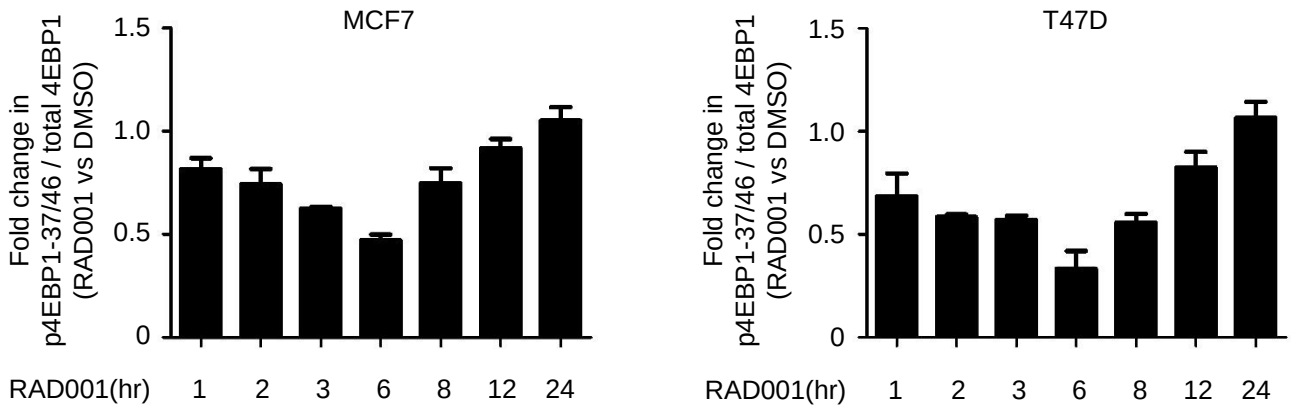
Supplemental Information

Supplemental Figures;

Supplemental Experimental Methods;

A


MCF7
T47D
Fold change in p4EBP1-37/46 / total 4EBP1 (RAD001 vs DMSO)
1.5
1.0
0.5
0
RAD001(hr) 1 2 3 6 8 12 24

B

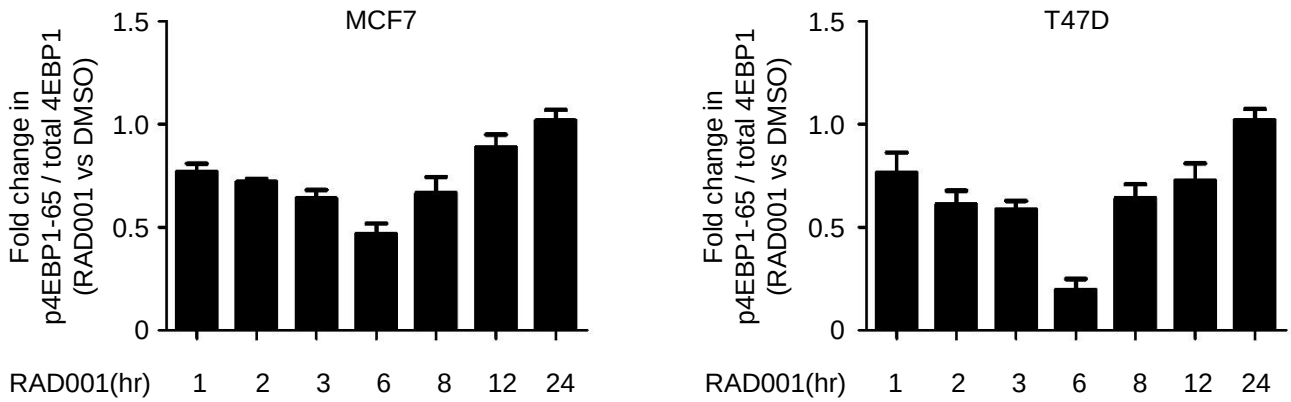

MCF7
T47D
Fold change in p4EBP1-65 / total 4EBP1 (RAD001 vs DMSO)
1.5
1.0
0.5
0
RAD001(hr) 1 2 3 6 8 12 24

Figure S1. The dynamic effects of rapamycin treatment on the 4EBP1 phosphorylation. (A and B) Relative expression of (A) p4EBP-37/46 and (B) p4EBP1-65 after the indicated times of treatment with RAD001 was quantified by calculating the densitometry of p4EBP versus total 4EBP1. The results are expressed as the fold change in RAD001 treated groups at indicated times relative to the DMSO-treated controls and presented as means ± S.E.M. (n = 3).

Supplemental Figures

Figure S2

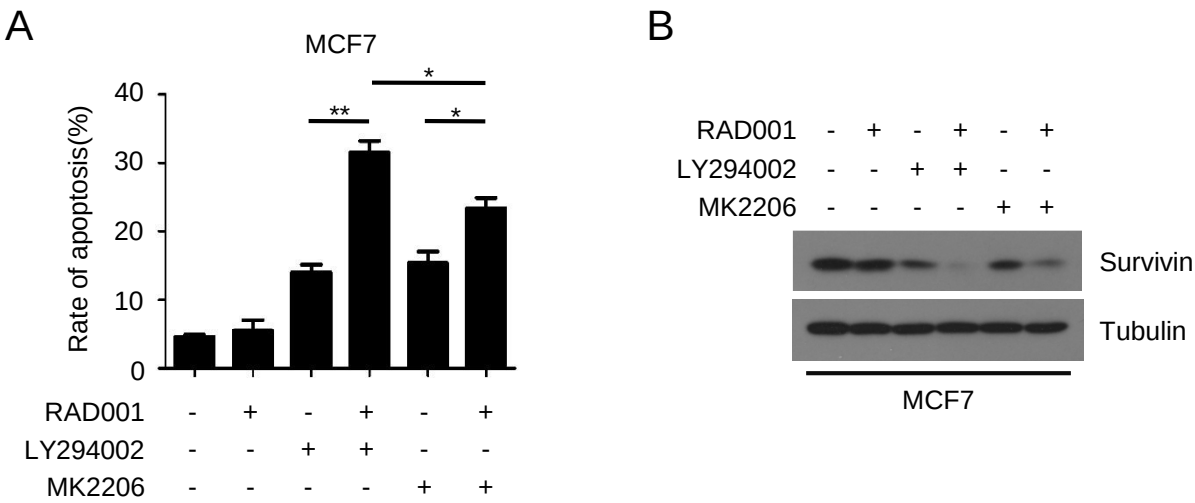


Figure S2. LY294002 showed the more potent role than MK2206 in enhancing the effect of RAD001 on cell apoptosis. (A and B) MCF7 cells were treated with 2 nM RAD001, 2 μM LY294002 or 0.5 μM MK2206, alone or in combination for 24 h. (A) The rate of cell apoptosis in the indicated groups. The results were presented as means ± S.E.M. (n = 3) * $P < 0.05$, ** $P < 0.01$. Values represent means ± SEM (n = 3). (B) Western blot of survivin expression in the indicated groups.

Supplementary Experimental Methods

Apoptosis assay

Cell apoptosis was measured using Annexin V-FITC-/PI apoptosis kit (Keygen biotech Co., Ltd, Nanjing, China) following the manufacturer's instructions. Briefly, cells were collected with trypsin, washed two times with ice-cold PBS, and then resuspended in binding buffer. Thereafter, Annexin V-FITC and PI were added and incubated for 15 min in the dark. The rate of apoptotic cells was measured analyzed on a flow cytometer (BD Biosciences). Data was analyzed with Flowjo software.