Supplemental data

Src promotes estrogen dependent ERα proteolysis in human breast cancer

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Supplemental Methods

QPCR quantitation of ERα mRNA

The reactions contained ERα-specific exon 5 5'CTCCTAACTTGCTCTTGGACAG 3' and the exon 7 sequence 5'TCGGTTCCGC ATGATGAATC 3' primers, 200ng RNA template, 0.1U uracil DNA glycosylase (Epicentre), 10μl QuantiTect SYBR Green RT-PCR Master Mix (Qiagen Inc.), 0.2 μl QuantiTect RT Mix (Qiagen Inc.) and 3.5mMl $MgCl_2$. After incubation at 50°C for 20 minutes and initial activation at 95°C for 15 minutes, 45 cycles of 94°C for 15 seconds, 60°C for 20 seconds, 72°C for 10 seconds were carried out. A program for the melting curve analysis was set at 95°C with a slope of 20°C per sec, 68°C with a slope of 20°C per second, and 95°C with a slope of 0.1°C.

QPCR quantitation of GREB1 and pS2 mRNA

PCR reactions were at 95°C 30 s, followed by 60°C 60 s for 40 cycles. The primers used to detect ER target genes GREB1 and pS2 are as the followings: GREB1 forward ATCAGCTGCTCGGACTTGCTG and GREB1 reverse TGAGCTCCGGTCCTGACAGATG; pS2 forward

GCGCCCTGGTCCTGGTGTCCAT and pS2 reverse GAAACCACAATTCTGTCTTTCAC. GAPDH was used as an internal control: forward primer GAAGGTGAAGGTCGGAGTC and reverse primer GAAGATGGTGATGGGATTTC.

Supplemental Figure 1

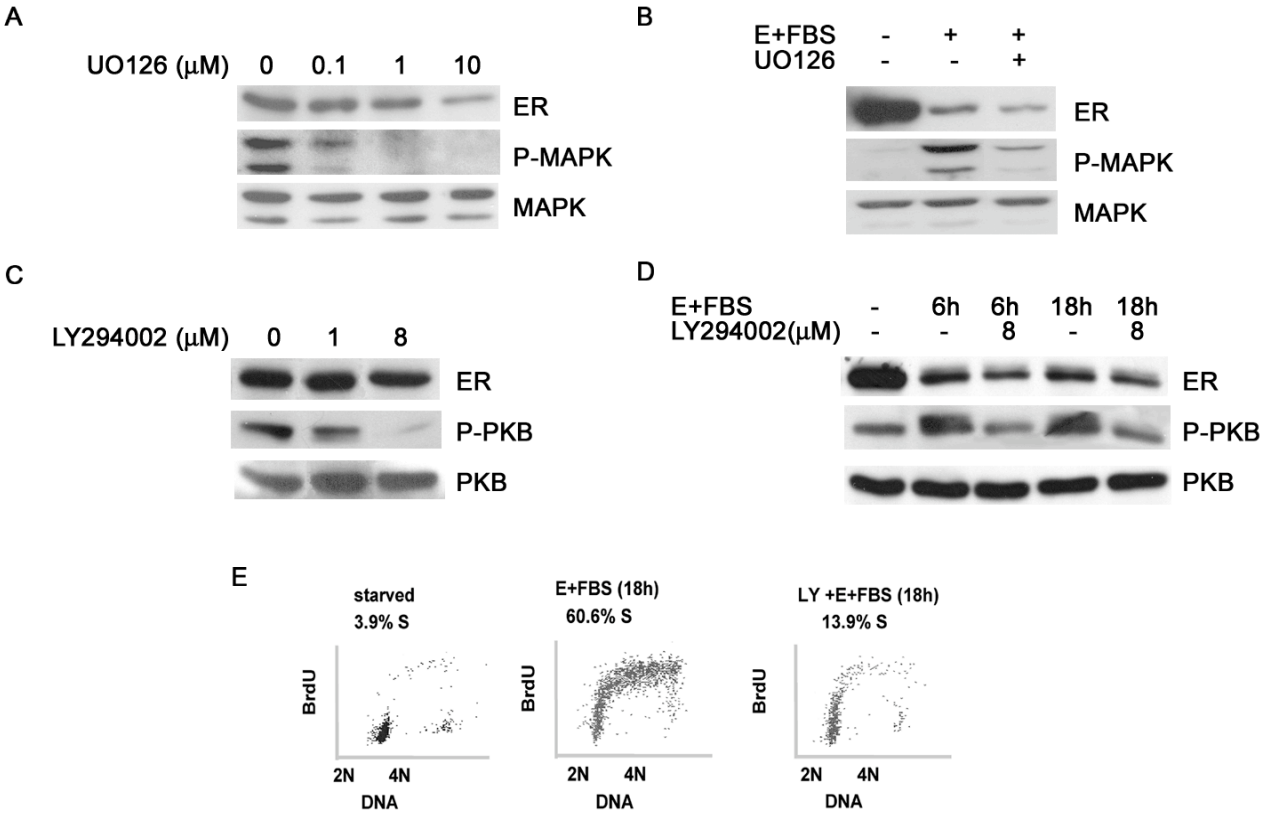
MEK and PI3K do not stimulate estrogen dependent ER degradation.

A: asynchronous MCF-7 cultures were treated with MEK inhibitor, UO126 for 48 hr prior to immunoblotting for ER and activated phosphorylated MAPK (MAPK-P).

B: serum and E-deprived MCF-7 were treated with E + 5% FBS with or without 10 μM UO126 for 6 hr and ER levels assayed.

C: ER and activated PKB (P-PKB) in MCF-7 treated with PI3K inhibitor, LY294002 for 48 hr.

D and E: E and serum deprived MCF-7 cells were stimulated with E + 5%FBS or E + 5%FBS together with 8 μM LY294002 for 6 and 18 hr followed by ER and PKB immunoblotting. Cell cycle analysis by flow cytometry at t=0 and 18 hr is shown.


A
UO126 (μM) 0 0.1 1 10
ER
P-MAPK
MAPK
B
E+FBS - + +
UO126 - - +
ER
P-MAPK
MAPK
C
LY294002 (μM) 0 1 8
ER
P-PKB
PKB
D
E+FBS - 6h 6h 18h 18h
LY294002(μM) - - 8 - 8
ER
P-PKB
PKB
E
starved
3.9% S
E+FBS (18h)
60.6% S
LY +E+FBS (18h)
13.9% S
BrdU
2N 4N
DNA