Supplemental Information

Phosphatidylinositol-3 Kinase Inhibitors, Buparlisib and Alpelisib, Sensitize Estrogen Receptor-positive Breast Cancer Cells to Tamoxifen

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Supplemental Figure 1: Median Effect Analysis and western blotting results in MCF-7 cells

MCF-7 cells were treated with (A) T + BYL, (B) T + BKM, and (C) T + everolimus for 72 hours. Immunoblotting was performed at 3, 8, and 24 hours after control (CTL), tamoxifen (T), alpelisib (BYL), and T+BYL in (D) or buparlisib (BKM) and T+BKM in (E).

Supplemental Figure 2:

ER expression level in MCF-7 cells treated with (A) CTL, T, BYL, T + BYL or (B) CTL, T, BKM, and T + BKM at 3, 8, 24, 48, and 72 hours.

Supplemental Figure 3:

(A) Proportion of apoptotic cells in T47D cells treated with CTL, T, BYL, T+BYL, BKM, and T + BKM for 72 hours. (B) MCF-7 cells transfected with myristolyated AKT. Clones 5 and 8 were chosen for subsequent experiments. Median effect analysis for tamoxifen-resistant MCF-7 (MCF-7/TamR) treated with T + BYL (C) or T + BKM (D) for 72 hours.

Supplemental Figure 4:

(A) Knockdown efficiency of PI3K-p110α and PI3K-p110β by different siRNAs, (1) wild type (2) siRNA-control (3) siRNA-PIK3CA and (4) siRNA-PIK3CB, in T47D cells. (B) Knockdown efficiency of PI3K-p110α by (1) wild type (2) siRNA-control (3) siRNA-PIK3CA, in MCF7 cells. Median effect analysis for T+BYL in T47D cells: (C) wild type (D) siRNA-control (E) siRNA-PIK3CA (F) siRNA-PIK3CB or in MCF-7 cells: (K) wild type (L) siRNA-PIK3CA. Median effect analysis for T+BKM in T47D cells: (G) wild type (H) siRNA-control (I) siRNA-PIK3CA (J) siRNA-PIK3CB or in MCF-7 cells: (M) wild type (N) siRNA-PIK3CA. All

of the siRNA were given at 200pM according to manufacturer's manual. Both the western blotting and median effect analysis were performed after 72 hours of treatment.

Supplemental Figure 5: MTT Assay of Alpelisib or Buparlisib in Different ER Mutant MCF-7 Cells

MCF-7 cells transfected with Y537S (MCF-7/Y537S) or Y537C (MCF-7/Y537C) mutant plasmids were treated with (A) alpelisib or (B) buparlisib.

DNA sequencing analysis to confirm the presence of the mutant plasmid (TAT to AGT) in MCF7 cells (C) or ZR 75-1 cells (E). Detection of GFP under fluorescence microscopy (Leica, 200x) in MCF-7 cells (D) or ZR 75-1 cells (F)

Supplemental Figure 6: Uncropped western blot figures for Figure 2A and 2B

Uncropped original western blot figures for Figure 2A (A-C) and Figure 2B (D-F)

Supplemental Figure 7: Uncropped western blot figures for Figure 4B

The western blots that were cropped into Figure 4B were marked in blue square.

Supplemental Figure 8: Uncropped western blot figures for Supplemental Figure 1D, 1E, 2A, and 2B

Uncropped original western blot figures for Supplemental Figure 1D (A), Supplemental Figure 1E (B), Supplemental Figure 2A (C), and Supplemental Figure 2B (D)

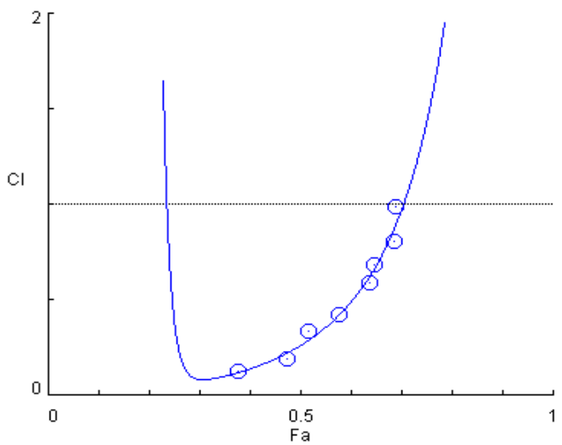
Supplemental Figure 9: Uncropped western blot figures for Supplemental Figure 3B, 4A, and 4B

Uncropped original western blot figures for Supplemental Figure 3B (A), Supplemental Figure 4A (B), and Supplemental Figure 4B (C)

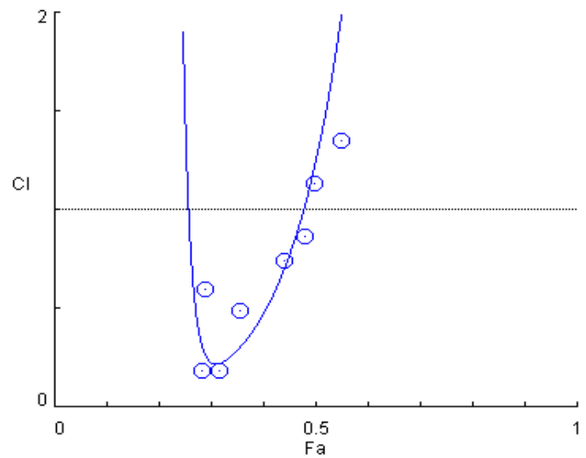
Supplement Table 1: Tumor Size Comparison Between Treatment Groups

Unpaired t-test was used to compare treatment effects between groups. p-value is listed in the table.

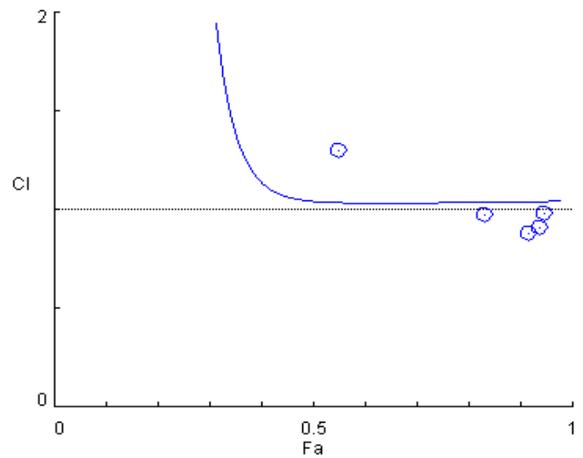
(A)


T+BYL
CI
2
0
0
0.5
1
Fa

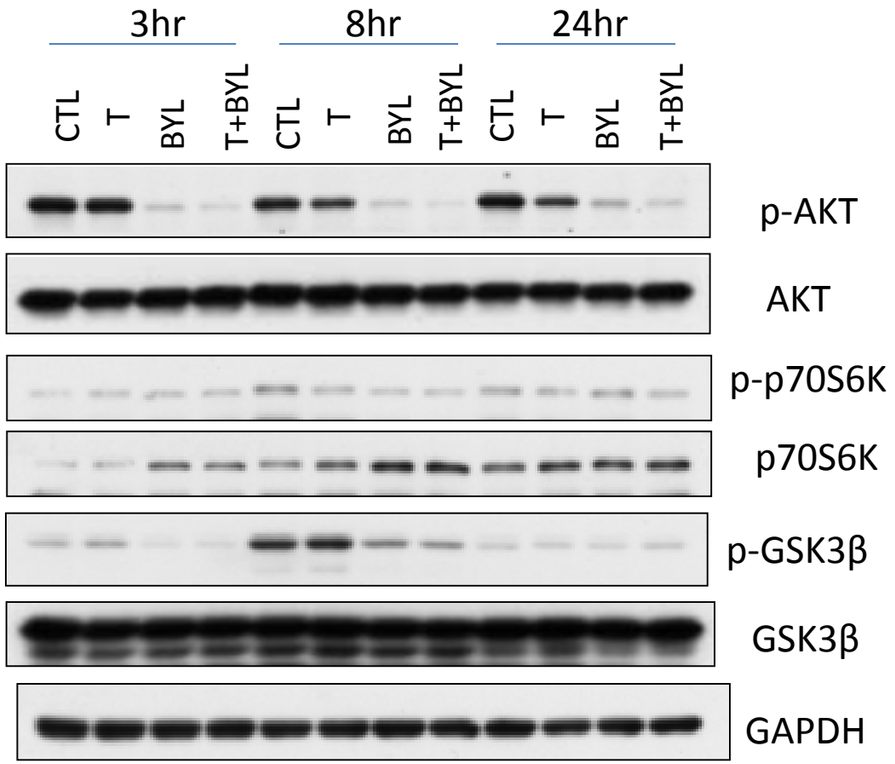
(B)


T+BKM
CI
2
0
0
0.5
1
Fa

(C)


T+ Eve
CI
2
0
0
0.5
1
Fa

(D)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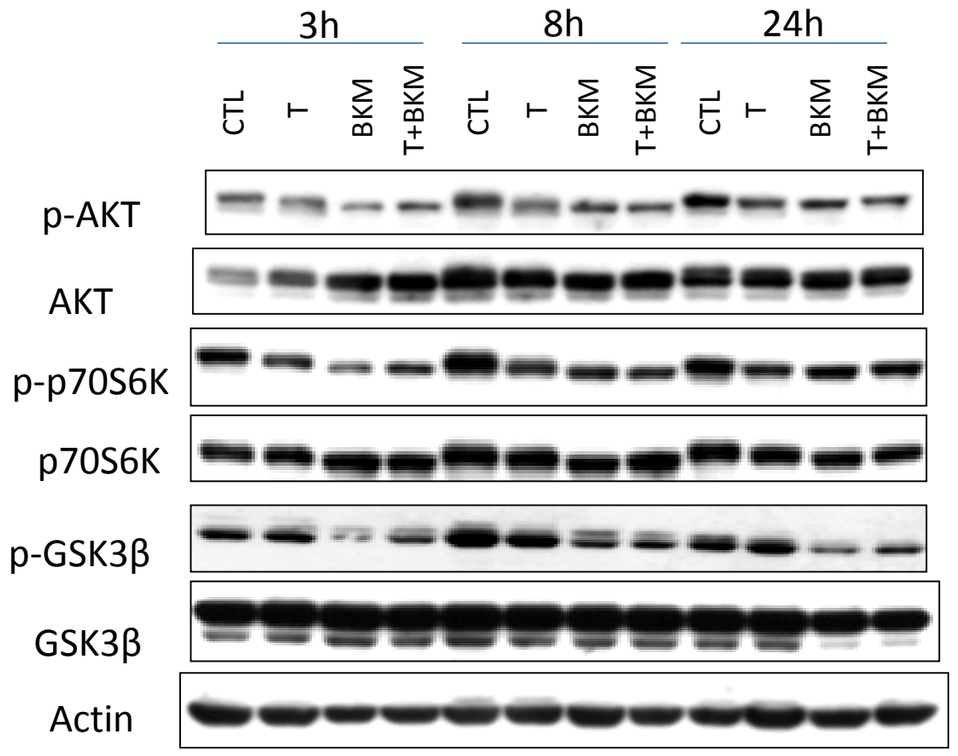
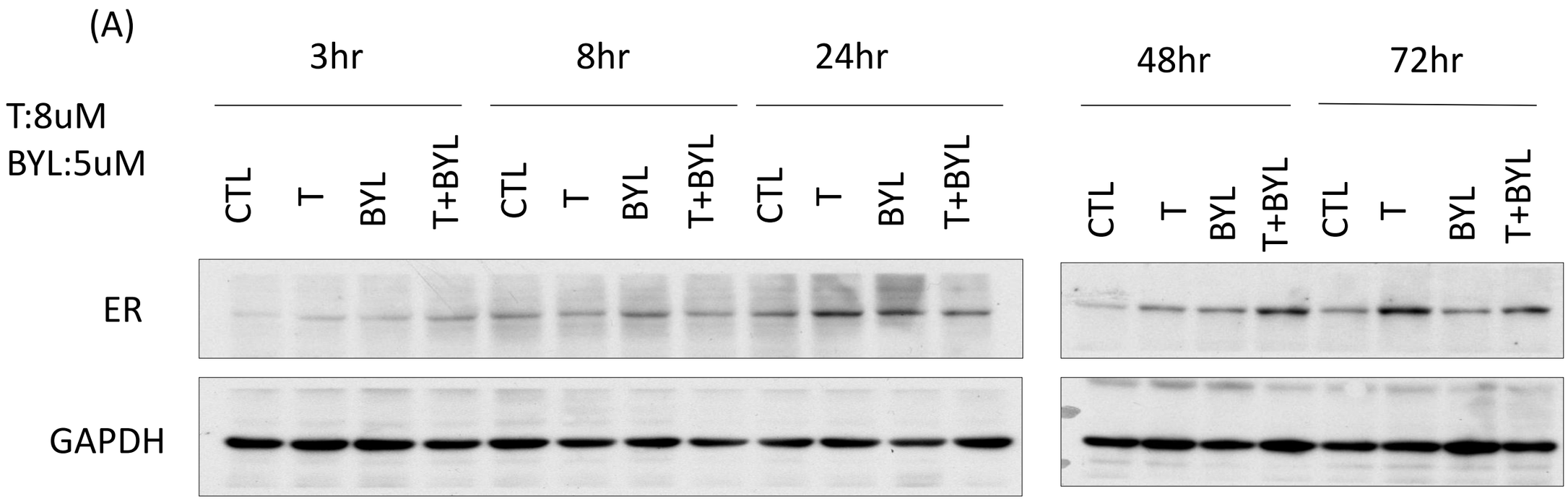
8hr
24hr
CTL
T
BYL
T+BYL
CTL
T
BYL
T+BYL
CTL
T
BYL
T+BYL
p-AKT
AKT
p-p70S6K
p70S6K
p-GSK3β
GSK3β
GAPDH

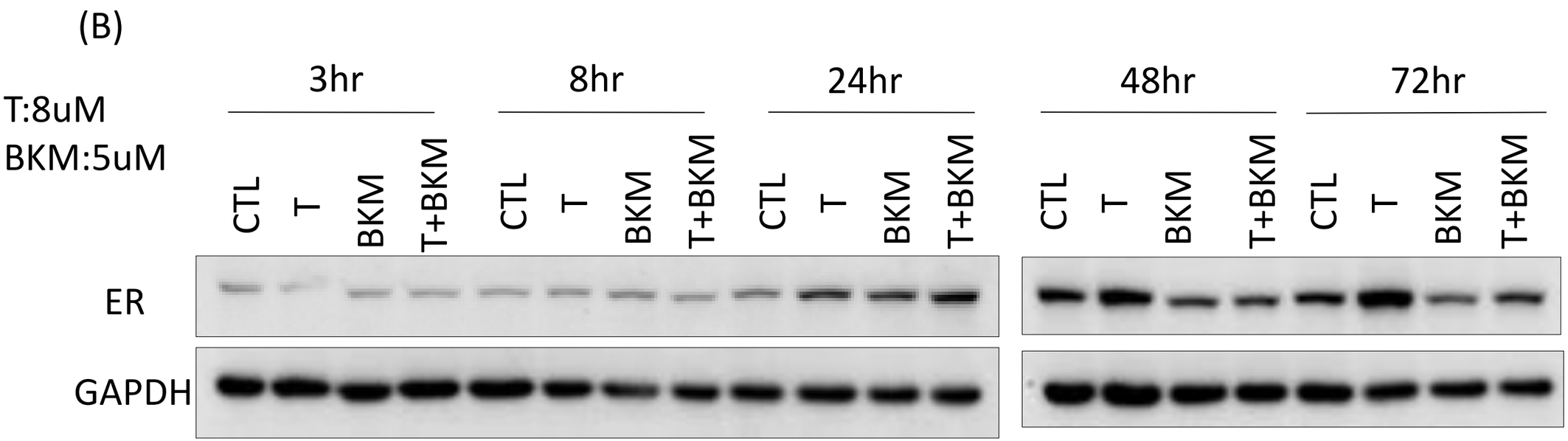
(E)


3h
8h
24h
CTL
T
BKM
T+BKM
CTL
T
BKM
T+BKM
CTL
T
BKM
T+BKM
p-AKT
AKT
p-p70S6K
p70S6K
p-GSK3β
GSK3β
Actin

(A)


3hr
8hr
24hr
48hr
72hr
T:8uM
BYL:5uM
CTL
T
BYL
T+BYL
ER
GAPDH

(B)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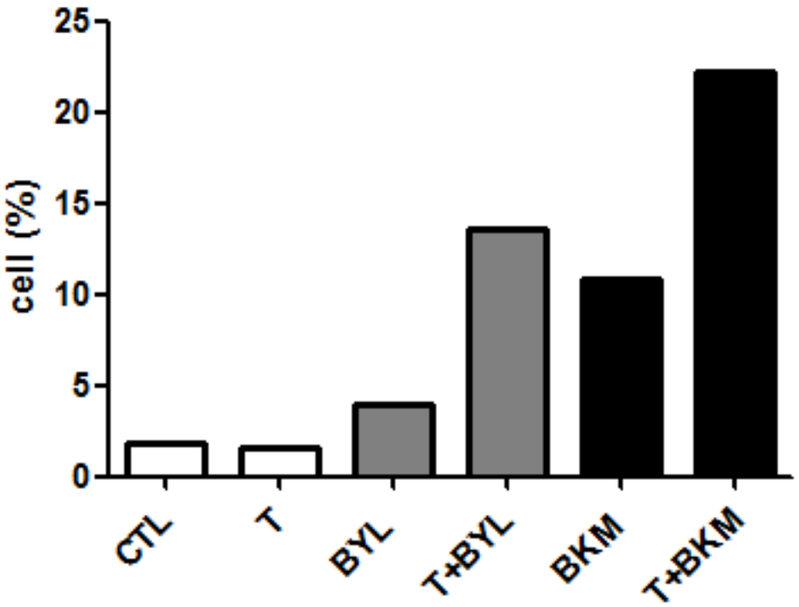
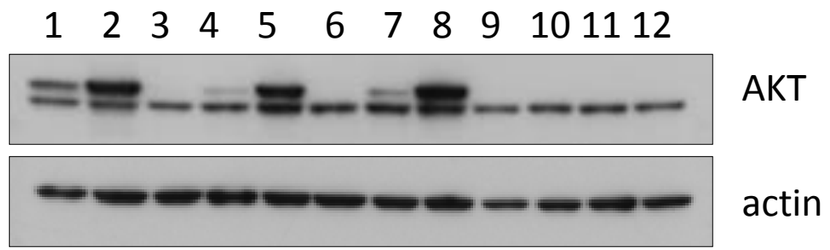
BKM:5uM
CTL
T
BKM
T+BKM
ER
GAPDH

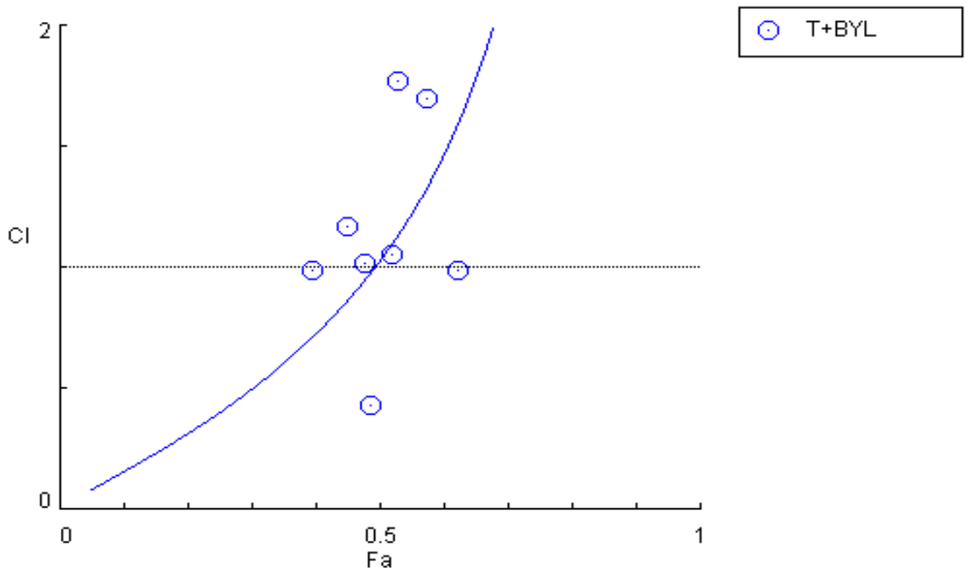
(A)


25
20
15
10
5
0
cell (%)
CTL
T
BYL
T+BYL
BKM
T+BKM

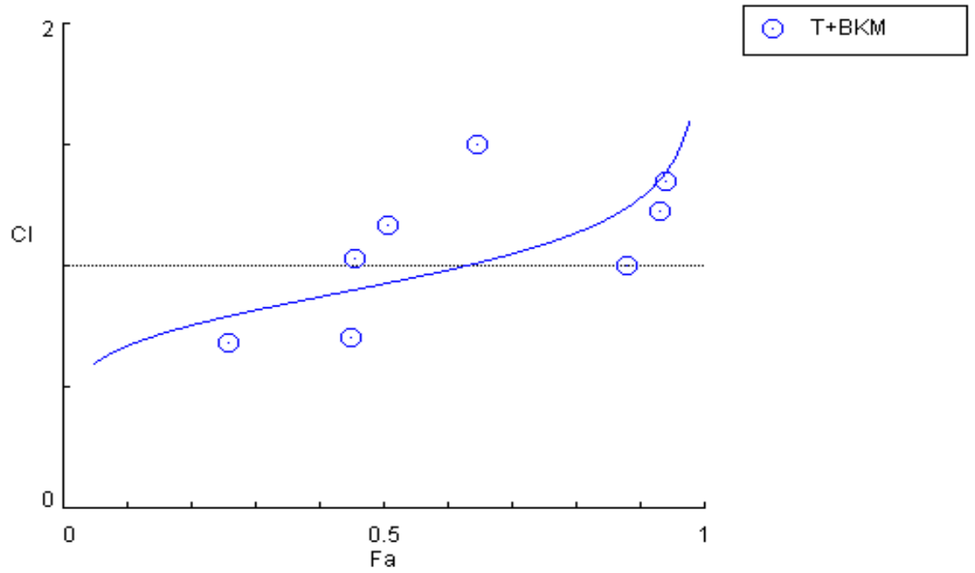
(B)


1 2 3 4 5 6 7 8 9 10 11 12
AKT
actin

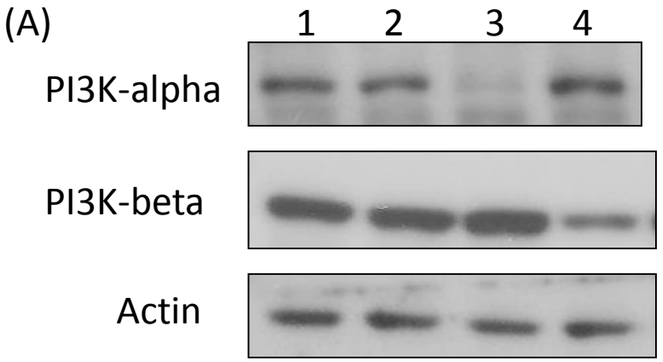
(C)


T+BYL
2
CI
0
0
0.5
1
Fa

(D)


T+BKM
2
CI
0
0
0.5
1
Fa

(A)



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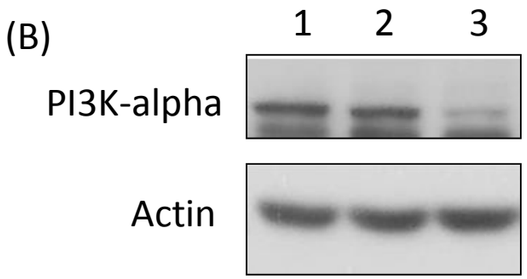

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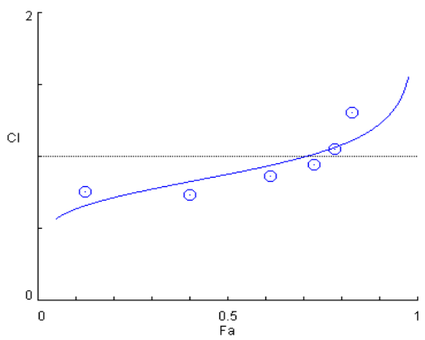

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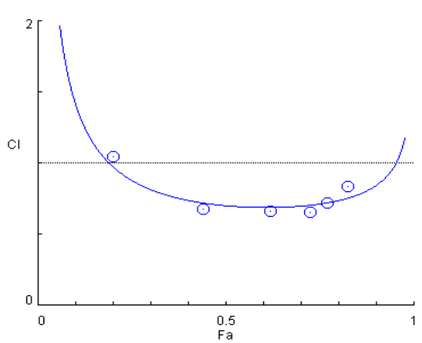

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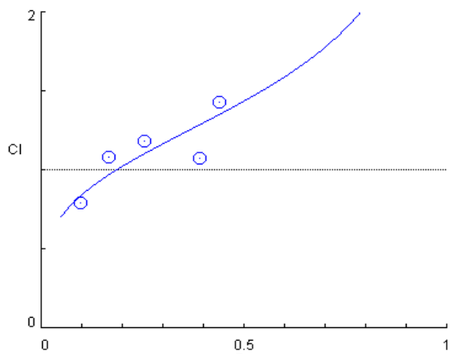

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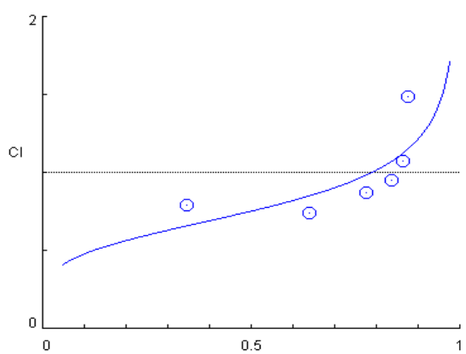

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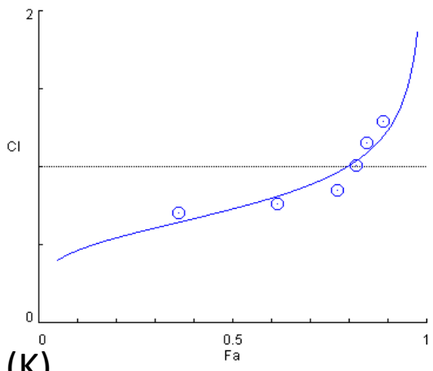

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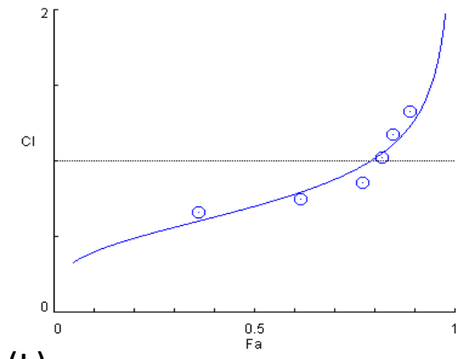

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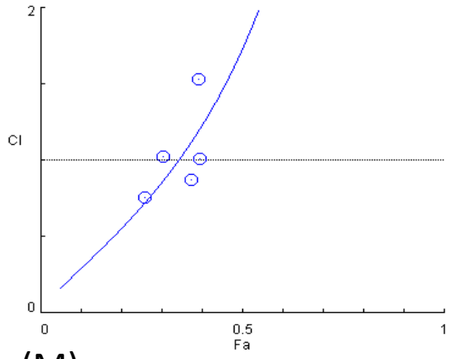

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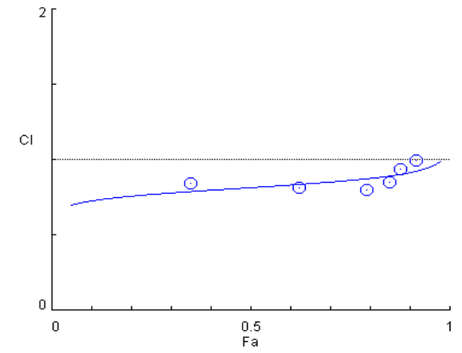

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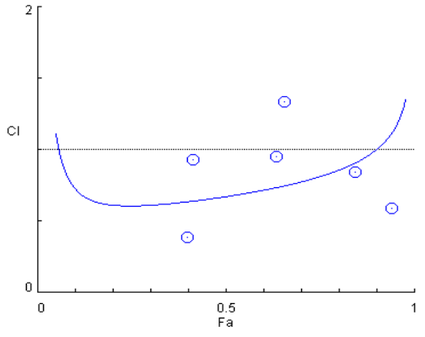

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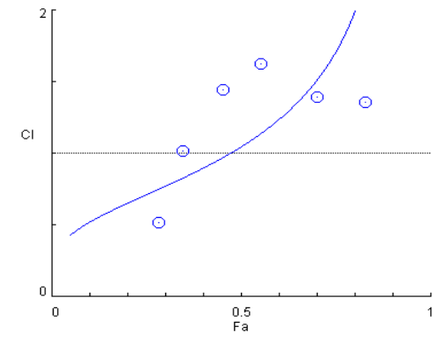

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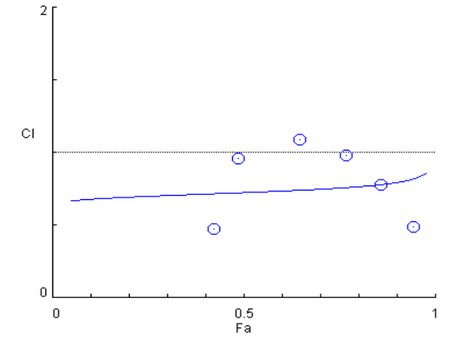

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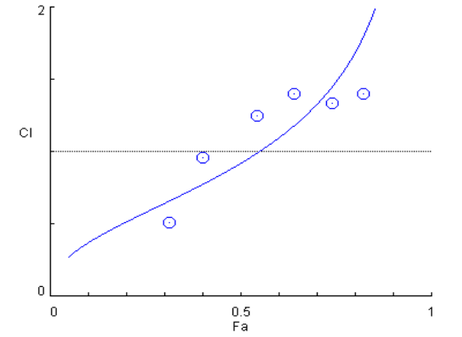

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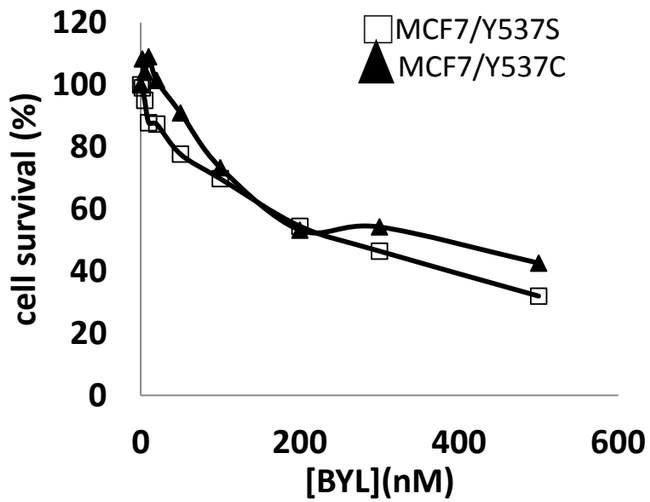
MCF7/Y537S
MCF7/Y537C
cell survival (%)
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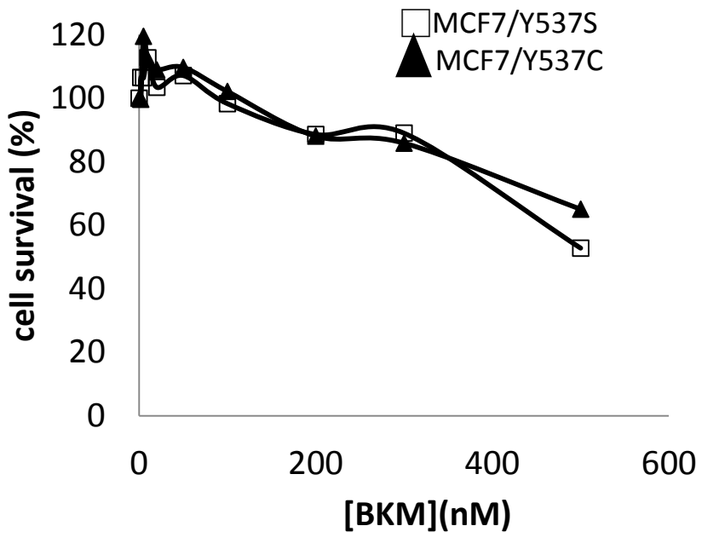
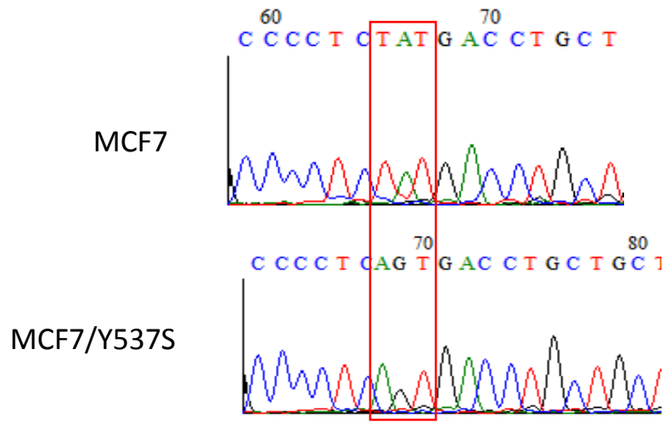
100
80
60
40
20
0
0
200
400
600
[BYL](nM)

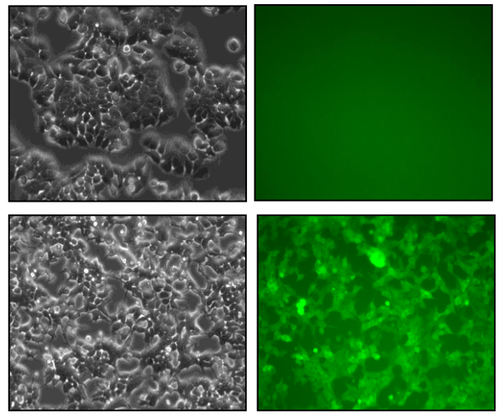
(B)


MCF7/Y537S
MCF7/Y537C
cell survival (%)
120
100
80
60
40
20
0
0
200
400
600
[BKM](nM)

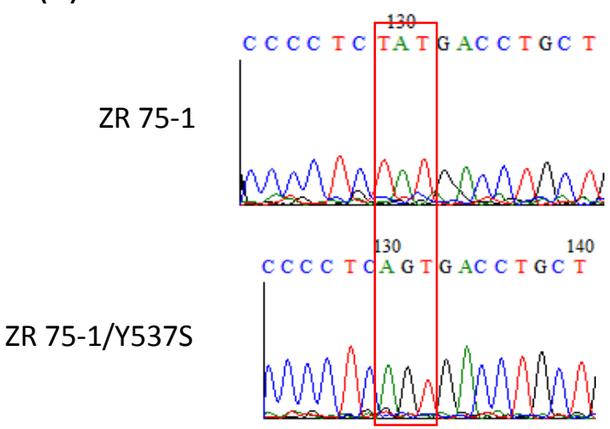
(C)


60
70
C C C C T C T A T G A C C T G C T
MCF7
70
80
C C C C T C A G T G A C C T G C T G C T
MCF7/Y537S

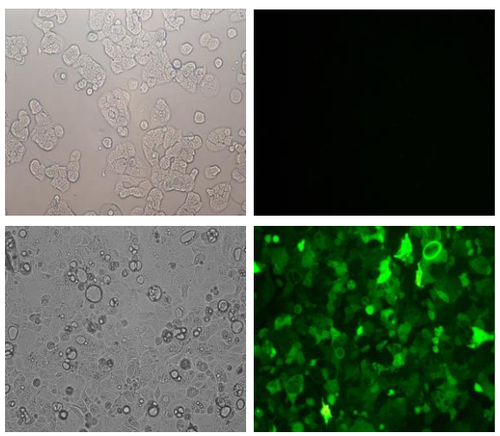
(D)

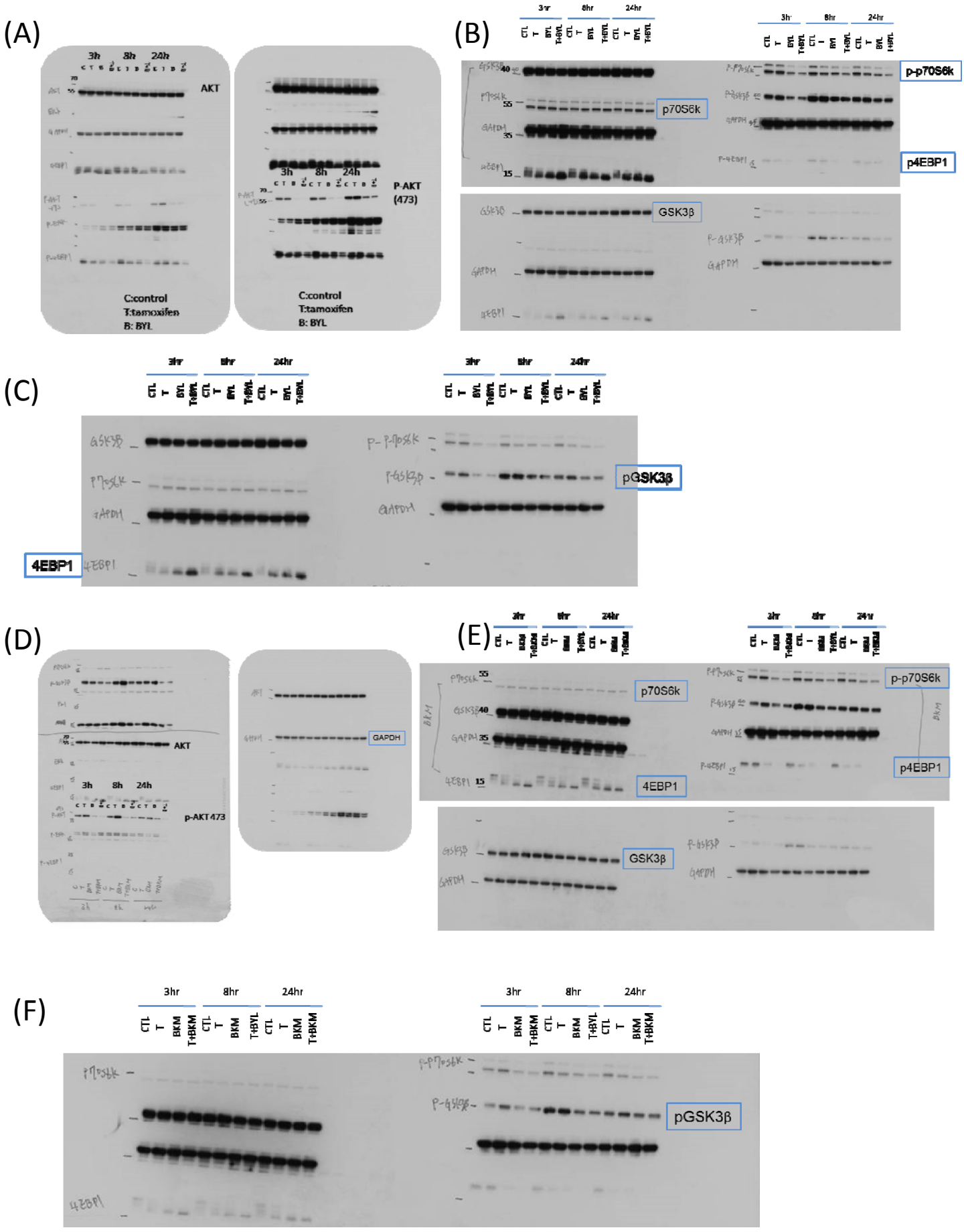

MCF7
MCF7/Y537S

(E)

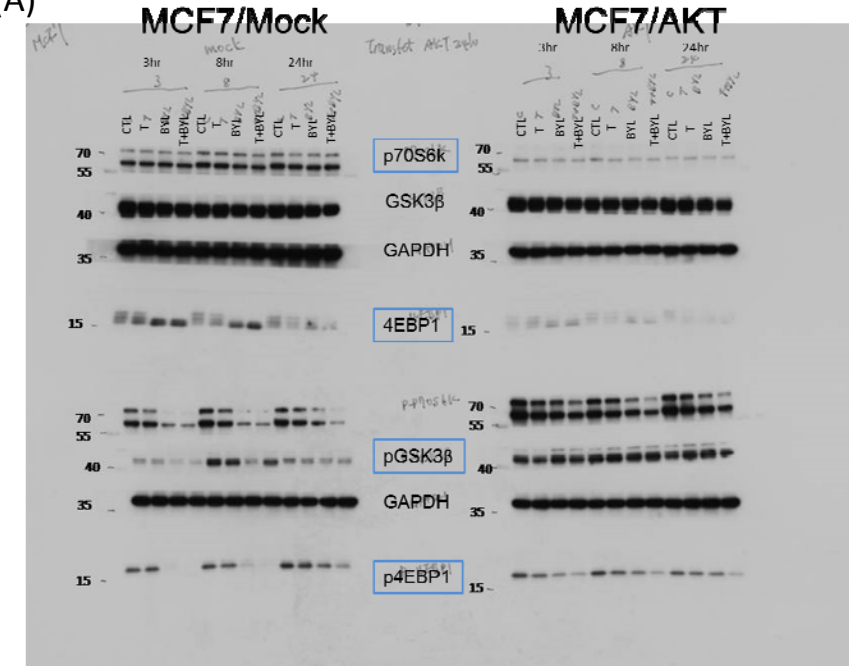

130
C C C C T C T A T G A C C T G C T
ZR 75-1
130
140
C C C C T C A G T G A C C T G C T
ZR 75-1/Y537S

(F)

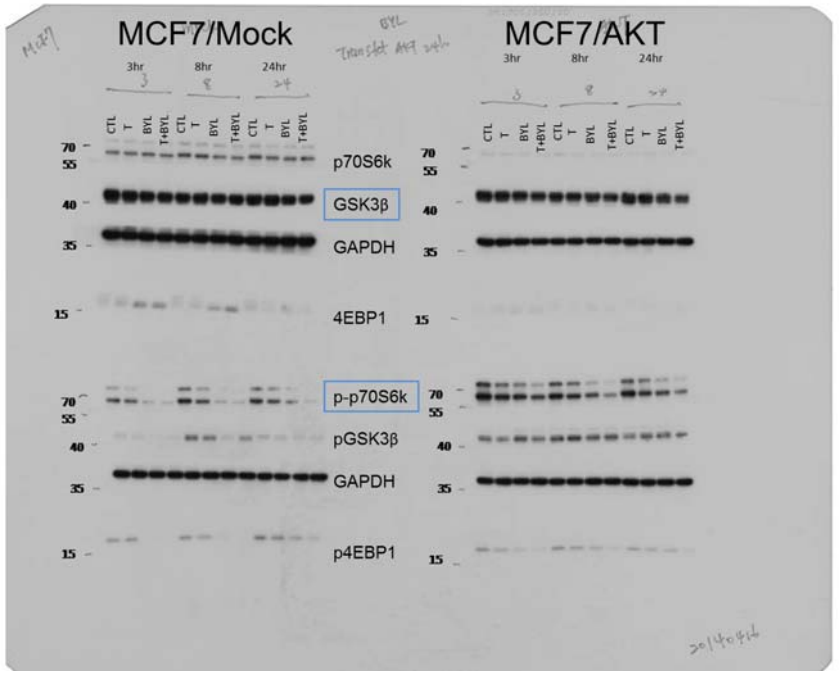

ZR 75-1
ZR 75-1/Y537S


(A)
3h 8h 24h
AKT
C:control
T:tamoxifen
B: BYL
P-AKT (473)
(B)
3hr 8hr 24hr
CTL T BYL T+BYL
p70S6k
p-p70S6k
p4EBP1
GSK3β
GAPDH
(C)
pGSK3β
4EBP1
(D)
AKT
p-AKT 473
GAPDH
(E)
p70S6k
p-p70S6k
p4EBP1
4EBP1
GSK3β
(F)
CTL T BKM T+BKM
pGSK3β

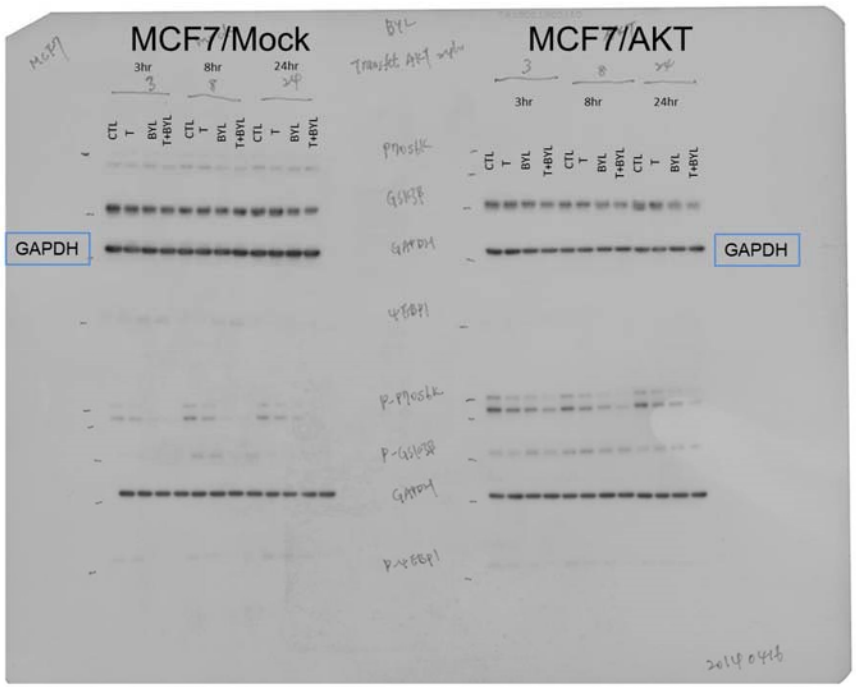
(A)


MCF7/Mock
MCF7/AKT
3hr
8hr
24hr
CTL
T
BYL
T+BYL
70
55
40
35
15
p70S6k
GSK3β
GAPDH
4EBP1
pGSK3β
p4EBP1

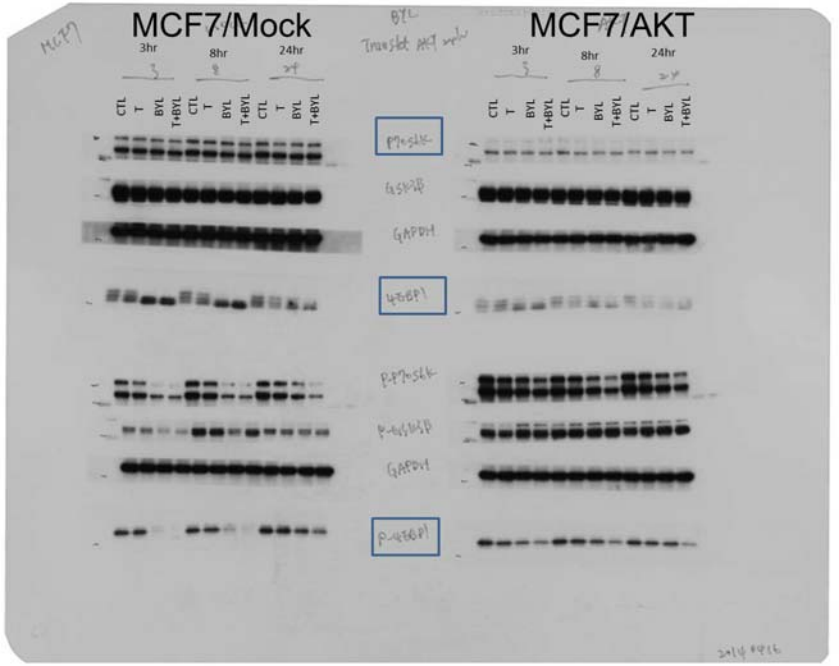
(B)


MCF7/Mock
MCF7/AKT
3hr
8hr
24hr
CTL
T
BYL
T+BYL
70
55
40
35
15
p70S6k
GSK3β
GAPDH
4EBP1
p-p70S6k
pGSK3β
p4EBP1

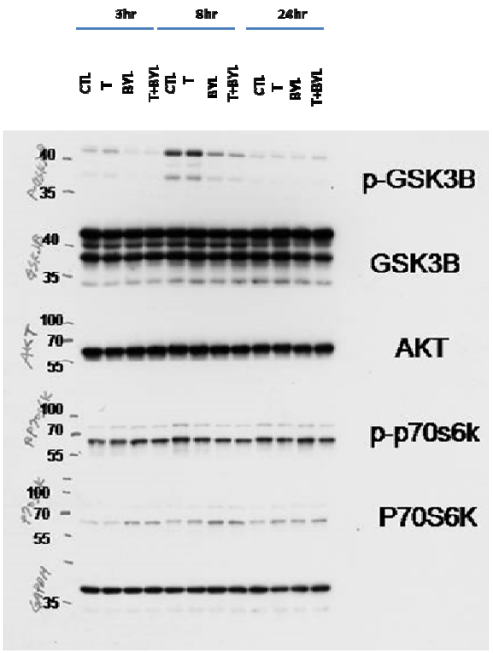
(C)

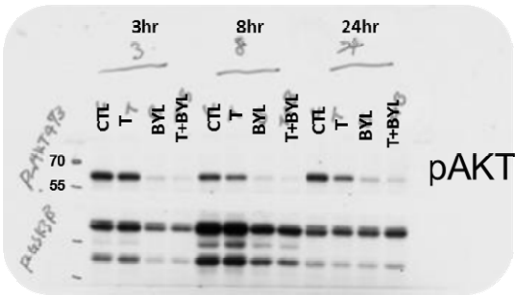

MCF7/Mock
MCF7/AKT
3hr
8hr
24hr
CTL
T
BYL
T+BYL
GAPDH
GAPDH

(D)


MCF7/Mock
MCF7/AKT
3hr
8hr
24hr
CTL
T
BYL
T+BYL

(A)


3hr
8hr
24hr
CTL
T
BYL
T+BYL
p-GSK3B
GSK3B
AKT
p-p70s6k
P70S6K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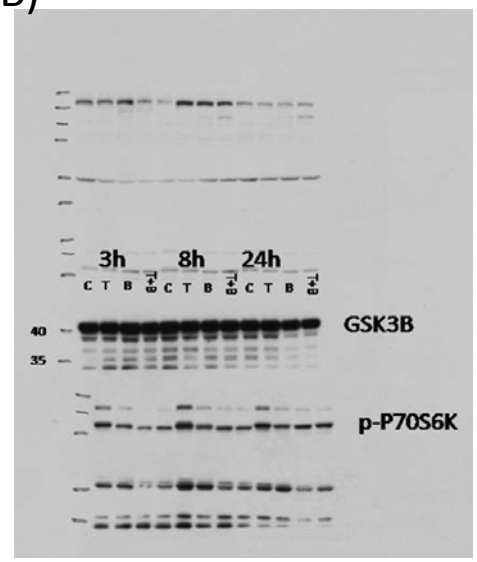
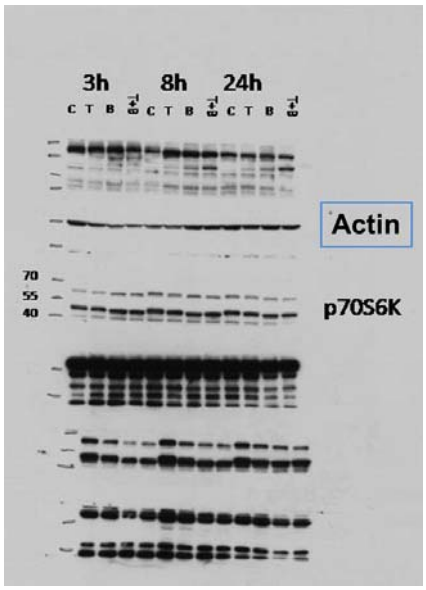
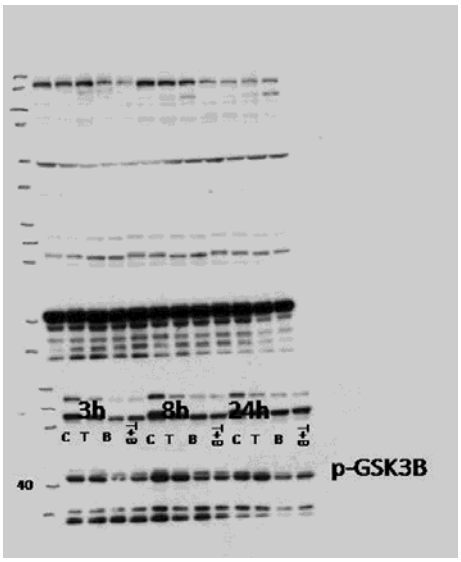
24hr
CTL
T
BYL
T+BYL
pAKT

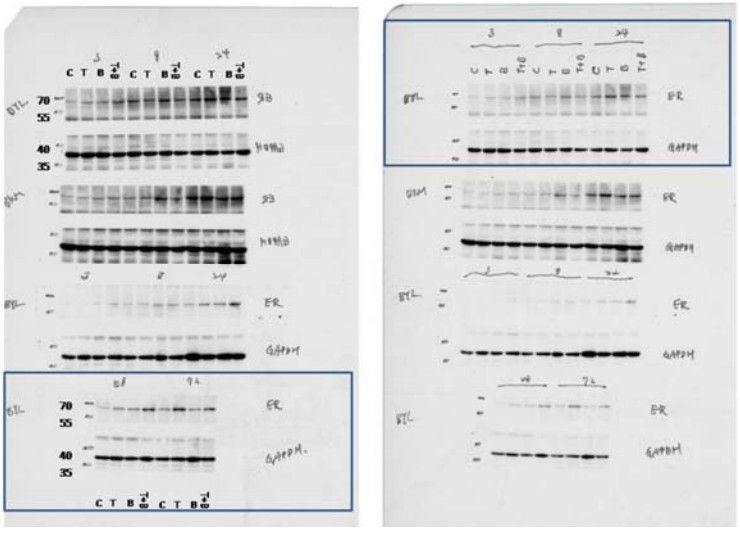
(B)


3h
8h
24h
GSK3B
p-P70S6K

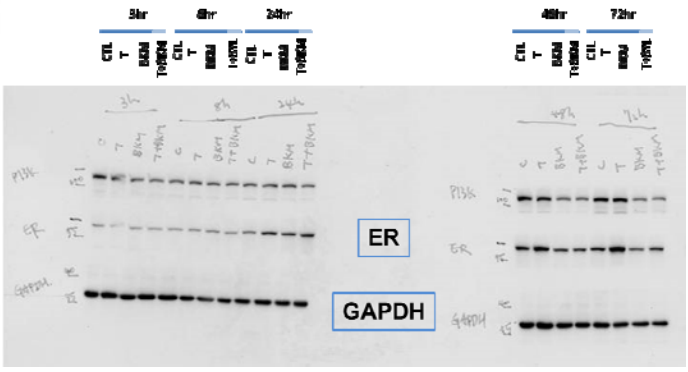

3h
8h
24h
Actin
p70S6K


3h
8h
24h
p-GSK3B

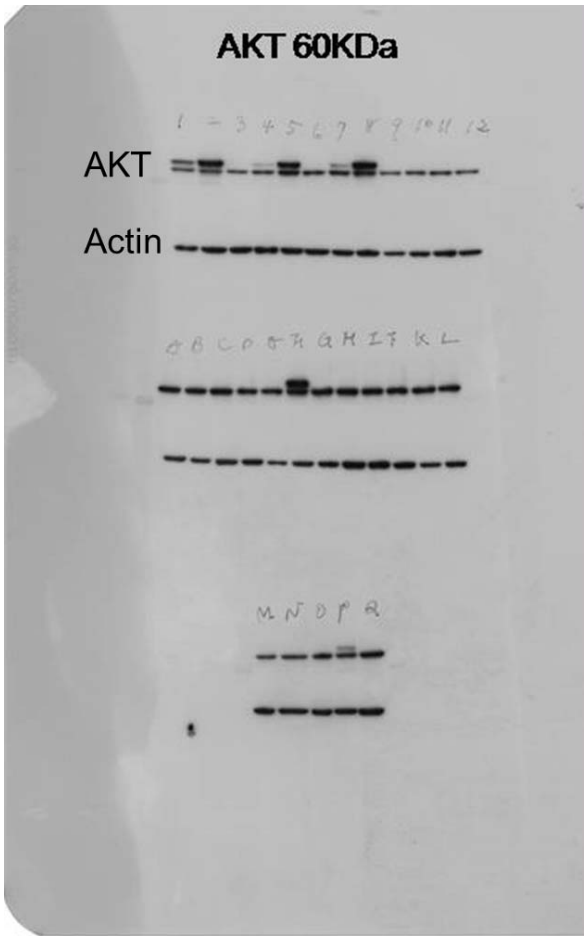
(C)


ER
GAPDH

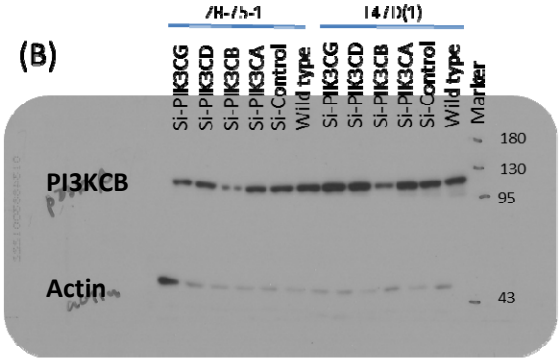
(D)

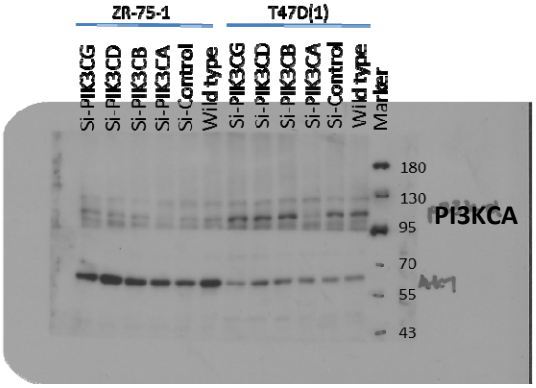

3hr
8hr
24hr
48hr
72hr
ER
GAPDH

(A)

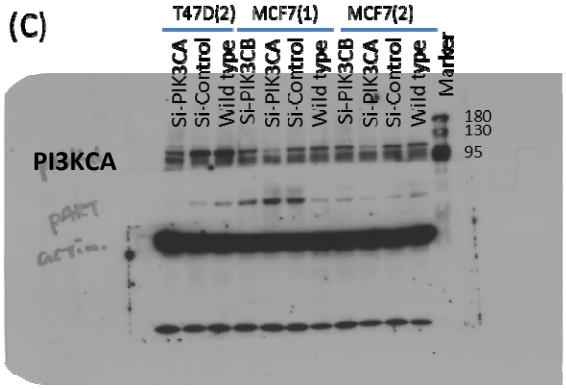

AKT 60KDa
AKT
Actin

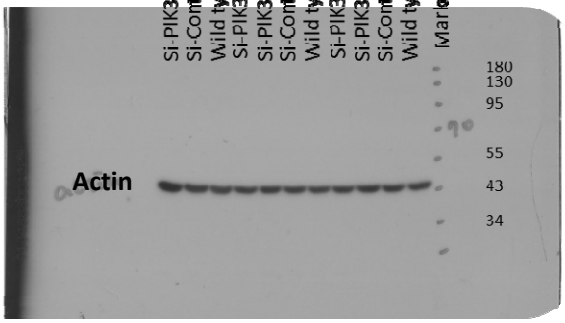
(B)


ZR-75-1
T47D(1)
Si-PIK3CG
Si-PIK3CD
Si-PIK3CB
Si-PIK3CA
Si-Control
Wild type
Marker
180
130
95
43
PI3KCB
Actin


ZR-75-1
T47D(1)
Si-PIK3CG
Si-PIK3CD
Si-PIK3CB
Si-PIK3CA
Si-Control
Wild type
Marker
180
130
95
70
55
43
PI3KCA

(C)


T47D(2)
MCF7(1)
MCF7(2)
Si-PIK3CA
Si-Control
Wild type
Si-PIK3CB
Si-PIK3CA
Si-Control
Wild type
Si-PIK3CB
Si-PIK3CA
Si-Control
Wild type
Marker
180
130
95
PI3KCA


T47D(2)
MCF7(1)
MCF7(2)
Si PIK3CA
Si-Control
Wild type
Si-PIK3CB
Si-PIK3CA
Si-Control
Wild type
Si-PIK3CB
Si-PIK3CA
Si-Control
Wild type
Marker
180
130
95
55
43
34
Actin

p-value	Control	T	BYL719	BKM120
T	0.002199			
BYL719	0.102642			
BKM120	0.01985			
T+BYL719	0.000921	0.001334	0.000815	
T+BKM120	0.000666	0.000134		0.003286