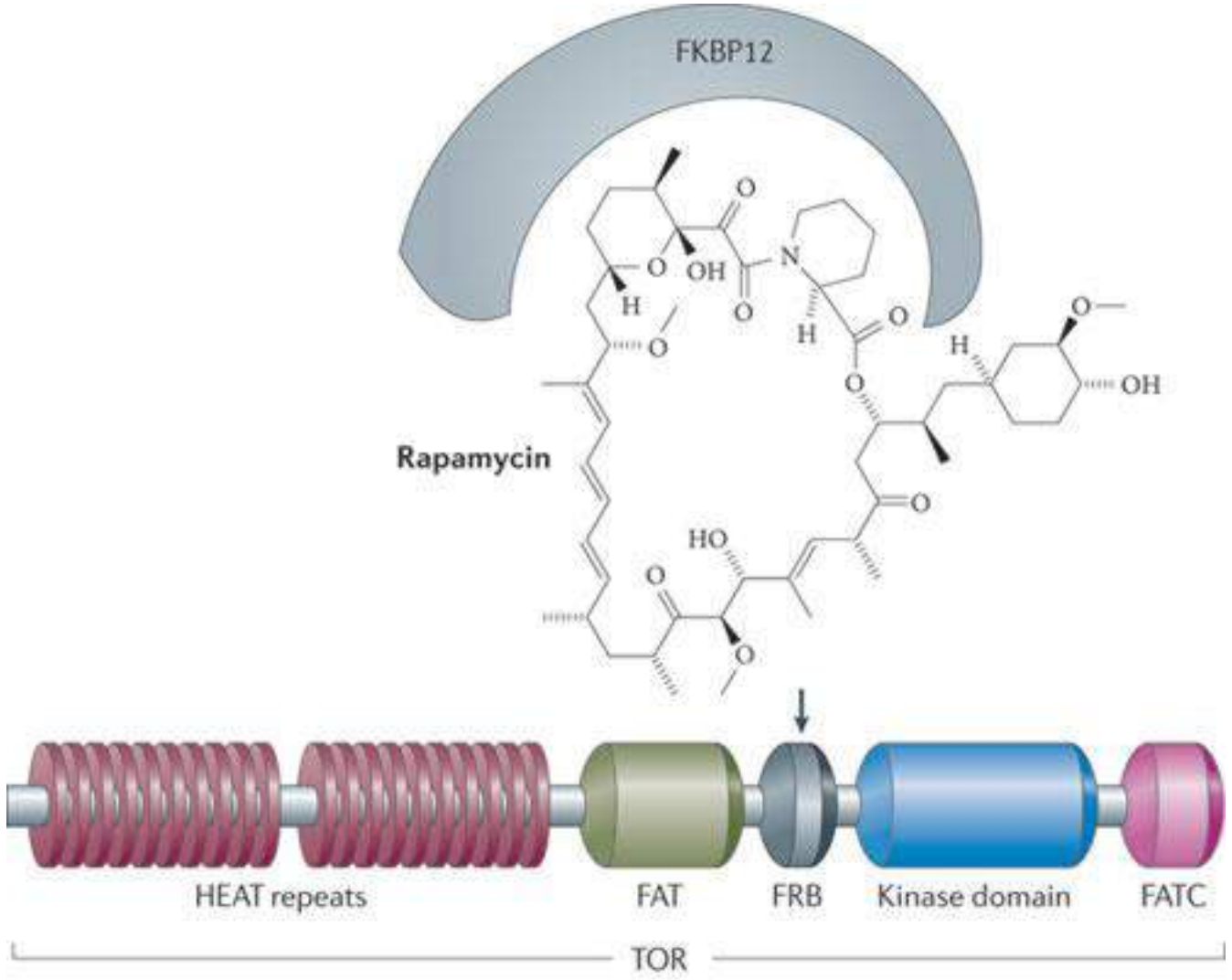


Rapamycin

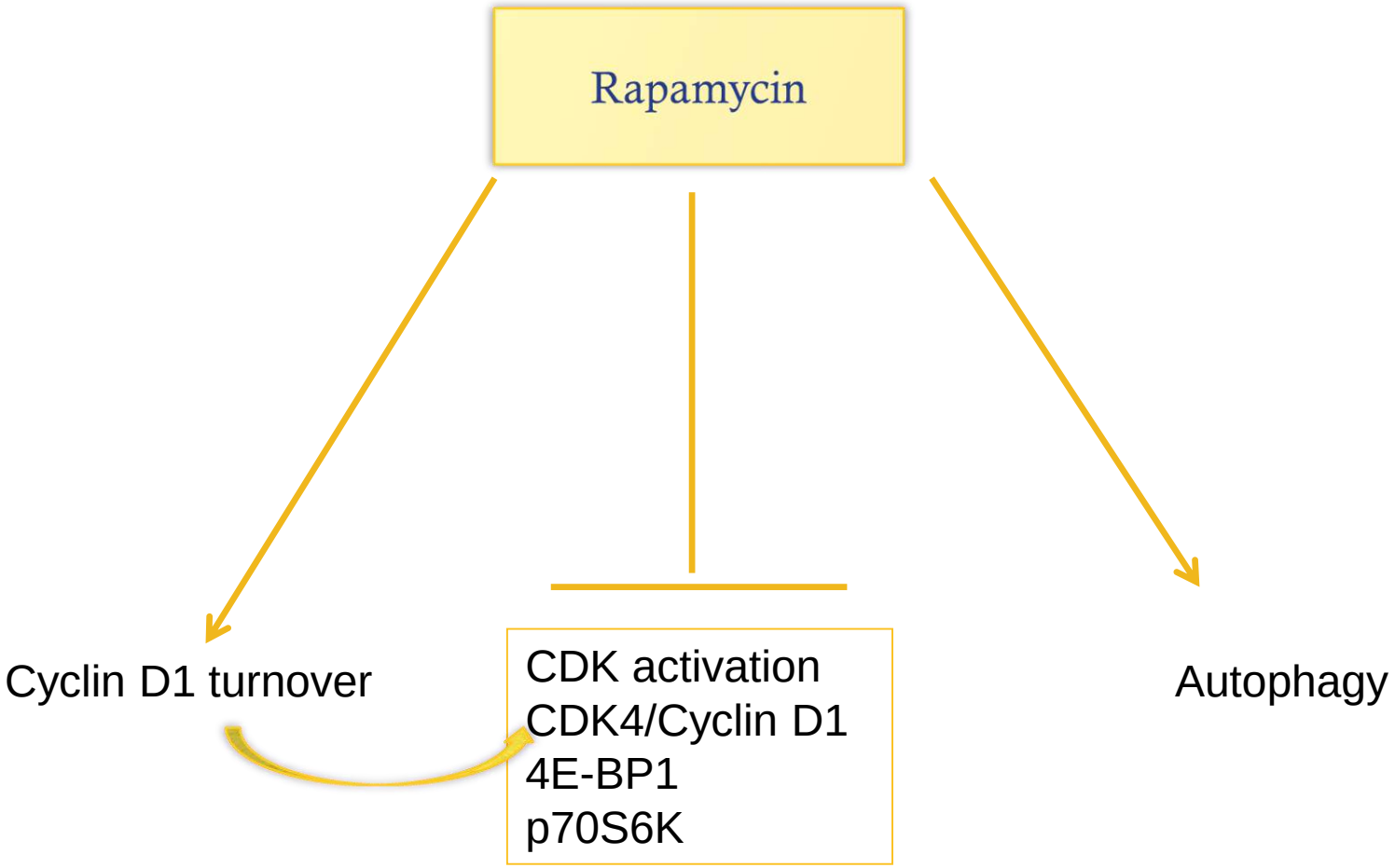
- Rapamycin (10 nM) is an antifungal agent that inhibits cell proliferation and induces autophagy/apoptosis
- More than 160 clinical trials with Rapalogs & Rapamycin in the treatment of metastatic RCC
  - Evorolimus (RAD001, Novartis), Temsirolimus (Torisel, Pfizer) etc.
- mTOR is a member of PIKK (DNA-PK, ATM, PI3K), which controls lipid and ribosomal biosynthesis, cell cycle progression, cell growth and differentiation, cytoskeletal arrangement
  - mTORC1: mTOR; raptor; GβL
  - mTORC2: mTOR; rictor; Sin-1 (MAPK interacting protein); GβL



Seth et al. Clinical Investigation, 2011



Nature Reviews | Drug Discovery



Result: Cell cycle arrest at G1/M

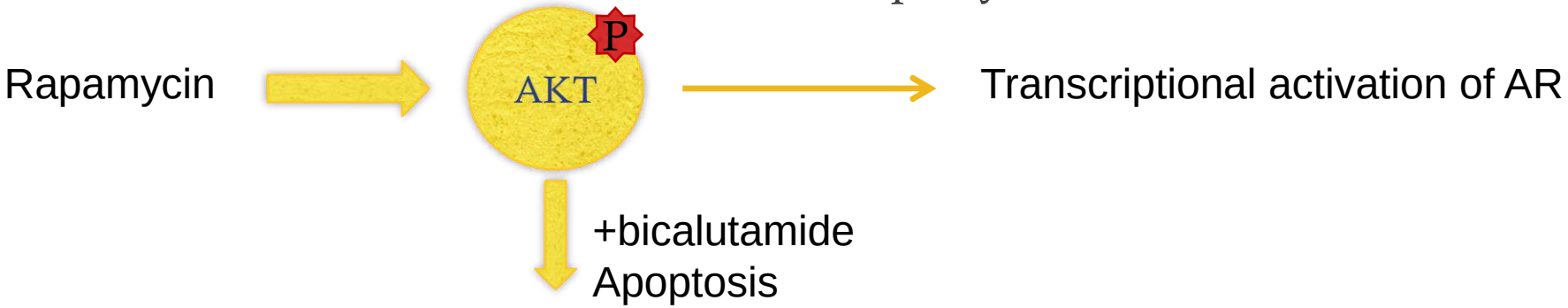
Strategic steps in rapamycin focused therapy models

1) Altered mTORC1 signalling cascade in AKT-independent manner (rapamycin sensitive pathway)

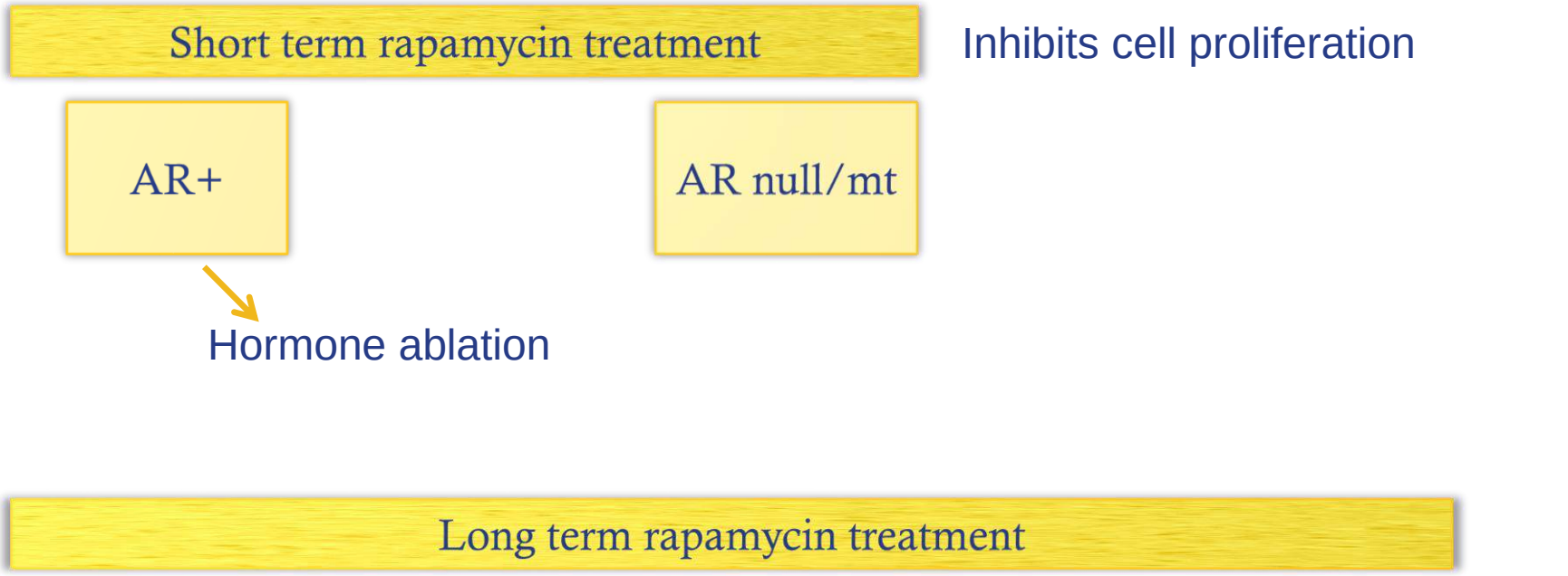
- Inhibits translation

2) AR dependent signalling cascade- mTORC2

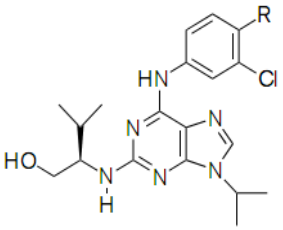
- Secondary phosphorylation of mTOR by AKT activation
- MAPK mediated Sin-1 crosstalk-rapamycin insensitive



Rapamycin monotherapy-Pca



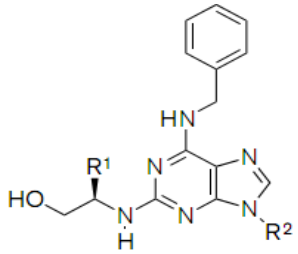
Purvalanol



Purvalanol A (R = H)

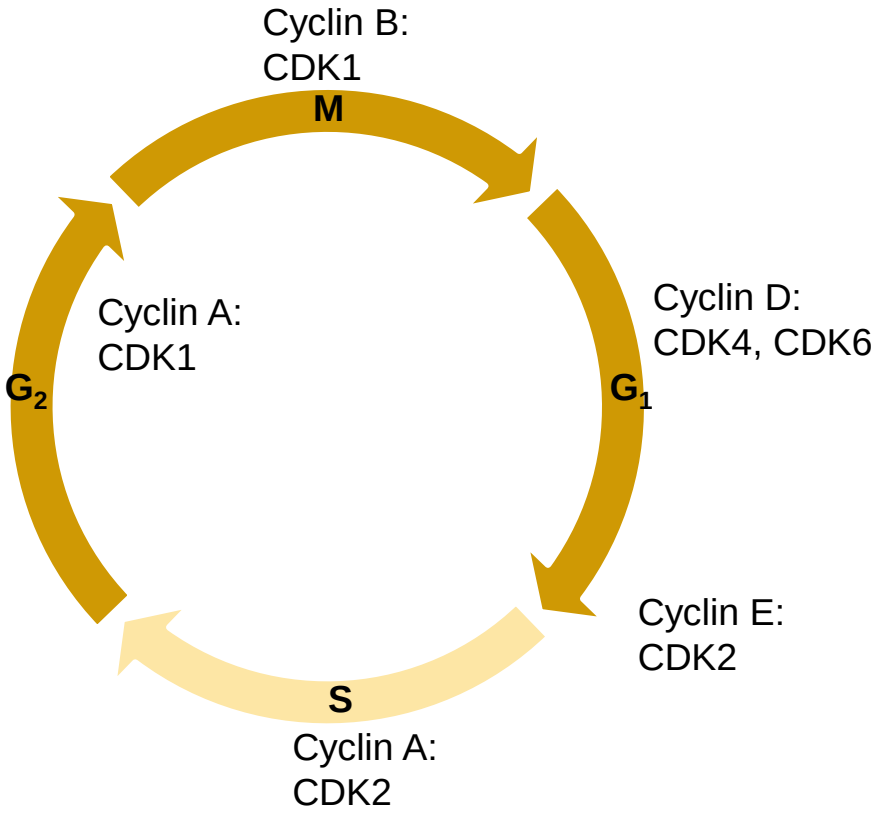
Purvalanol B (R = CO_2H)

Roscovitine

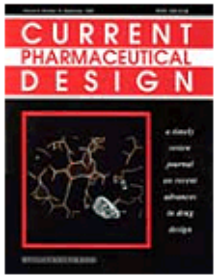


Olomoucin (R^1 = H, R^2 = CH_3)

Roscovitine (R^1 = CH_2CH_3, R^2 = $CH(CH_3)_2$)



Cell line	Metastasis	Androgen sensitve	PSA	PTEN
DU145	+	-	-	+/-
PC-3	++	-	+	-/-
LNCaP	++	++	+	-/-



CDK Inhibitors Induce Mitochondria-mediated Apoptosis Through the Activation of Polyamine Catabolic Pathway in LNCaP, DU145 and PC3 Prostate Cancer Cells

Authors: Damla Arisan, Elif; Obakan, Pinar; Coker-Gurkan, Ajda; Calcabrini, Annarica; Agostinelli, Enzo; Palavan Unsal, Narcin

Source: Current Pharmaceutical Design, Volume 20, Number 2, January 2014, pp. 180-188(9)

Publisher: Bentham Science Publishers

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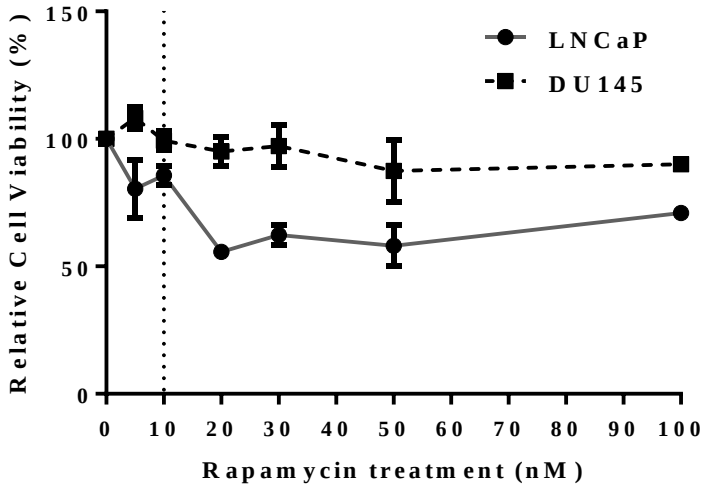
☆ ADD TO FAVOURITES

Abstract:

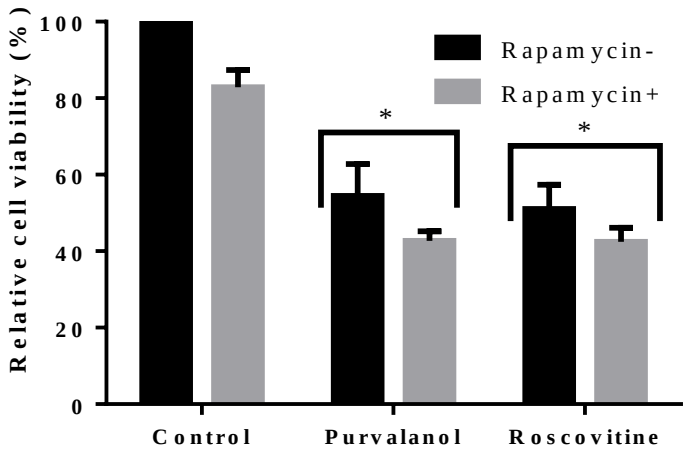
Androgen signaling is critical in prostate cancer development and progression. The co-existence of hormone responsive and irresponsive cells due to functional androgen receptor (AR) in prostate gland is the major obstacle in prostate cancer therapy models. Targeting aberrant cell cycle by novel cell cycle blocking agents is a promising strategy to treat various types of malignancies. Purvalanol and roscovitine are cyclin dependent kinase (CDK) inhibitors able to activate apoptotic cell death by inducing cell cycle arrest at G1/S and G2/M phases in cancer cells. Polyamines are unique cationic amine derivatives involved in the regulation of cell proliferation. Although the elevated intracellular level of polyamines (putrescine, spermidine and spermine) is typical for prostate gland, abnormal regulation of polyamine metabolism might result in rapid cell proliferation and, thus in prostate cancer progression. Therefore, treatment with drug-induced

Figure 1

A.


Relative Cell Viability (%)
150
100
50
0
LNCaP
DU145
0 10 20 30 40 50 60 70 80 90 100
Rapamycin treatment (nM)

B.


LNCaP
Relative cell viability (%)
100
80
60
40
20
0
Rapamycin-
Rapamycin+
*
*
Control
Purvalanol
Roscovitine

C.

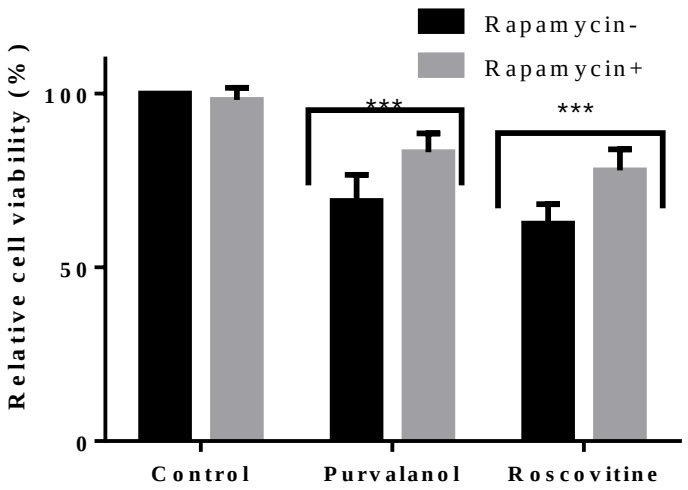
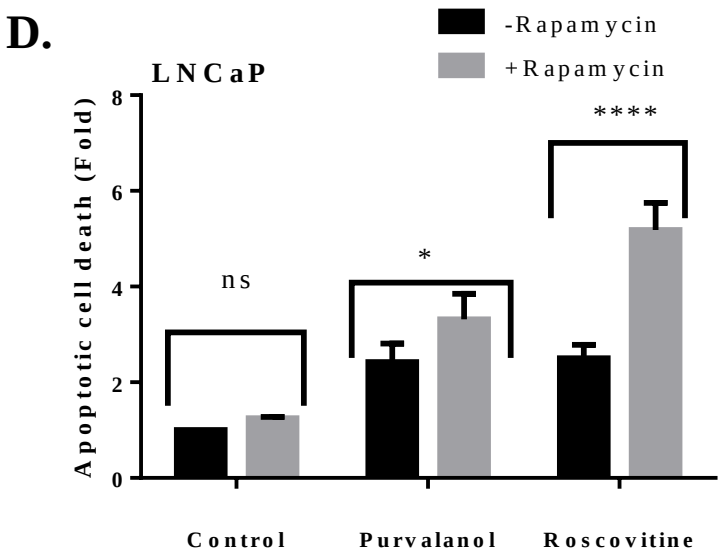
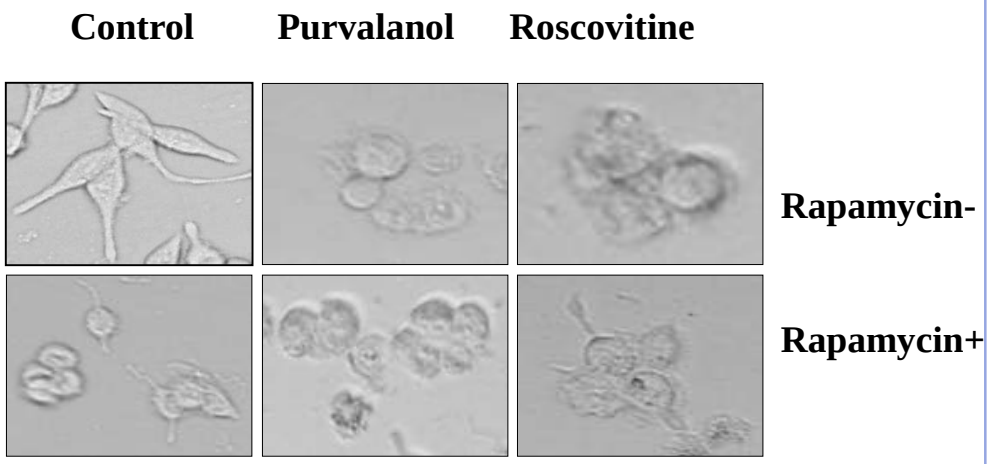
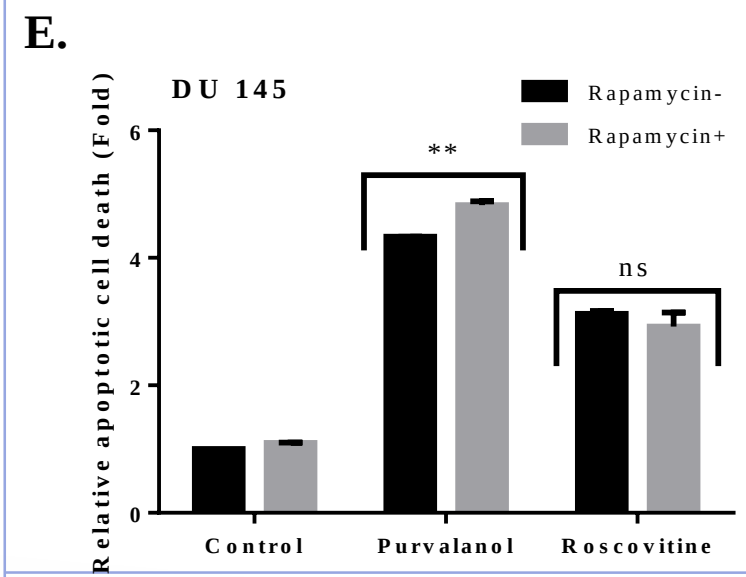

DU 145
Relative cell viability (%)
100
50
0
Rapamycin-
Rapamycin+
***
***
Control
Purvalanol
Roscovitine

Figure 1


D.
LNCaP
-Rapamycin
+Rapamycin
Apoptotic cell death (Fold)
0
2
4
6
8
ns
*
****
Control
Purvalanol
Roscovitine


Control
Purvalanol
Roscovitine
Rapamycin-
Rapamycin+


E.
DU 145
Rapamycin-
Rapamycin+
Relative apoptotic cell death (Fold)
0
2
4
6
**
ns
Control
Purvalanol
Roscovitine

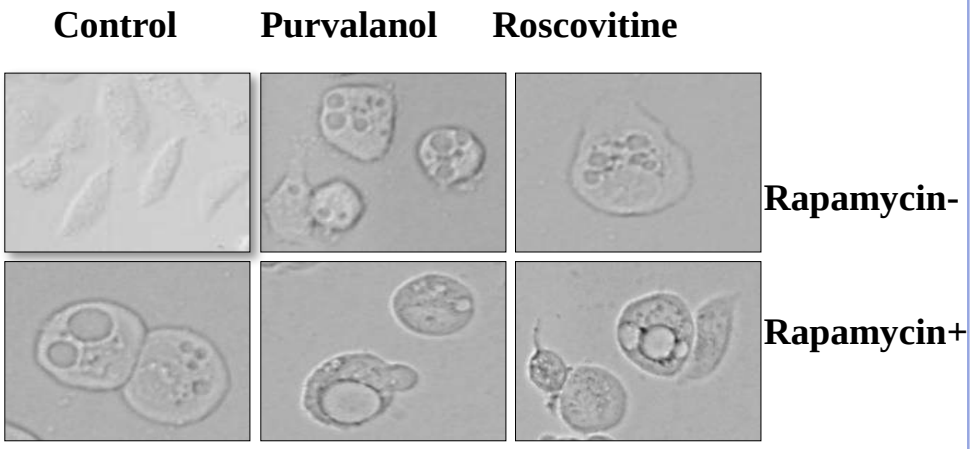

Control
Purvalanol
Roscovitine
Rapamycin-
Rapamycin+

Figure 2

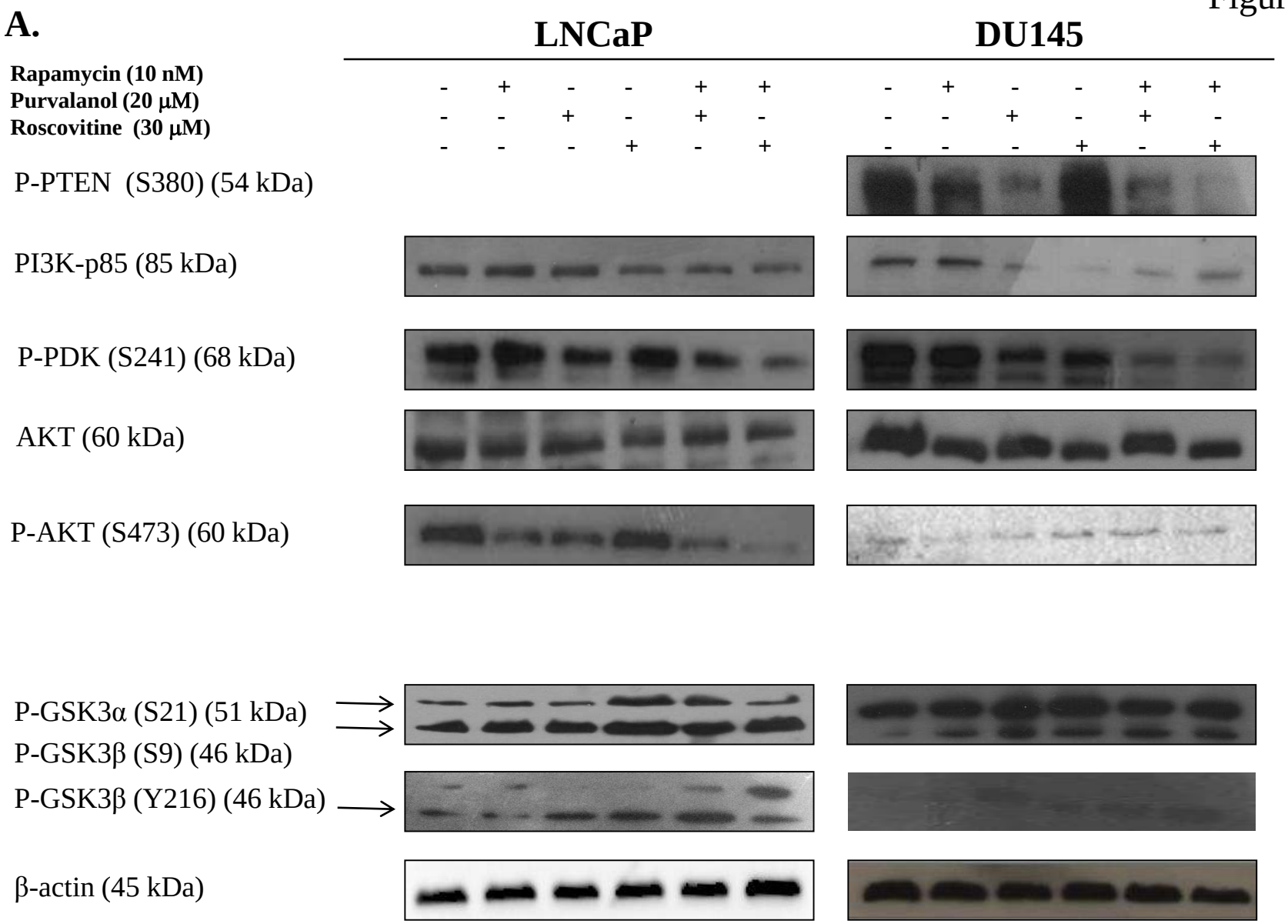

A.
LNCaP
DU145
Rapamycin (10 nM)
Purvalanol (20 μM)
Roscovitine (30 μM)
P-PTEN (S380) (54 kDa)
PI3K-p85 (85 kDa)
P-PDK (S241) (68 kDa)
AKT (60 kDa)
P-AKT (S473) (60 kDa)
P-GSK3α (S21) (51 kDa)
P-GSK3β (S9) (46 kDa)
P-GSK3β (Y216) (46 kDa)
β-actin (45 kDa)

Figure 2

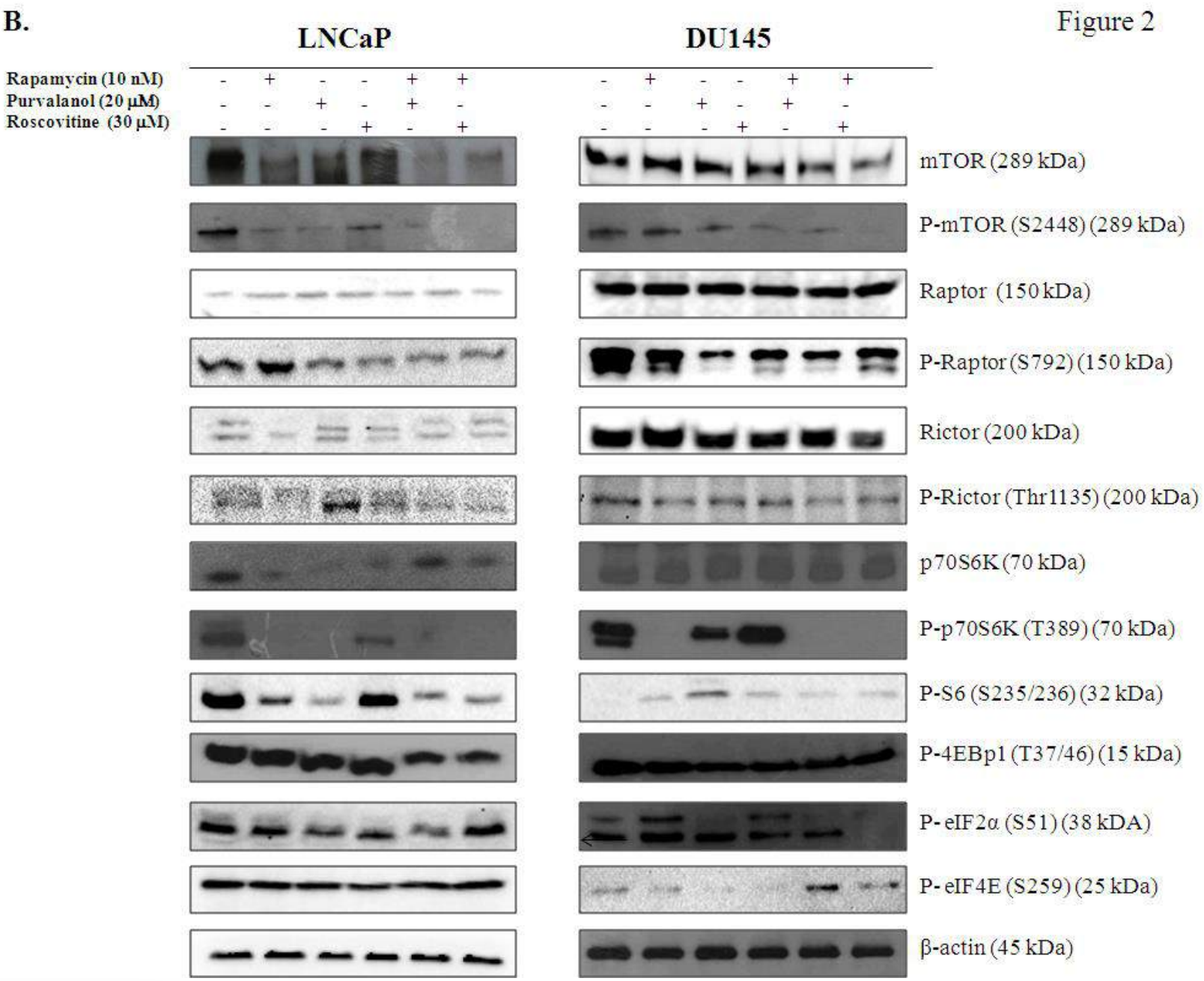
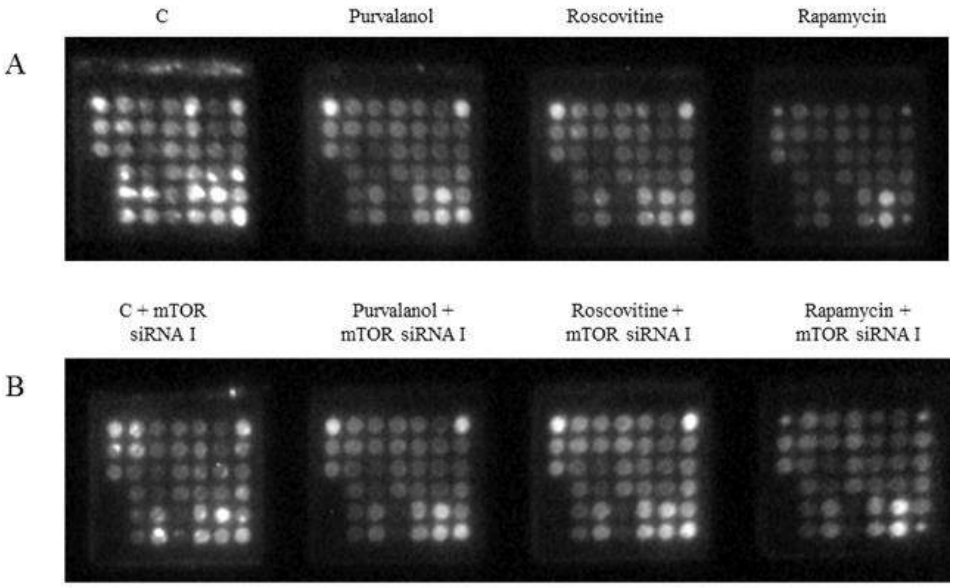

B.
LNCaP
DU145
Rapamycin (10 nM)
Purvalanol (20 μM)
Roscovitine (30 μM)
mTOR (289 kDa)
P-mTOR (S2448) (289 kDa)
Raptor (150 kDa)
P-Raptor (S792) (150 kDa)
Rictor (200 kDa)
P-Rictor (Thr1135) (200 kDa)
p70S6K (70 kDa)
P-p70S6K (T389) (70 kDa)
P-S6 (S235/236) (32 kDa)
P-4EBp1 (T37/46) (15 kDa)
P- eIF2α (S51) (38 kDA)
P- eIF4E (S259) (25 kDa)
β-actin (45 kDa)

Figure 3





	Target	Phosphorylation Site	Modification
1	Positive Control	N/A	N/A
2	Negative Control	N/A	N/A
3	ERK1/2	Thr202/Tyr204	Phosphorylation
4	Stat1	Tyr701	Phosphorylation
5	Stat3	Tyr705	Phosphorylation
6	Akt	Thr308	Phosphorylation
7	Akt	Ser473	Phosphorylation
8	AMPKα	Thr172	Phosphorylation
9	S6 Ribosomal Protein	Ser235/236	Phosphorylation
10	mTOR	Ser2448	Phosphorylation
11	HSP27	Ser78	Phosphorylation
12	Bad	Ser112	Phosphorylation
13	p70 S6 Kinase	Thr389	Phosphorylation
14	PRAS40	Thr246	Phosphorylation
15	p53	Ser15	Phosphorylation
16	p38	Thr180/Tyr182	Phosphorylation
17	SAPK/JNK	Thr183/Tyr185	Phosphorylation
18	PARP	Asp214	Cleavage
19	Caspase-3	Asp175	Cleavage
20	GSK-3β	Ser9	Phosphorylation

Figure 4

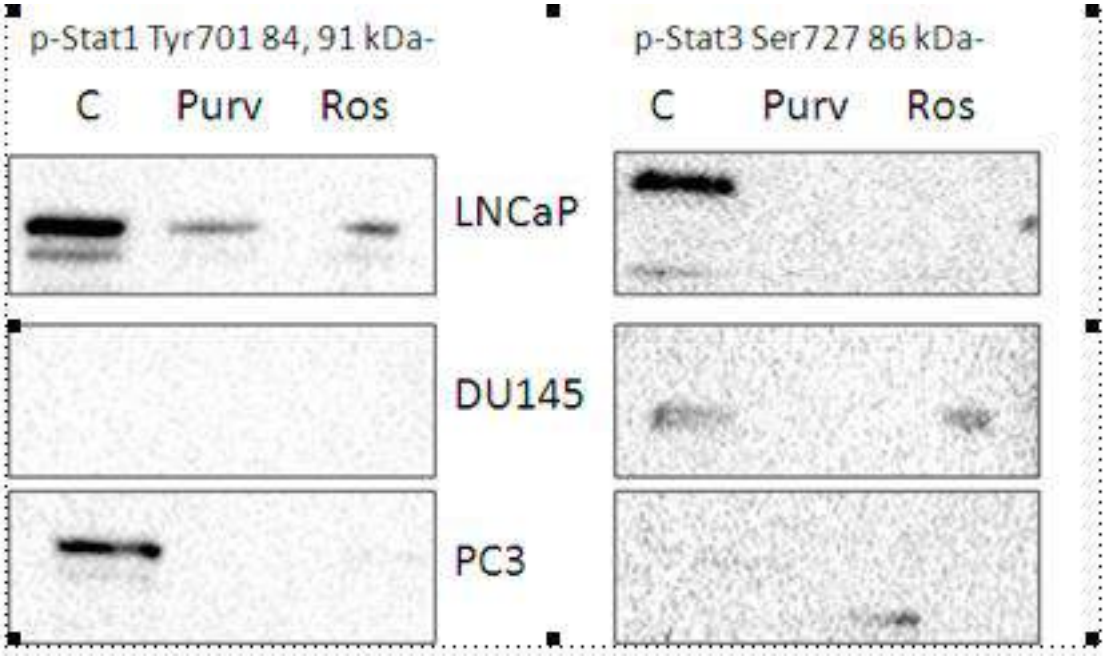
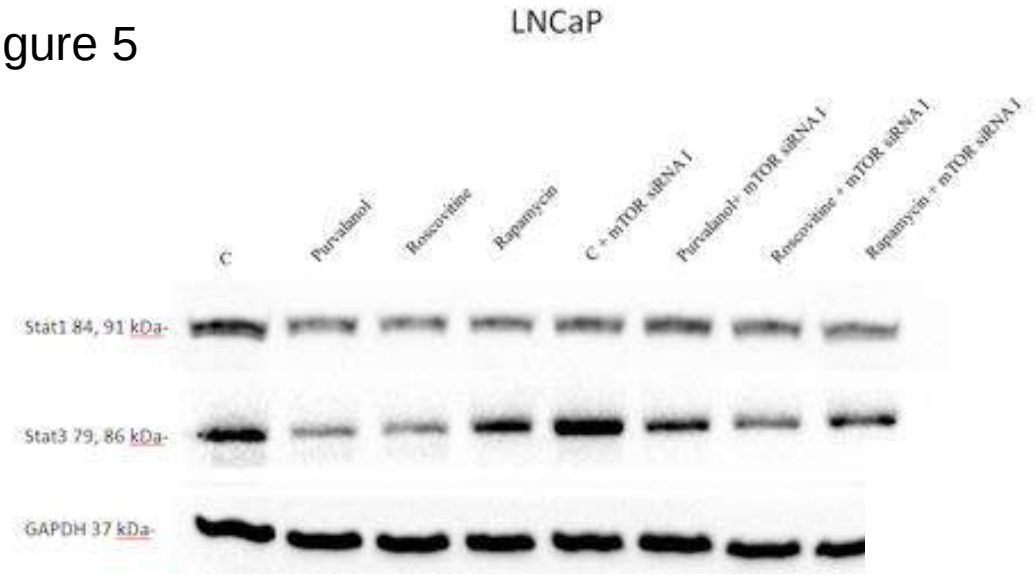
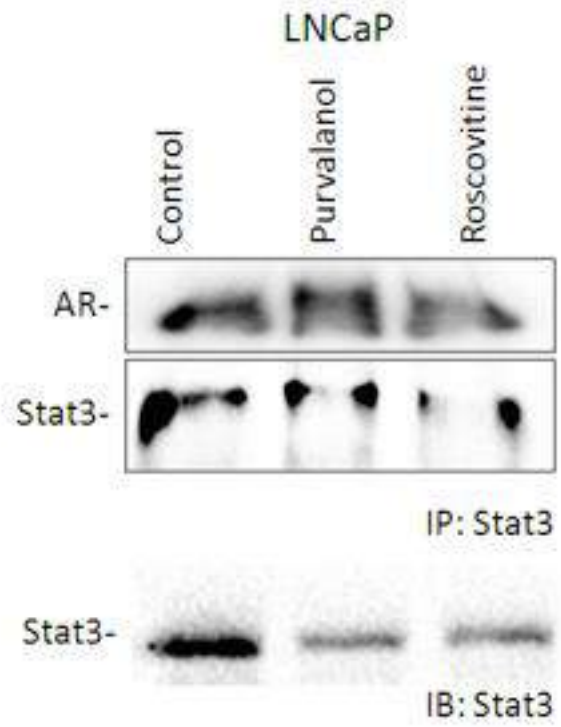

p-Stat1 Tyr701 84, 91 kDa-
C
Purv
Ros
p-Stat3 Ser727 86 kDa-
C
Purv
Ros
LNCaP
DU145
PC3

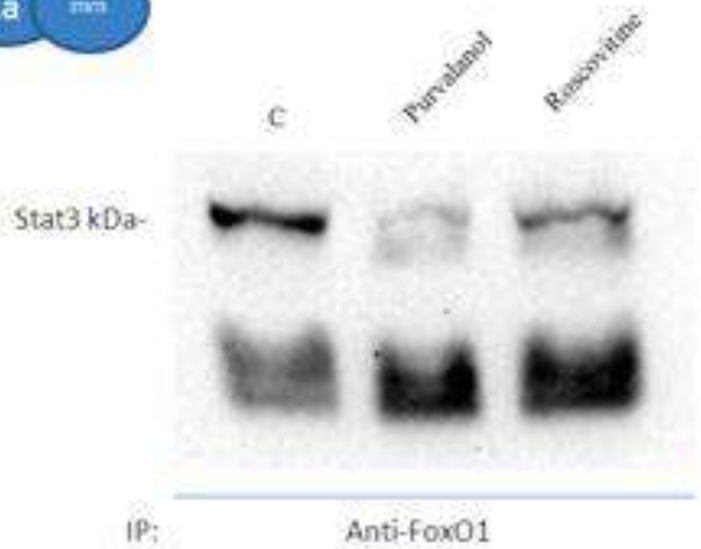
Figure 5

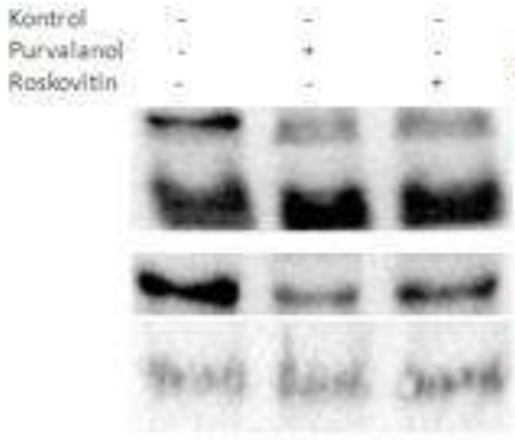

LNCaP
C
Purvalanol
Roscovitine
Rapamycin
C + mTOR siRNA I
Purvalanol+ mTOR siRNA I
Roscovitine + mTOR siRNA I
Rapamycin + mTOR siRNA I
Stat1 84, 91 kDa-
Stat3 79, 86 kDa-
GAPDH 37 kDa-


LNCaP
Control
Purvalanol
Roscovitine
AR-
Stat3-
IP: Stat3
Stat3-
IB: Stat3









LNCaP FoxO1 IP – Stat3 sonucu

FoxO1(78 kDA)

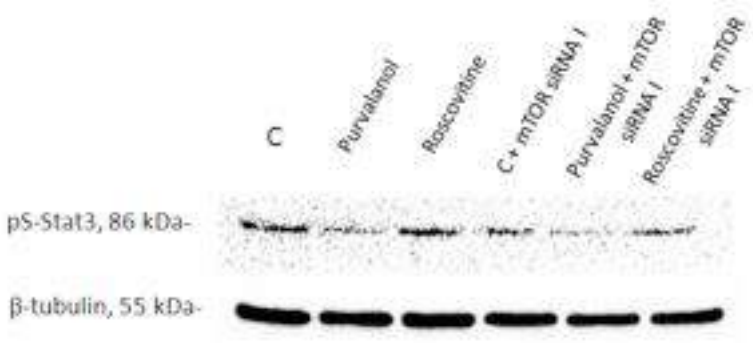
Akt (60 kDA)

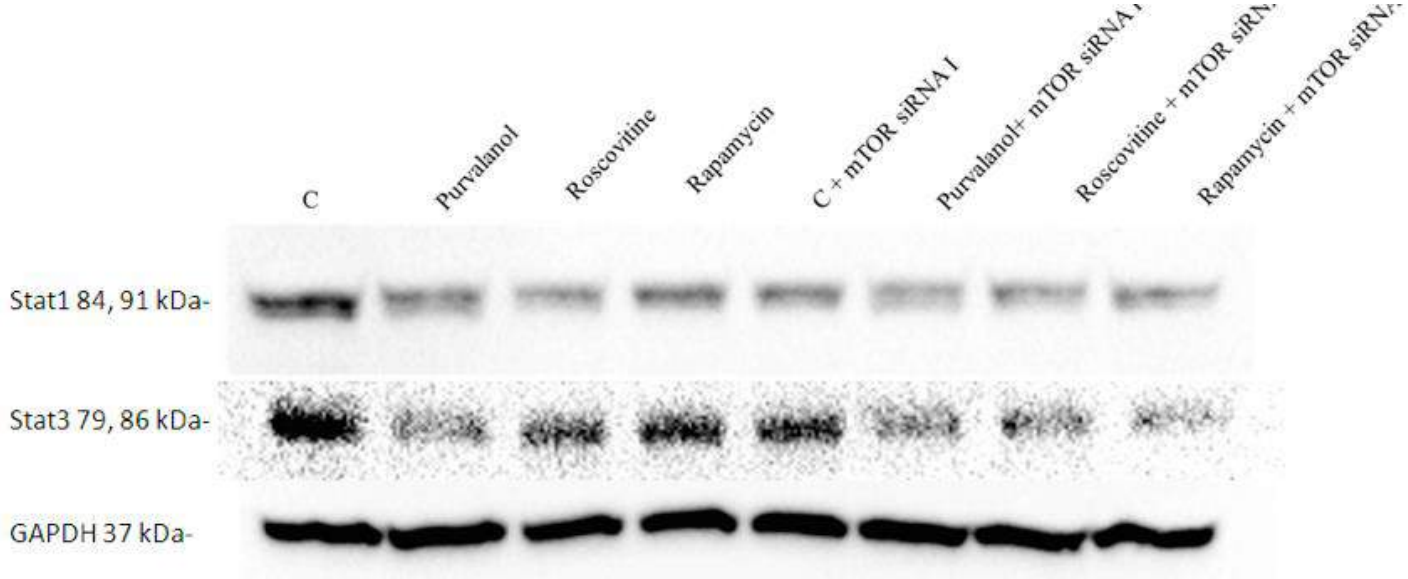
IP: FoxO1

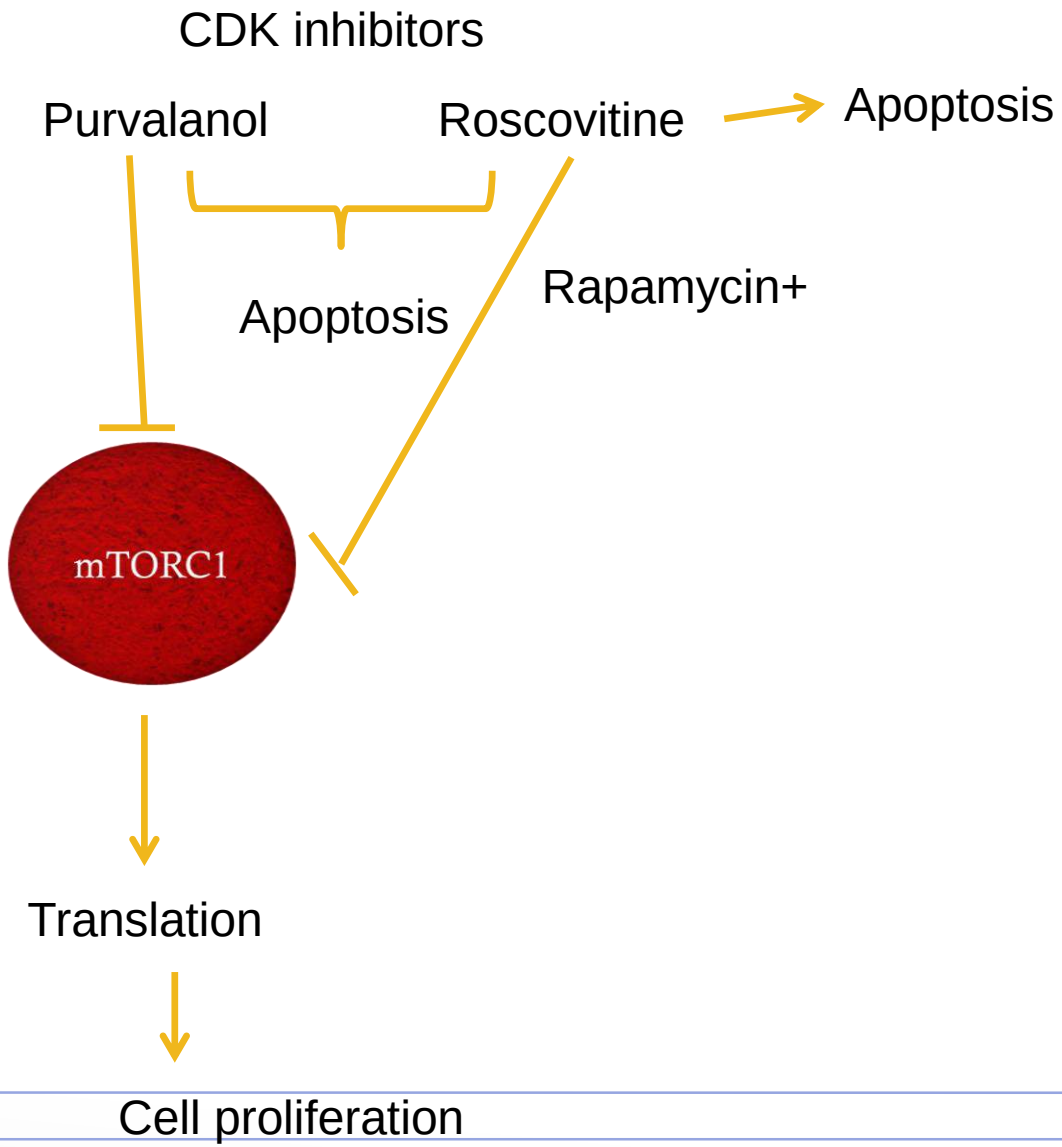
IB: Ubiquitin (10 kDA)

DU145

Figure 6






CDK inhibitors
Purvalanol
Roscovitine
Apoptosis
Apoptosis
Rapamycin+
mTORC1
Translation
Cell proliferation

CDK inhibitors

Purvalanol Roscovitine



Cell survival
suppression

&

Apoptosis

Apoptosis

Nuclear translocation of Foxo
Stat-AR interaction



#mobican at IKU
#ikumobigen



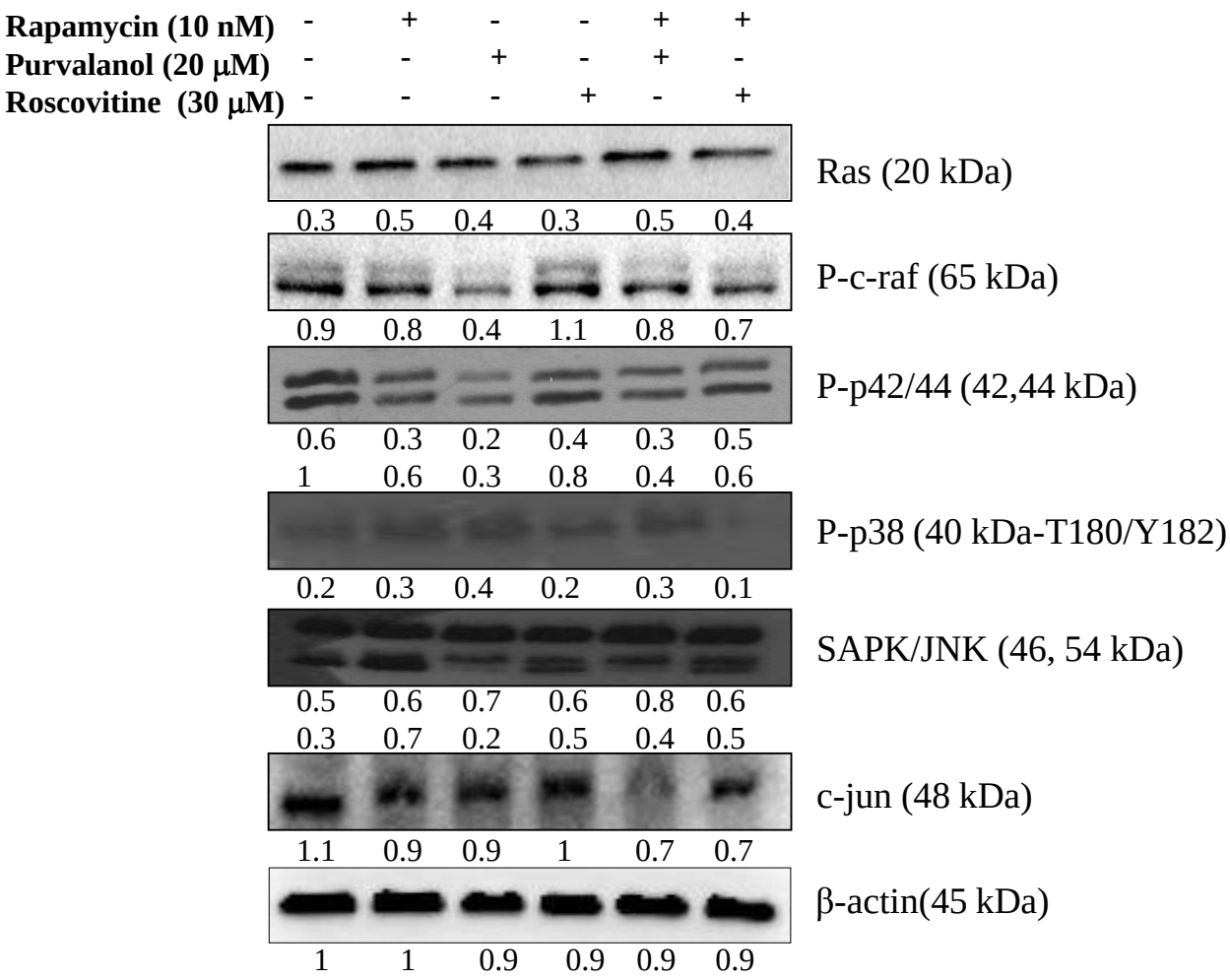
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Gülistan Borak
Aykut Özcan

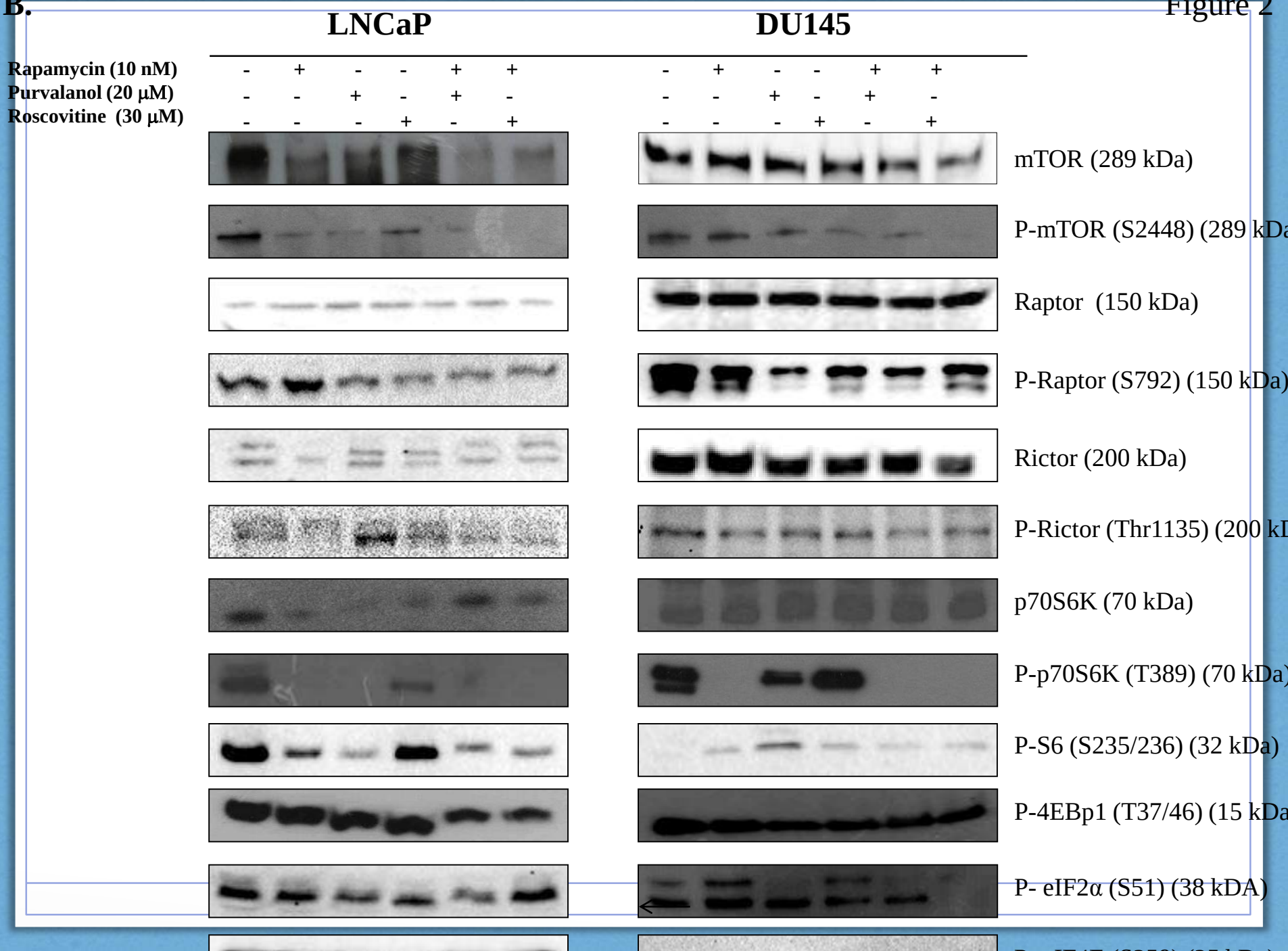


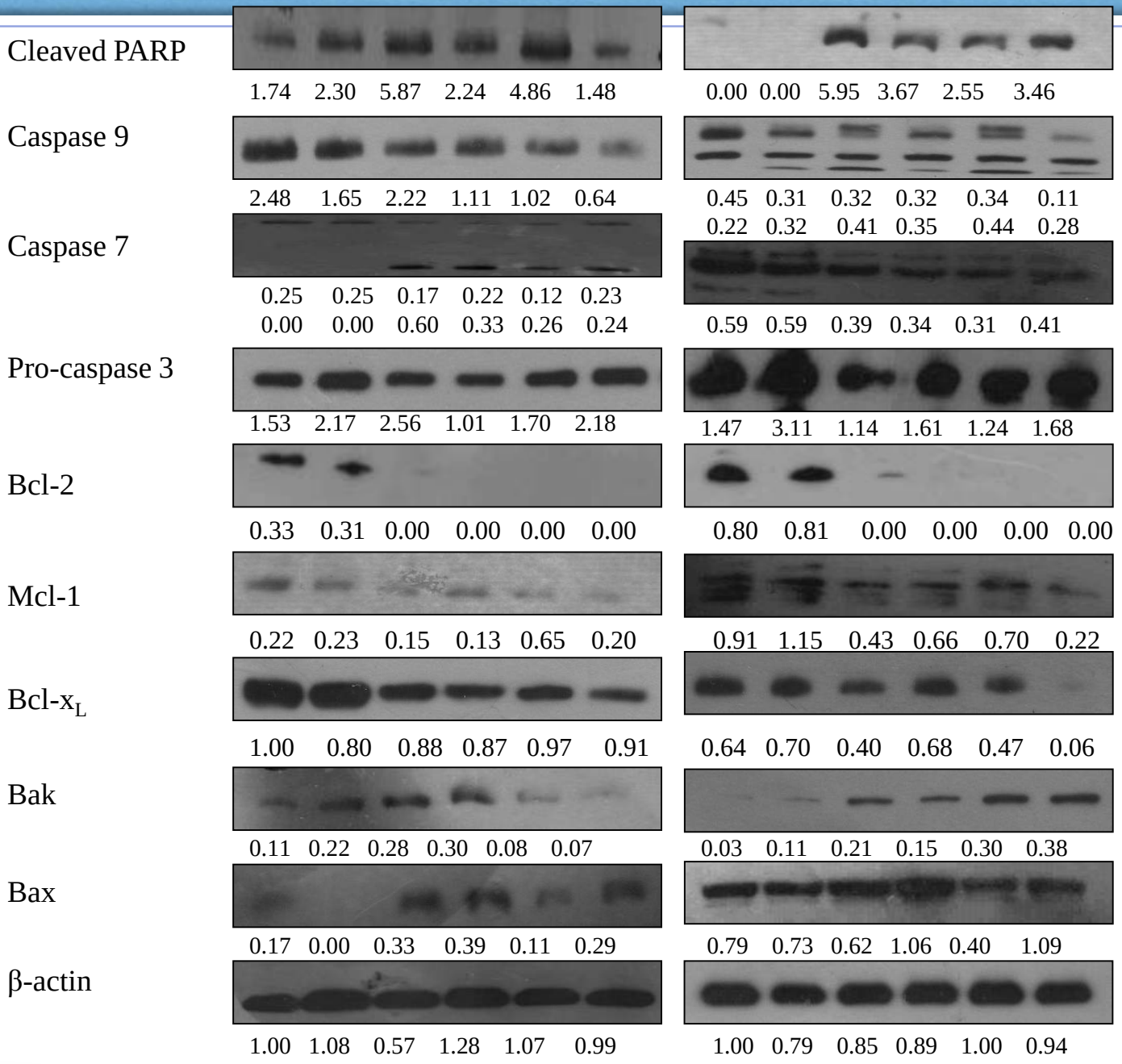
DU145

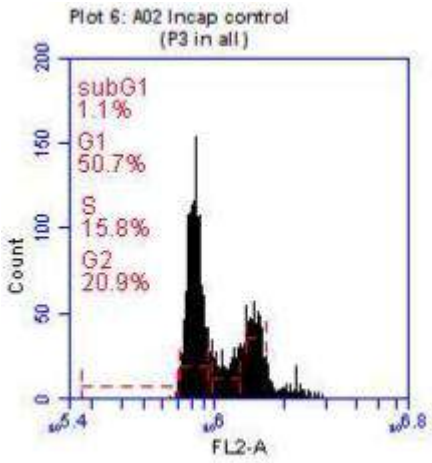
A.

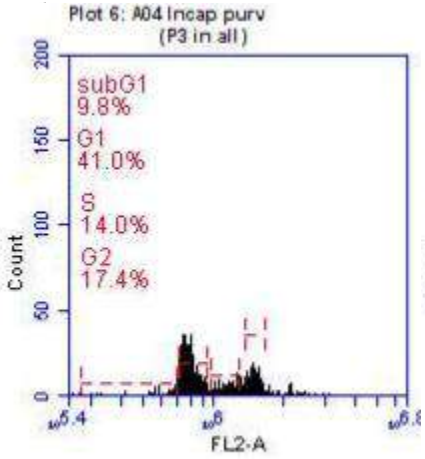

Rapamycin (10 nM) - + - - + +
Purvalanol (20 μM) - - + - + -
Roscovitine (30 μM) - - - + - +
Ras (20 kDa)
0.3 0.5 0.4 0.3 0.5 0.4
P-c-raf (65 kDa)
0.9 0.8 0.4 1.1 0.8 0.7
P-p42/44 (42,44 kDa)
0.6 0.3 0.2 0.4 0.3 0.5
1 0.6 0.3 0.8 0.4 0.6
P-p38 (40 kDa-T180/Y182)
0.2 0.3 0.4 0.2 0.3 0.1
SAPK/JNK (46, 54 kDa)
0.5 0.6 0.7 0.6 0.8 0.6
0.3 0.7 0.2 0.5 0.4 0.5
c-jun (48 kDa)
1.1 0.9 0.9 1 0.7 0.7
β-actin(45 kDa)
1 1 0.9 0.9 0.9 0.9

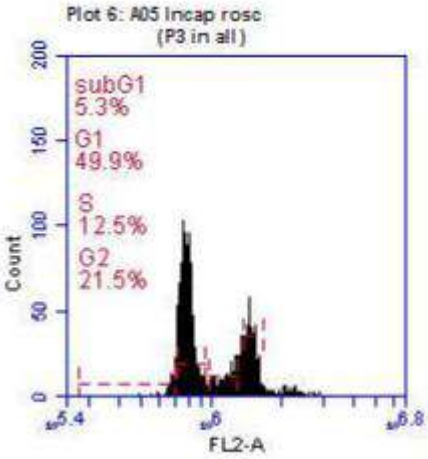
B.

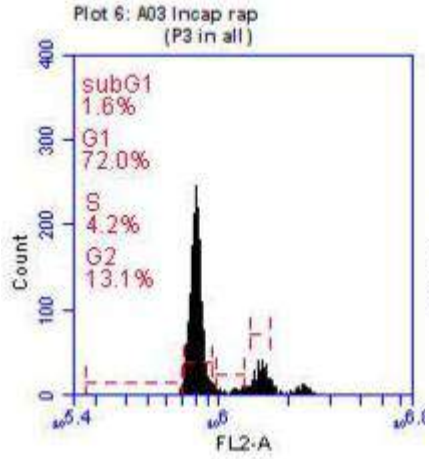

LNCaP
DU145
Rapamycin (10 nM)
Purvalanol (20 μM)
Roscovitine (30 μM)
- + - - + +
- - + - + -
- - - + - +
mTOR (289 kDa)
P-mTOR (S2448) (289 kDa
Raptor (150 kDa)
P-Raptor (S792) (150 kDa)
Rictor (200 kDa)
P-Rictor (Thr1135) (200 kD
p70S6K (70 kDa)
P-p70S6K (T389) (70 kDa)
P-S6 (S235/236) (32 kDa)
P-4EBp1 (T37/46) (15 kDa
P- eIF2α (S51) (38 kDA)

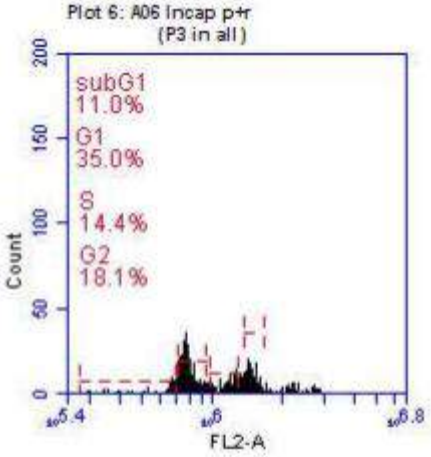

Cleaved PARP
1.74 2.30 5.87 2.24 4.86 1.48
0.00 0.00 5.95 3.67 2.55 3.46
Caspase 9
2.48 1.65 2.22 1.11 1.02 0.64
0.45 0.31 0.32 0.32 0.34 0.11
Caspase 7
0.22 0.32 0.41 0.35 0.44 0.28
0.25 0.25 0.17 0.22 0.12 0.23
0.00 0.00 0.60 0.33 0.26 0.24
0.59 0.59 0.39 0.34 0.31 0.41
Pro-caspase 3
1.53 2.17 2.56 1.01 1.70 2.18
1.47 3.11 1.14 1.61 1.24 1.68
Bcl-2
0.33 0.31 0.00 0.00 0.00 0.00
0.80 0.81 0.00 0.00 0.00 0.00
Mcl-1
0.22 0.23 0.15 0.13 0.65 0.20
0.91 1.15 0.43 0.66 0.70 0.22
Bcl-xL
1.00 0.80 0.88 0.87 0.97 0.91
0.64 0.70 0.40 0.68 0.47 0.06
Bak
0.11 0.22 0.28 0.30 0.08 0.07
0.03 0.11 0.21 0.15 0.30 0.38
Bax
0.17 0.00 0.33 0.39 0.11 0.29
0.79 0.73 0.62 1.06 0.40 1.09
β-actin
1.00 1.08 0.57 1.28 1.07 0.99
1.00 0.79 0.85 0.89 1.00 0.94

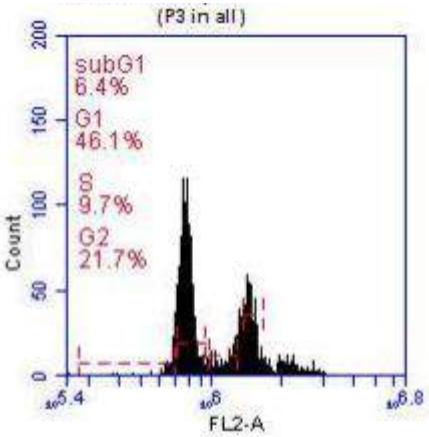

Plot 6: A02 Incap control
(P3 in all)
subG1
1.1%
G1
50.7%
S
15.8%
G2
20.9%
Count
FL2-A

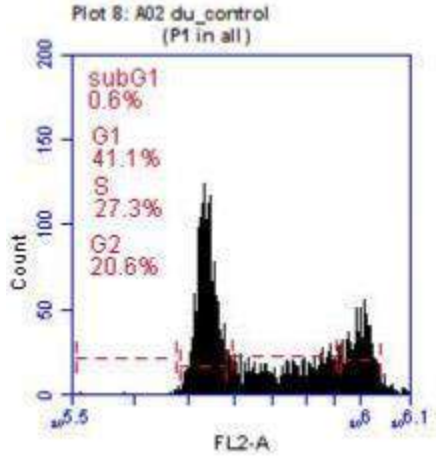

Plot 6: A04 Incap purv
(P3 in all)
subG1
9.8%
G1
41.0%
S
14.0%
G2
17.4%
Count
FL2-A

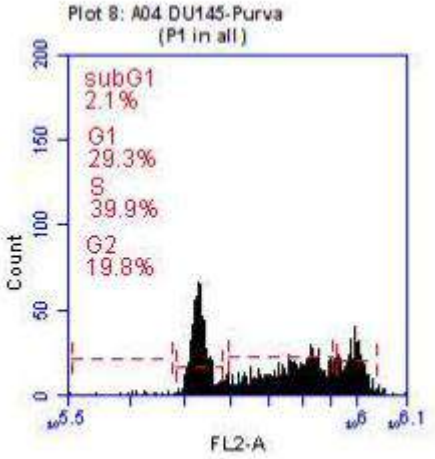

Plot 6: A05 Incap rosc
(P3 in all)
subG1
5.3%
G1
49.9%
S
12.5%
G2
21.5%
Count
FL2-A

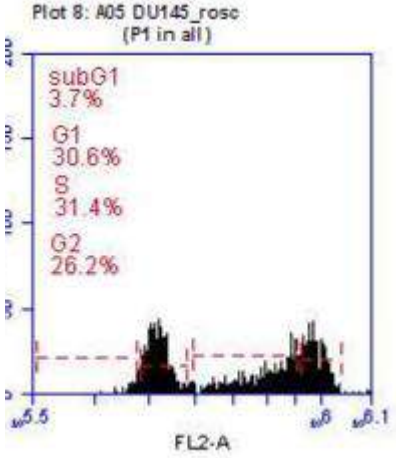

Plot 6: A03 Incap rap
(P3 in all)
subG1
1.6%
G1
72.0%
S
4.2%
G2
13.1%
Count
FL2-A

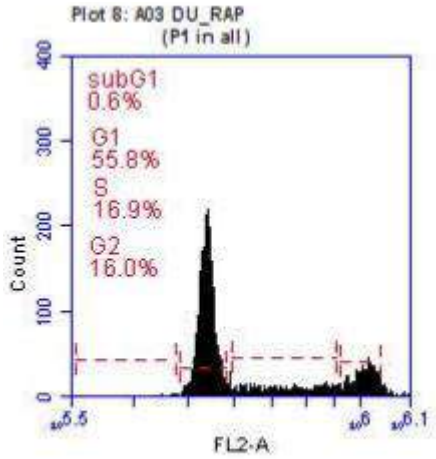

Plot 6: A06 Incap p+r
(P3 in all)
subG1
11.0%
G1
35.0%
S
14.4%
G2
18.1%
Count
FL2-A

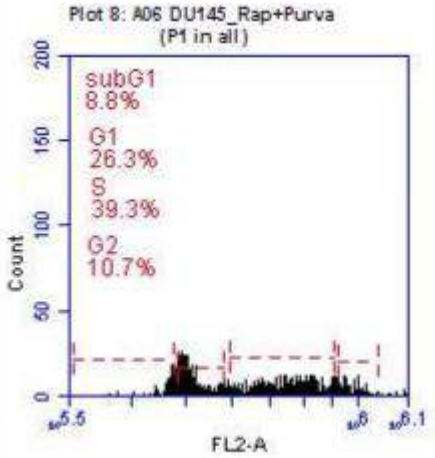

(P3 in all)
subG1
6.4%
G1
46.1%
S
9.7%
G2
21.7%
Count
FL2-A

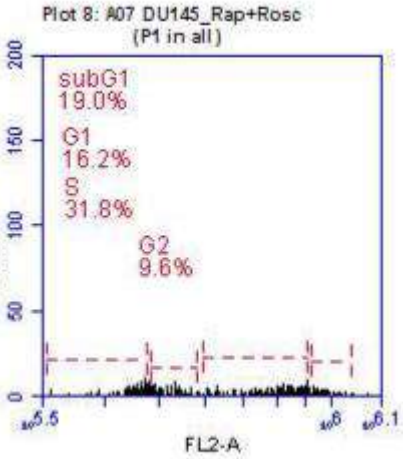

Plot 8: A02 du_control
(P1 in all)
subG1
0.6%
G1
41.1%
S
27.3%
G2
20.6%
Count
FL2-A


Plot 8: A04 DU145-Purva
(P1 in all)
subG1
2.1%
G1
29.3%
S
39.9%
G2
19.8%
Count
FL2-A


Plot 8: A05 DU145_rosc
(P1 in all)
subG1
3.7%
G1
30.6%
S
31.4%
G2
26.2%
FL2-A


Plot 8: A03 DU_RAP
(P1 in all)
subG1
0.6%
G1
55.8%
S
16.9%
G2
16.0%
Count
FL2-A


Plot 8: A06 DU145_Rap+Purva
(P1 in all)
subG1
8.8%
G1
26.3%
S
39.3%
G2
10.7%
Count
FL2-A


Plot 8: A07 DU145_Rap+Rosc
(P1 in all)
subG1
19.0%
G1
16.2%
S
31.8%
G2
9.6%
FL2-A

B.

Figure 3

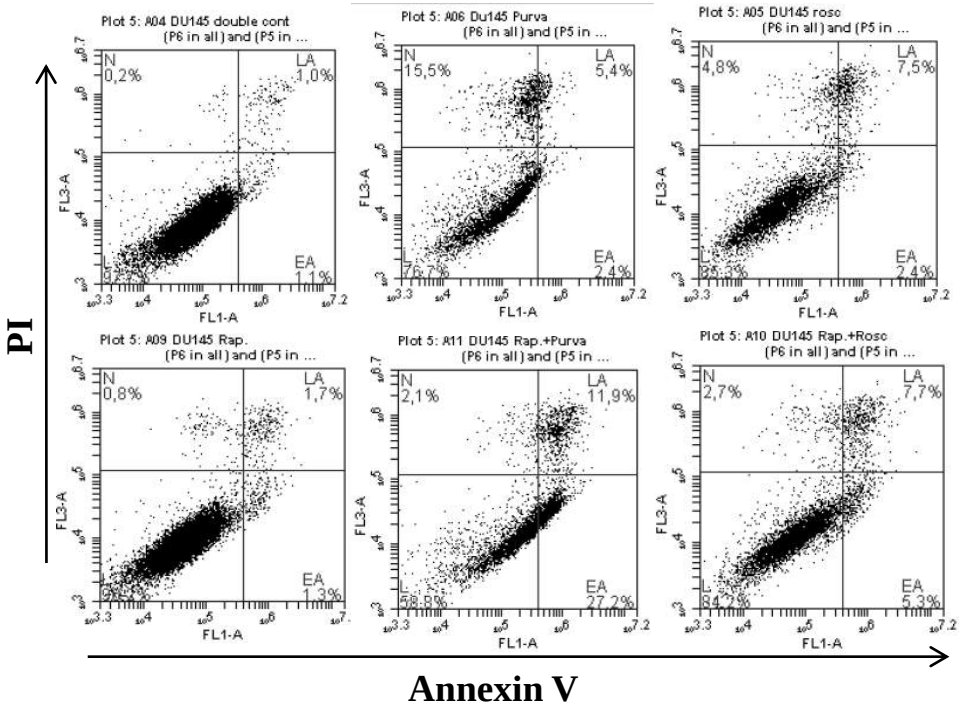

Plot 5: A04 DU145 double cont
(P6 in all) and (P5 in ...
N 0,2%
LA 1,0%
EA 1,1%
Plot 5: A06 Du145 Purva
(P6 in all) and (P5 in ...
N 15,5%
LA 5,4%
EA 2,4%
Plot 5: A05 DU145 roso
(P6 in all) and (P5 in ...
N 4,8%
LA 7,5%
EA 2,4%
Plot 5: A09 DU145 Rap.
(P6 in all) and (P5 in ...
N 0,8%
LA 1,7%
EA 1,3%
Plot 5: A11 DU145 Rap.+Purva
(P6 in all) and (P5 in ...
N 2,1%
LA 11,9%
EA 27,2%
Plot 5: A10 DU145 Rap.+Roso
(P6 in all) and (P5 in ...
N 2,7%
LA 7,7%
EA 5,3%
FL3-A
FL1-A
PI
Annexin V

Figure 4

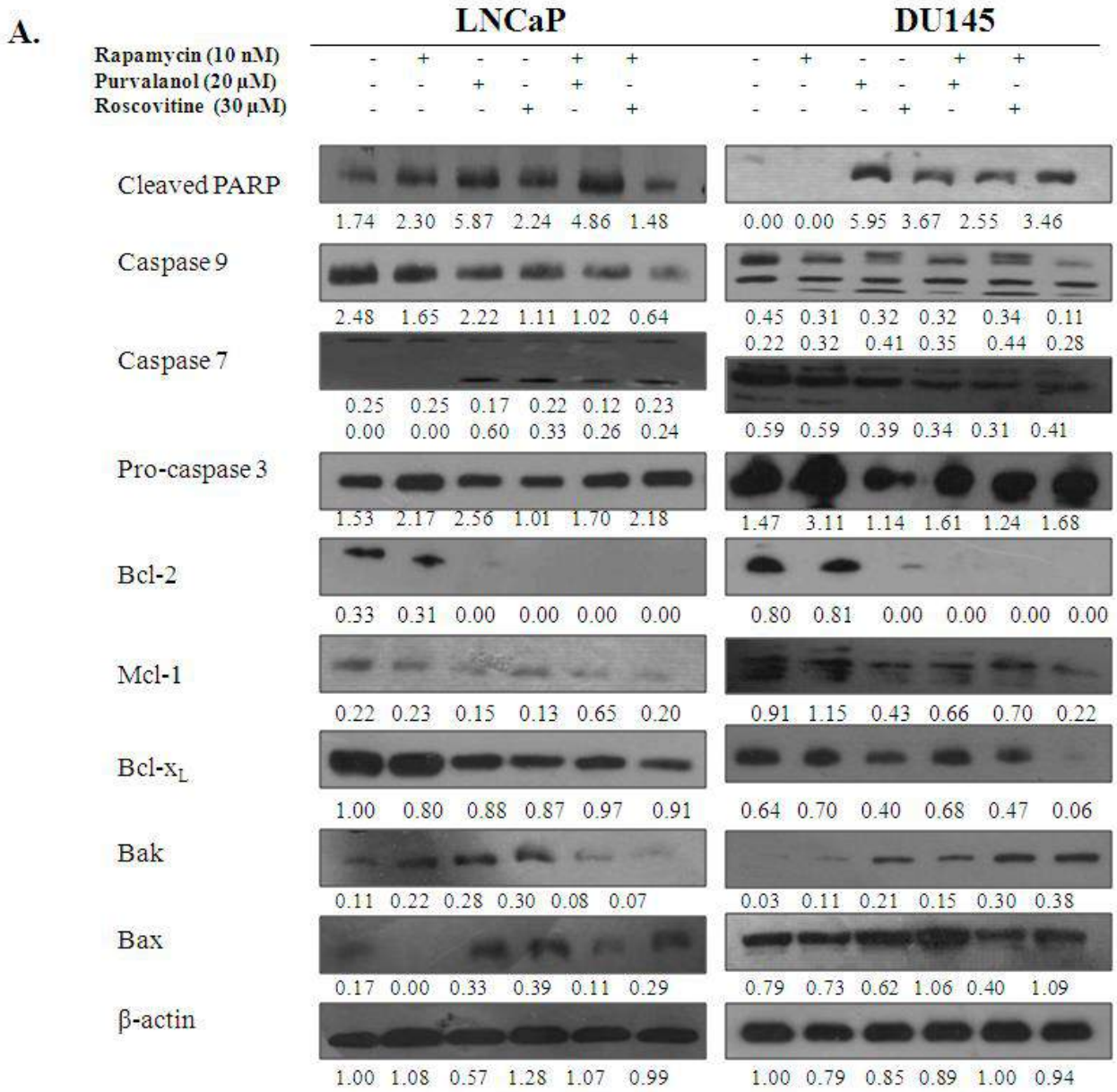
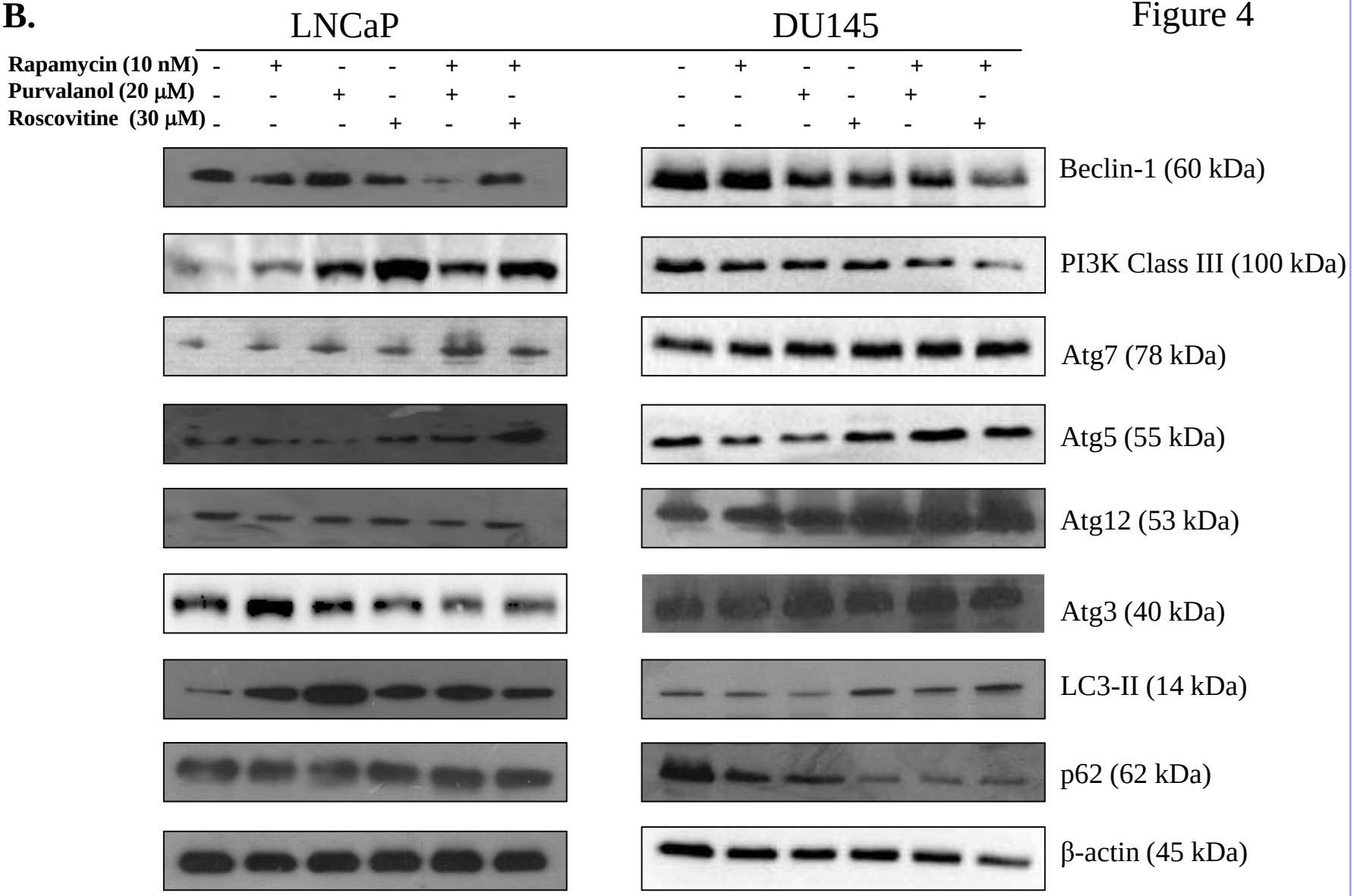
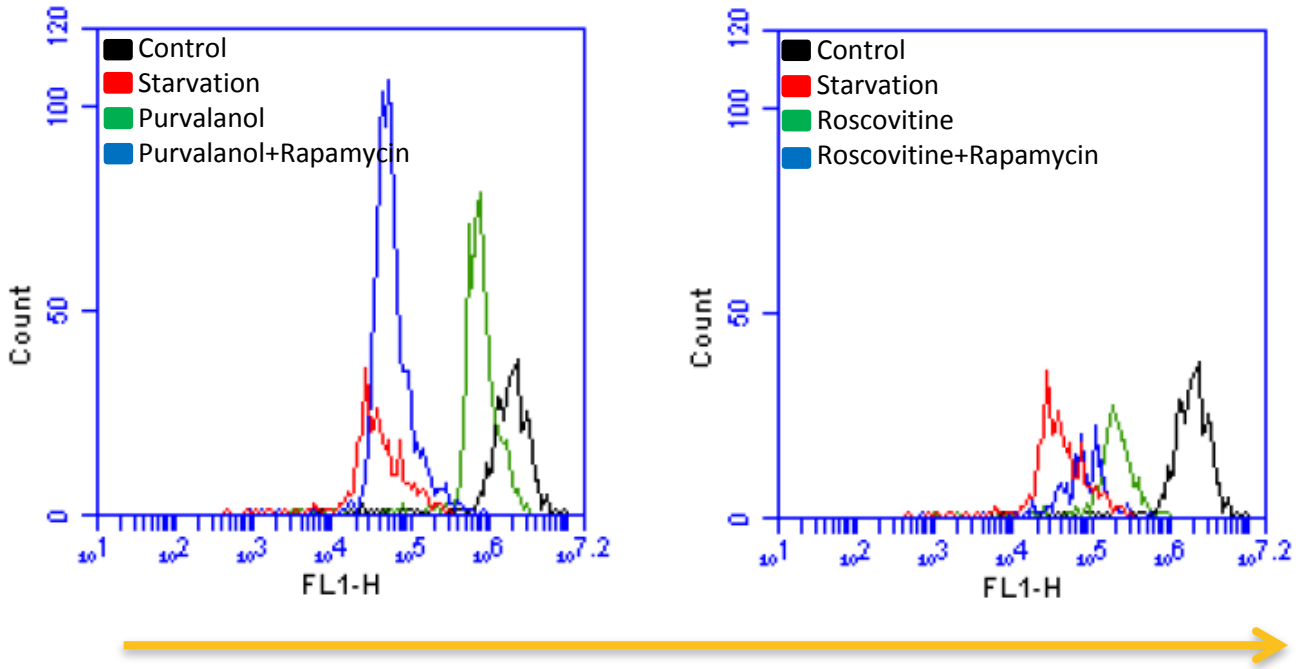

A.
LNCaP
DU145
Rapamycin (10 nM)
Purvalanol (20 μM)
Roscovitine (30 μM)
Cleaved PARP
1.74 2.30 5.87 2.24 4.86 1.48
0.00 0.00 5.95 3.67 2.55 3.46
Caspase 9
2.48 1.65 2.22 1.11 1.02 0.64
0.45 0.31 0.32 0.32 0.34 0.11
Caspase 7
0.25 0.25 0.17 0.22 0.12 0.23
0.00 0.00 0.60 0.33 0.26 0.24
0.22 0.32 0.41 0.35 0.44 0.28
0.59 0.59 0.39 0.34 0.31 0.41
Pro-caspase 3
1.53 2.17 2.56 1.01 1.70 2.18
1.47 3.11 1.14 1.61 1.24 1.68
Bcl-2
0.33 0.31 0.00 0.00 0.00 0.00
0.80 0.81 0.00 0.00 0.00 0.00
Mcl-1
0.22 0.23 0.15 0.13 0.65 0.20
0.91 1.15 0.43 0.66 0.70 0.22
Bcl-xL
1.00 0.80 0.88 0.87 0.97 0.91
0.64 0.70 0.40 0.68 0.47 0.06
Bak
0.11 0.22 0.28 0.30 0.08 0.07
0.03 0.11 0.21 0.15 0.30 0.38
Bax
0.17 0.00 0.33 0.39 0.11 0.29
0.79 0.73 0.62 1.06 0.40 1.09
β-actin
1.00 1.08 0.57 1.28 1.07 0.99
1.00 0.79 0.85 0.89 1.00 0.94

Figure 4

B.


LNCaP
DU145
Rapamycin (10 nM) - + - - + + - + - - + +
Purvalanol (20 μM) - - + - + - - - + - + -
Roscovitine (30 μM) - - - + - + - - - + - +
Beclin-1 (60 kDa)
PI3K Class III (100 kDa)
Atg7 (78 kDa)
Atg5 (55 kDa)
Atg12 (53 kDa)
Atg3 (40 kDa)
LC3-II (14 kDa)
p62 (62 kDa)
β-actin (45 kDa)

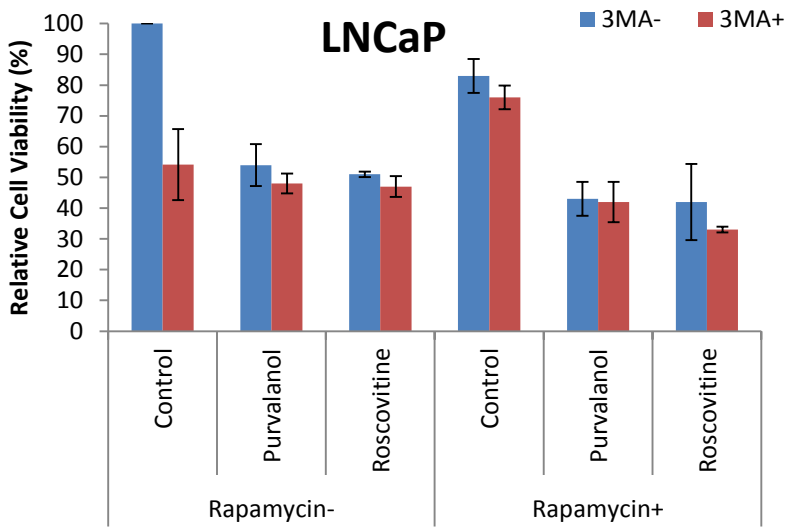
C.

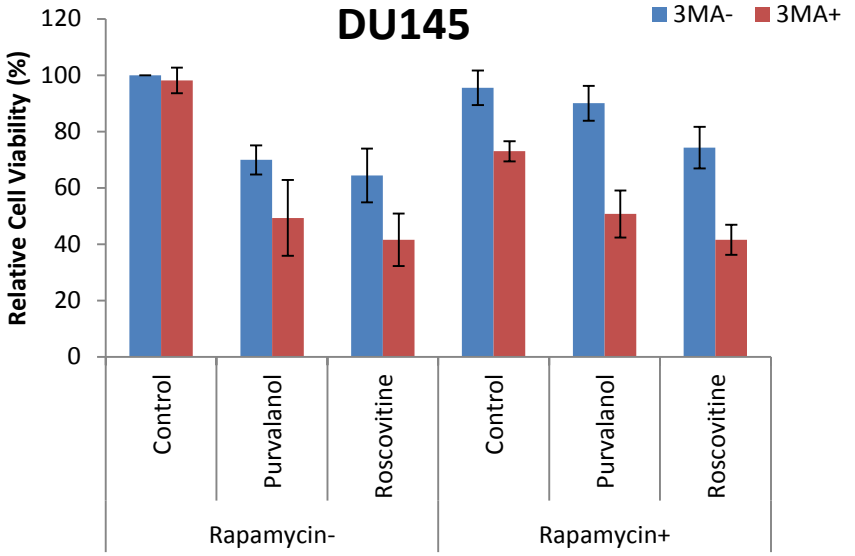


LC3-488 Alexa Flour

A.

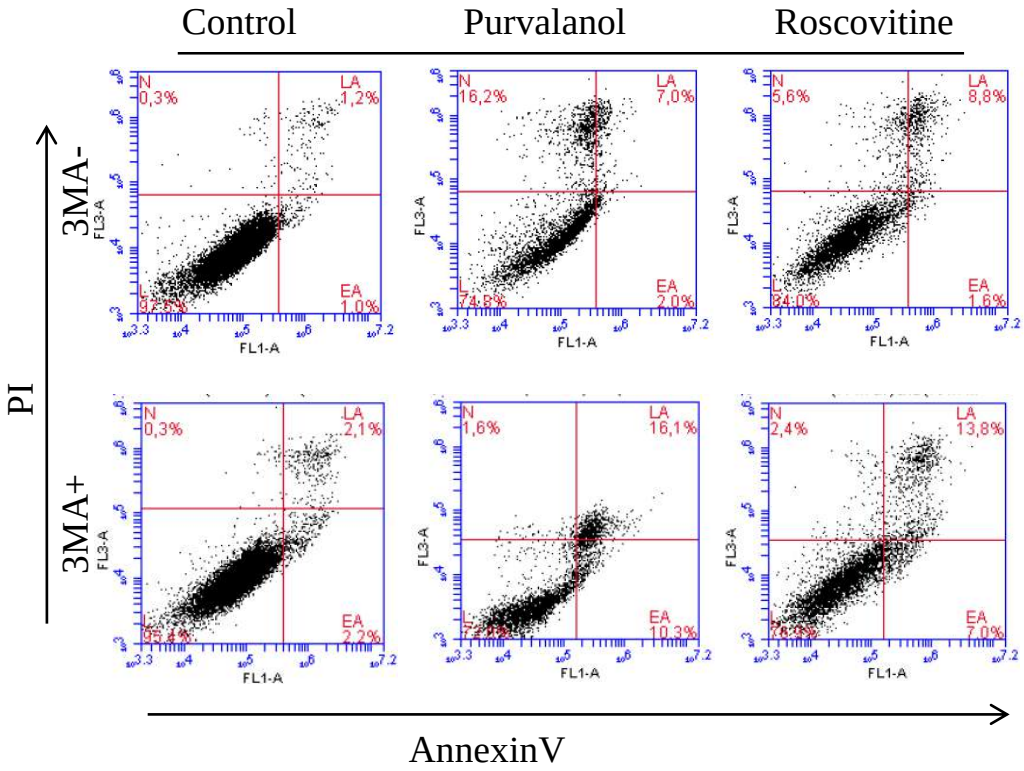
Figure 5


LNCaP
3MA-
3MA+
Relative Cell Viability (%)
100
90
80
70
60
50
40
30
20
10
0
Control
Purvalanol
Roscovitine
Control
Purvalanol
Roscovitine
Rapamycin-
Rapamycin+

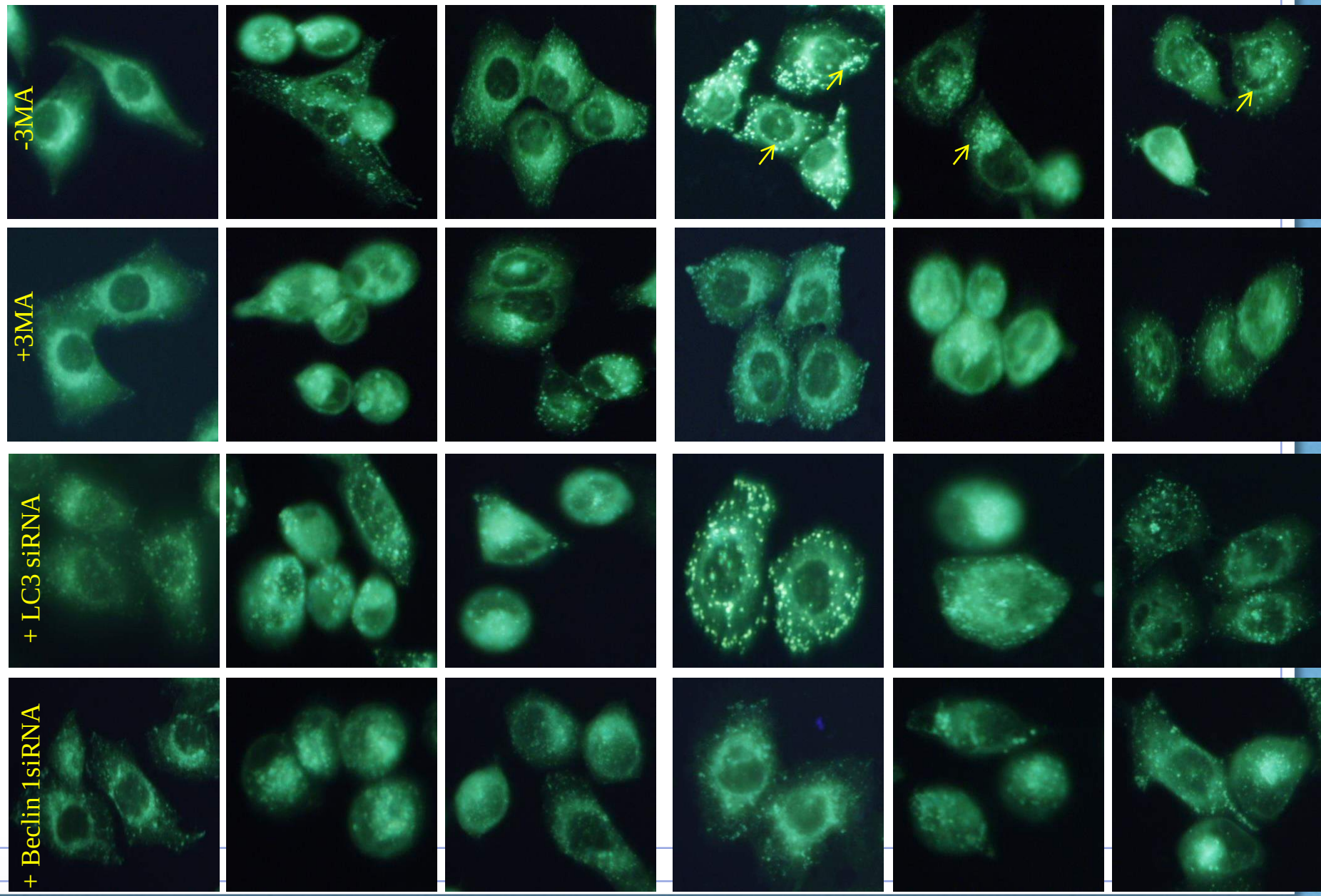

DU145
3MA-
3MA+
Relative Cell Viability (%)
120
100
80
60
40
20
0
Control
Purvalanol
Roscovitine
Control
Purvalanol
Roscovitine
Rapamycin-
Rapamycin+

B.

DU145

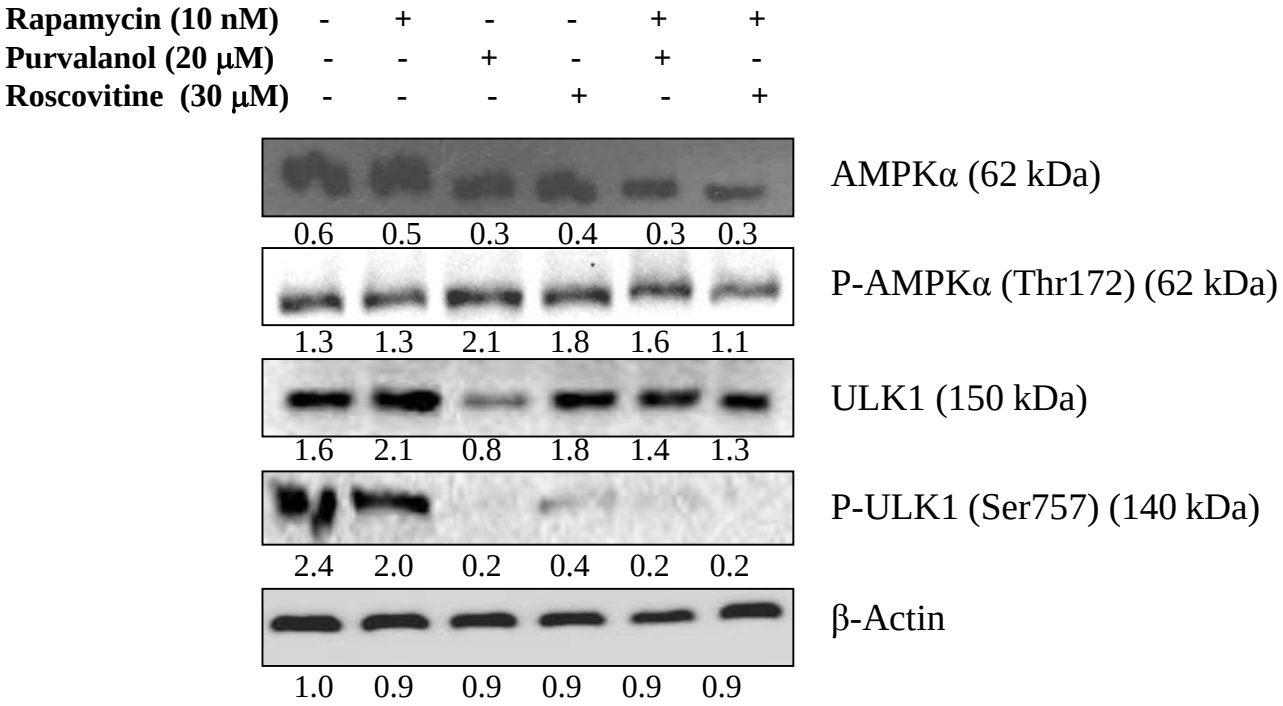

Control
Purvalanol
Roscovitine
3MA-
3MA+
PI
AnnexinV
FL1-A
FL3-A
N 0,3%
LA 1,2%
EA 1,0%
N 16,2%
LA 7,0%
EA 2,0%
N 5,6%
LA 8,8%
EA 1,6%
N 0,3%
LA 2,1%
EA 2,2%
N 1,6%
LA 16,1%
EA 10,3%
N 2,4%
LA 13,8%
EA 7,0%

DU145


- Rapamycin
+ Rapamycin
-
+ Purvalanol
+ Roscovitine
-
+ Purvalanol
+Roscovitine
-3MA
+3MA
+ LC3 siRNA
+ Beclin 1siRNA

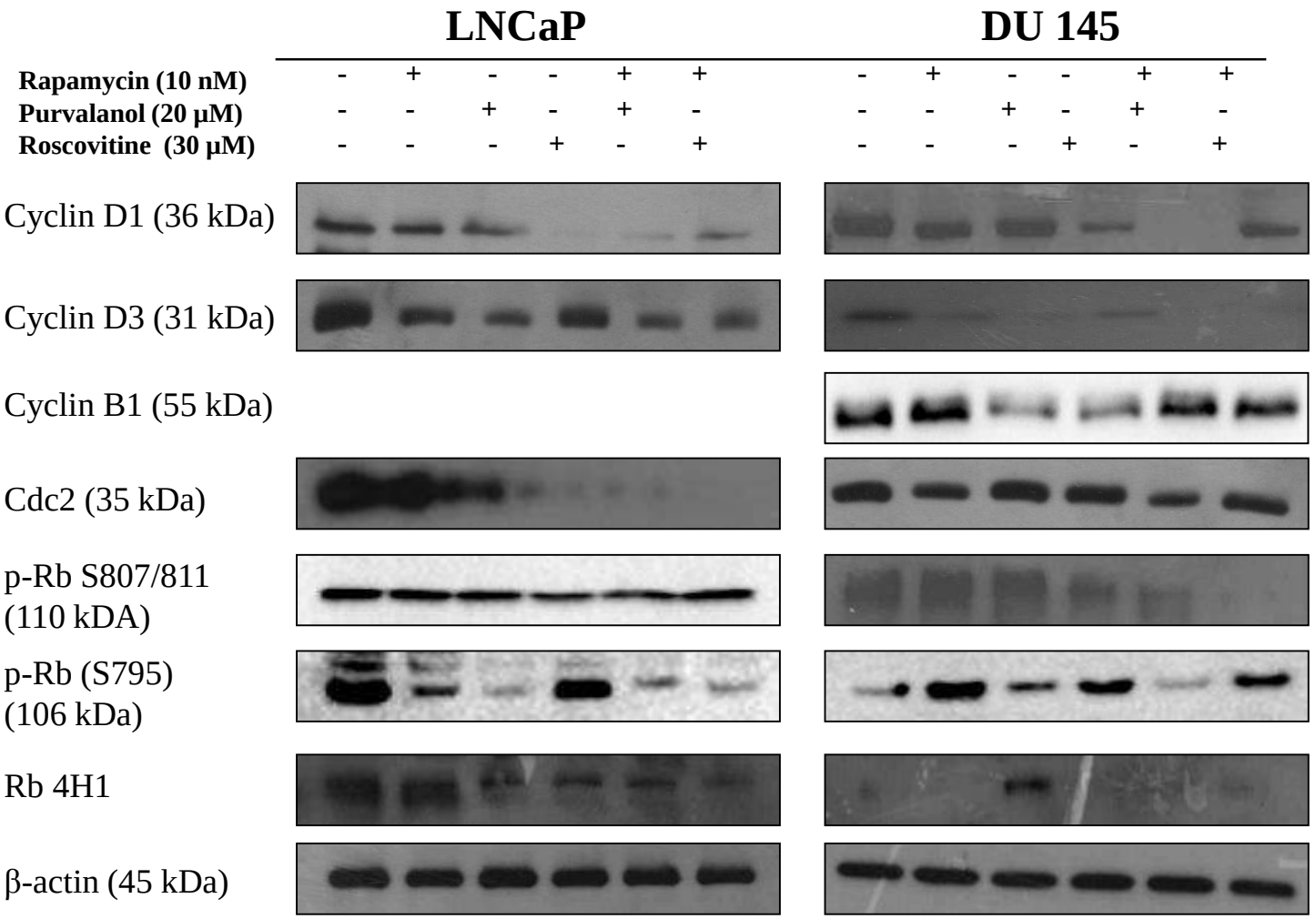
B.

Figure 6


DU145
Rapamycin (10 nM) - + - - + +
Purvalanol (20 μM) - - + - + -
Roscovitine (30 μM) - - - + - +
AMPKα (62 kDa)
0.6 0.5 0.3 0.4 0.3 0.3
P-AMPKα (Thr172) (62 kDa)
1.3 1.3 2.1 1.8 1.6 1.1
ULK1 (150 kDa)
1.6 2.1 0.8 1.8 1.4 1.3
P-ULK1 (Ser757) (140 kDa)
2.4 2.0 0.2 0.4 0.2 0.2
β-Actin
1.0 0.9 0.9 0.9 0.9 0.9

A.

Figure 3


LNCaP
DU 145
Rapamycin (10 nM)
Purvalanol (20 µM)
Roscovitine (30 µM)
- + - - + + - + - - + +
- - + - + - - - + - + -
- - - + - + - - - + - +
Cyclin D1 (36 kDa)
Cyclin D3 (31 kDa)
Cyclin B1 (55 kDa)
Cdc2 (35 kDa)
p-Rb S807/811 (110 kDA)
p-Rb (S795) (106 kDa)
Rb 4H1
β-actin (45 kDa)