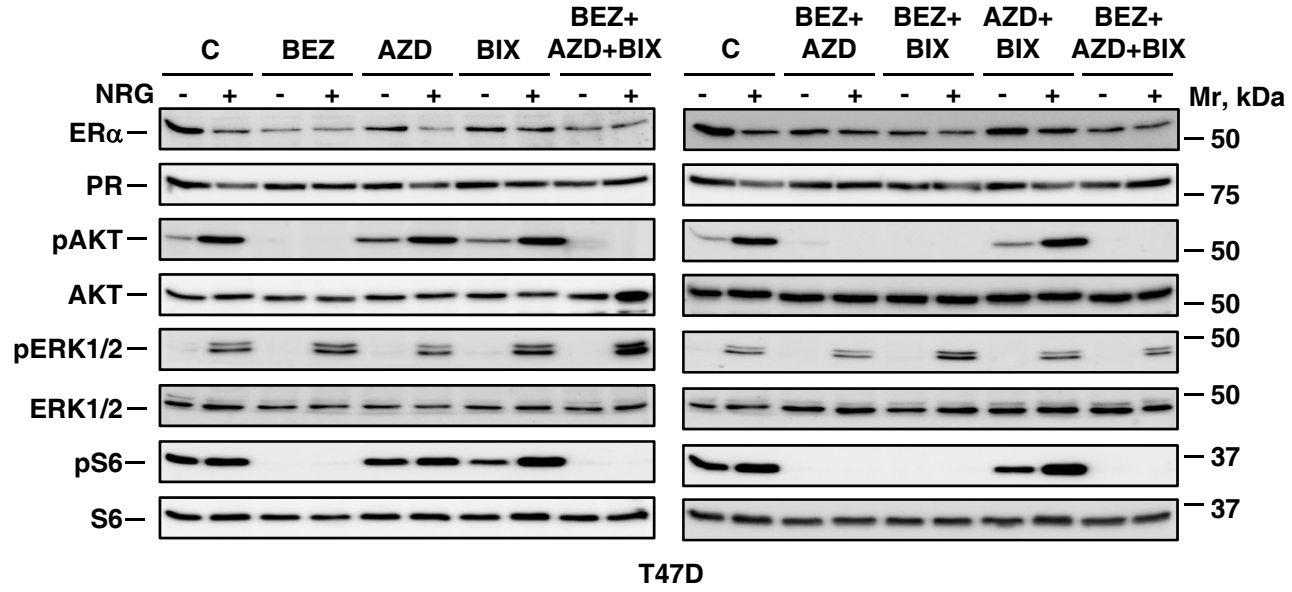
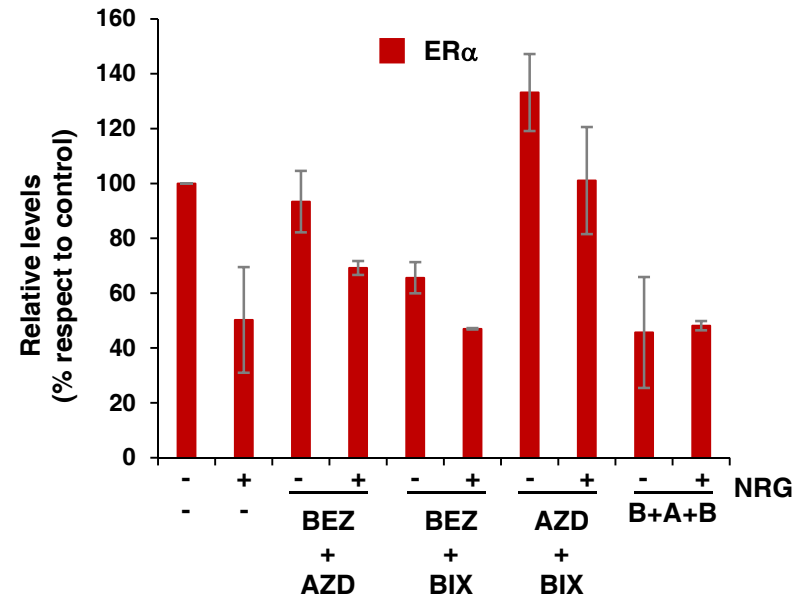
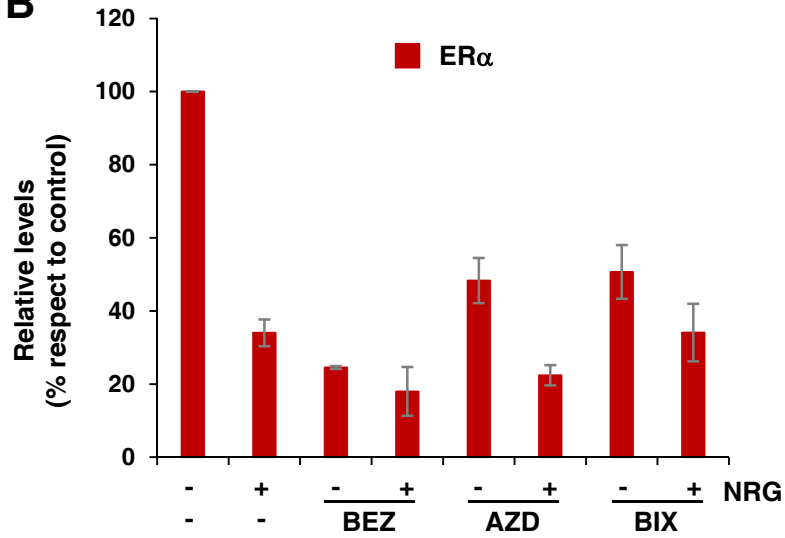
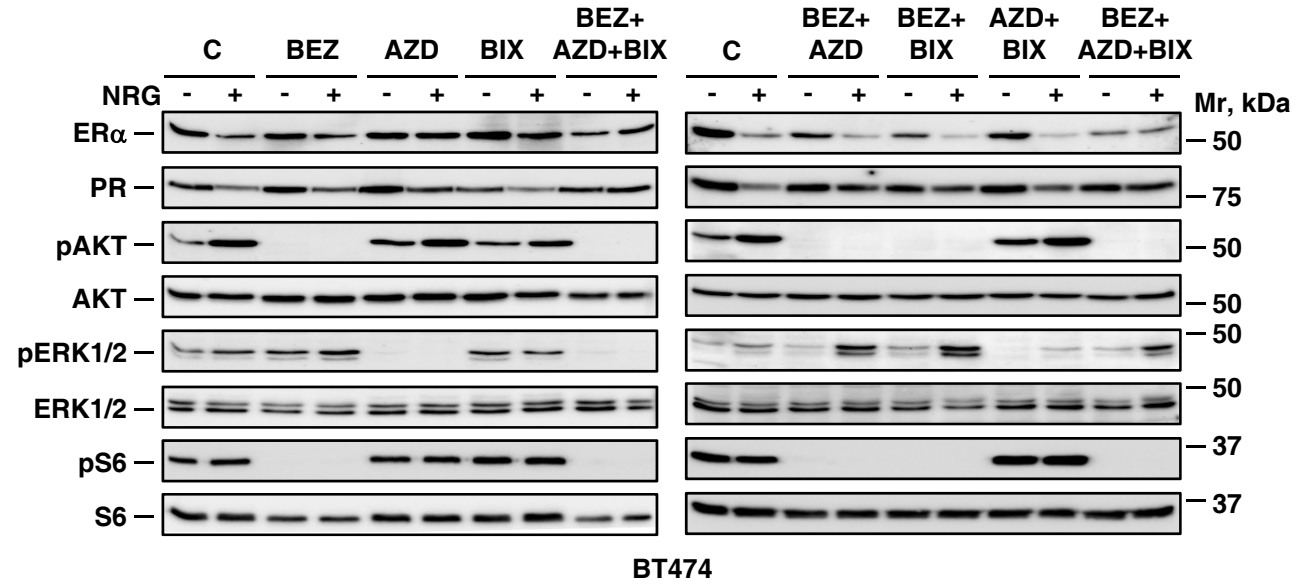
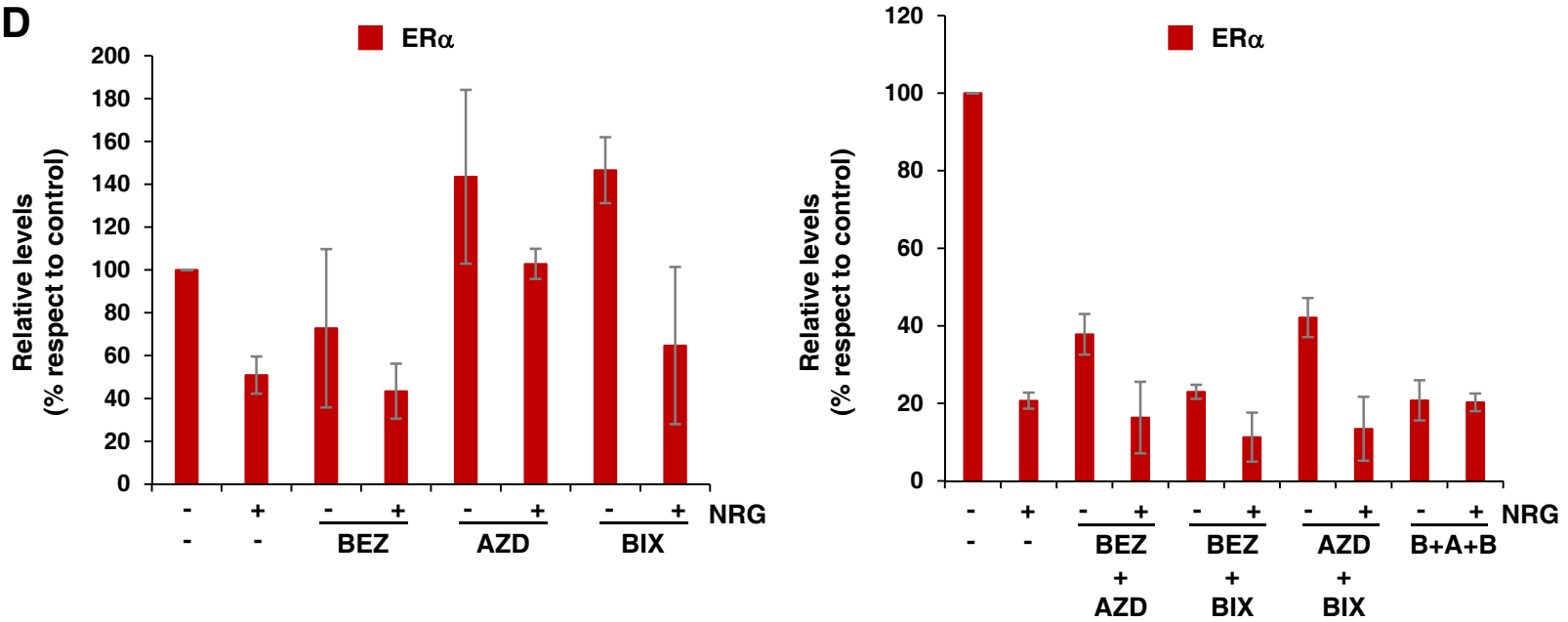


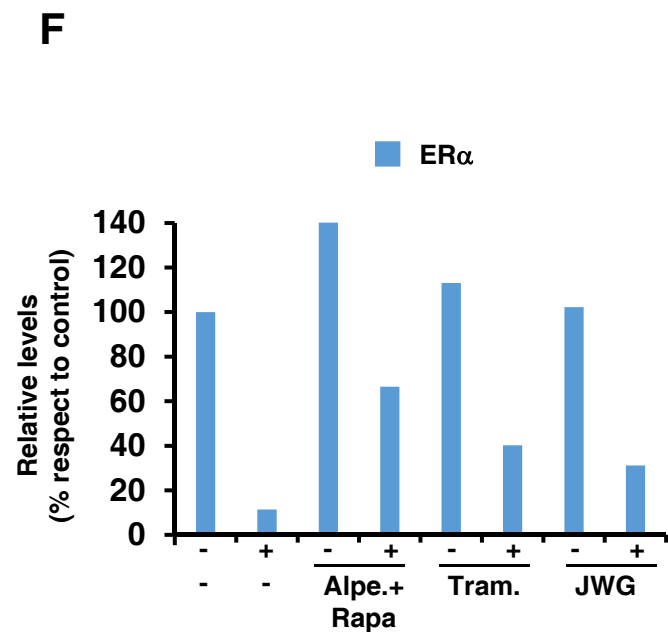
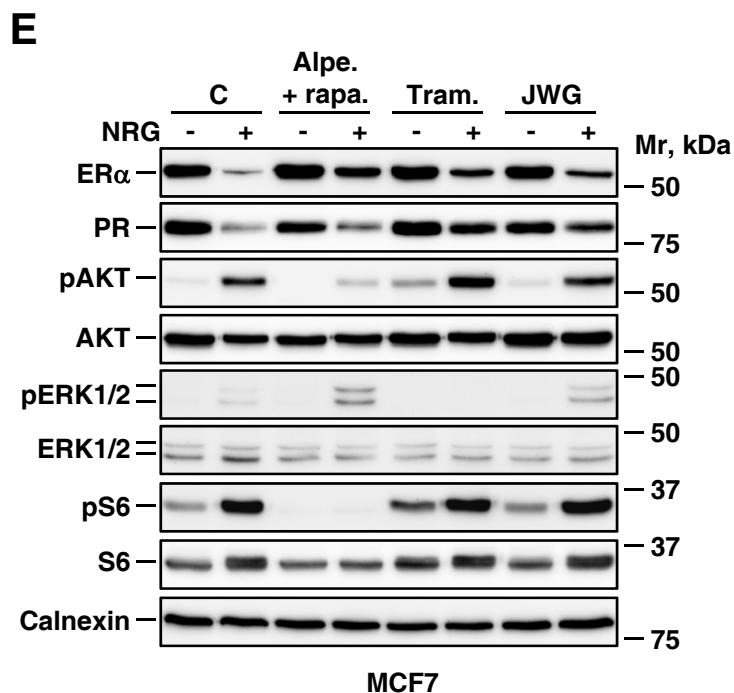
A**B**

C



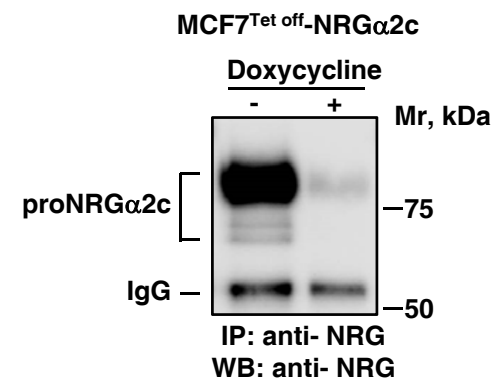
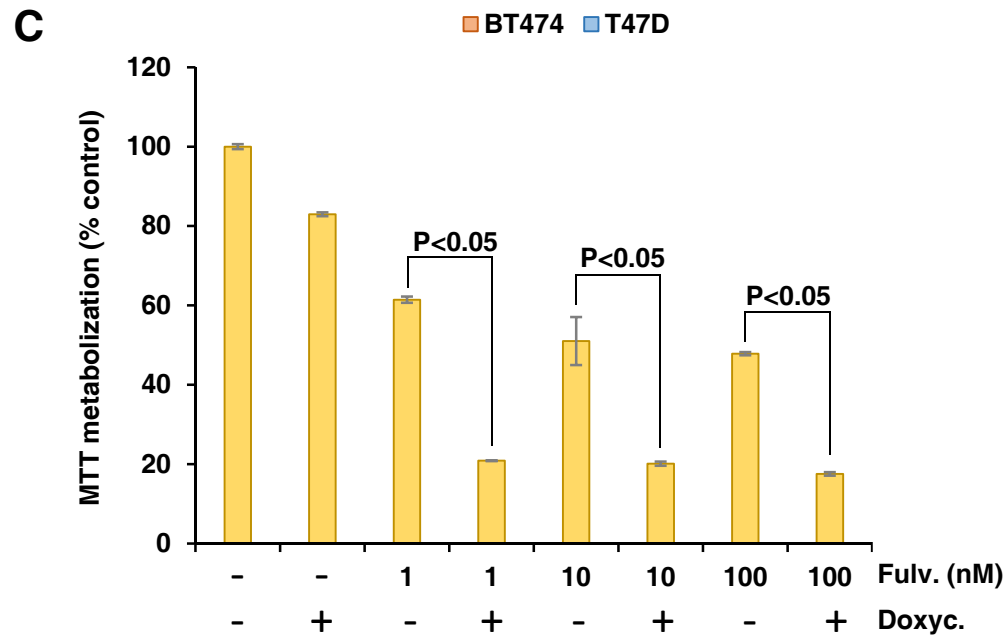
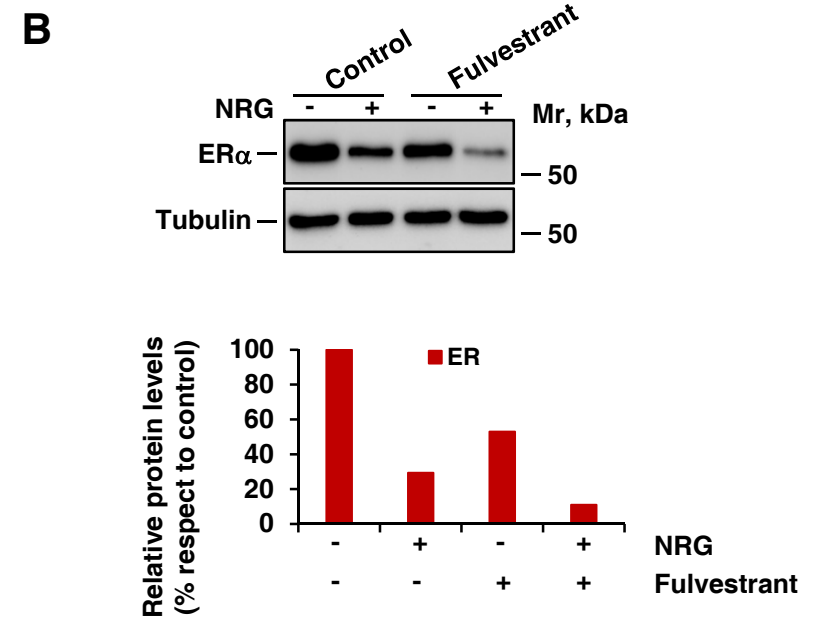
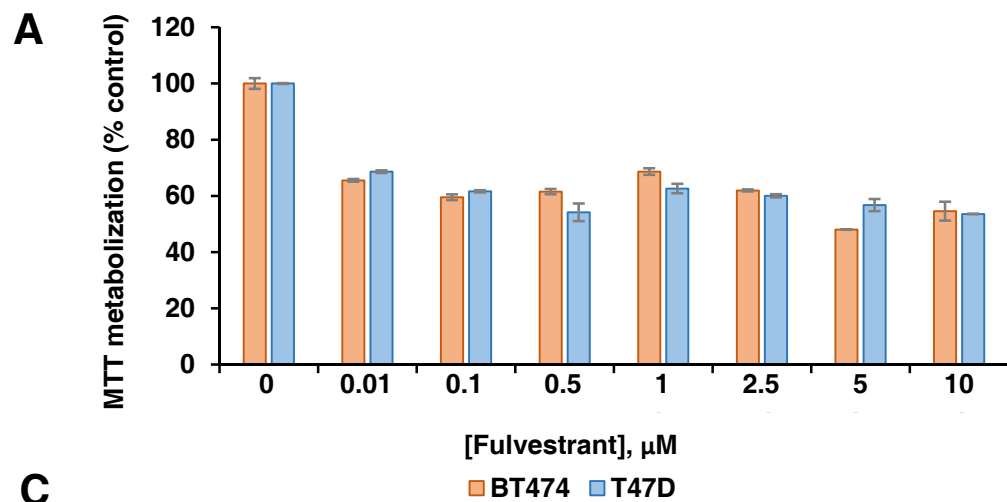
D





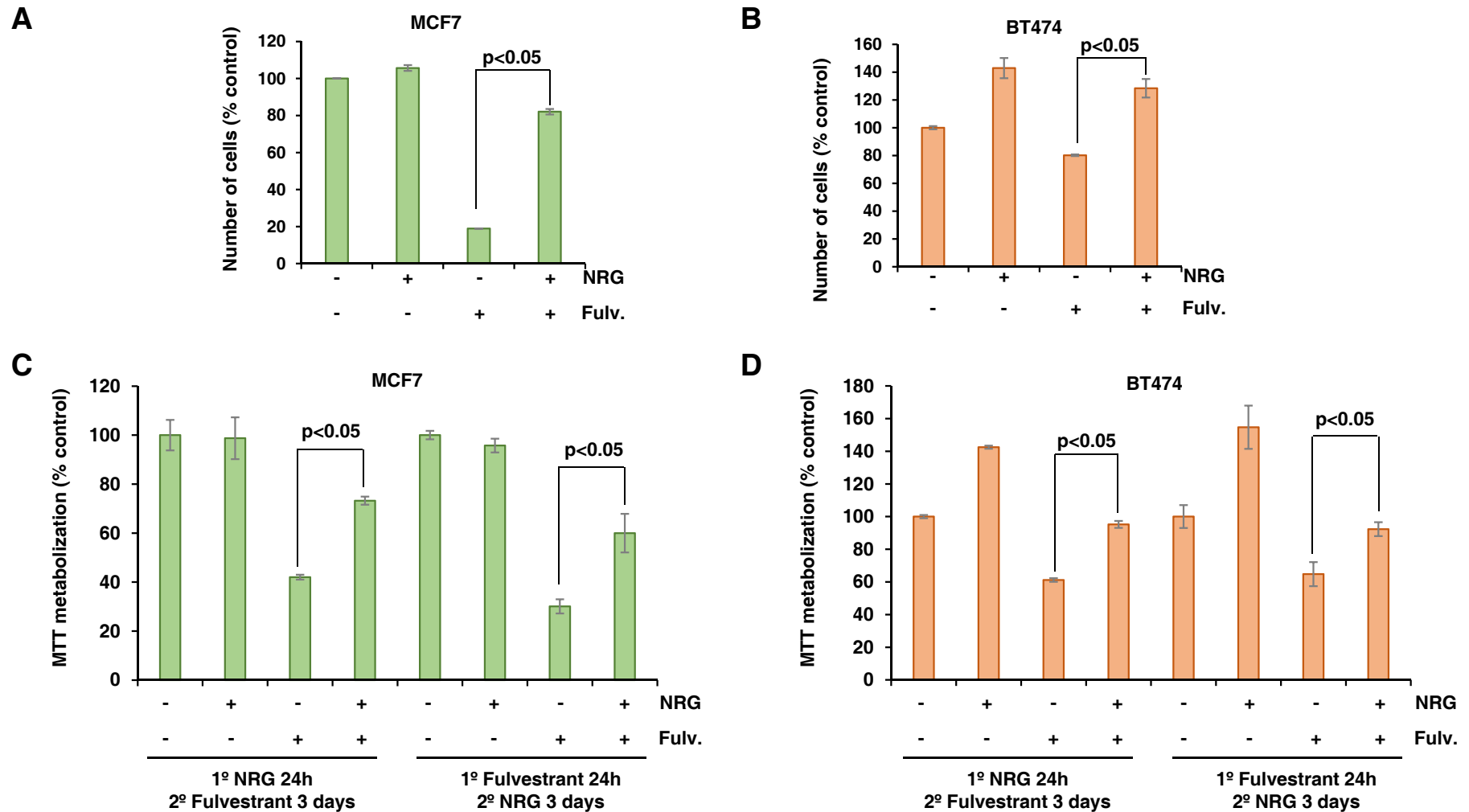
Supplementary Figure 1. T47D (A) and BT474 (C) cells were pretreated with a PI3K inhibitor (BEZ235, 1 μ M), a MEK1/2 inhibitor (AZD6244, 5 μ M), a MEK5 inhibitor (BIX02189, 10 μ M) and with double and triple combinations of these inhibitors for 2 hours. Later, they were then stimulated with NRG for 24 hours. The expression levels of ER α , PR and several of the downstream proteins involved in NRG receptor signaling were performed by Western blot. (B and D) The graphics represent the quantitation of ER α levels corresponding to the mean \pm SD of data from two independent experiments as performed in A and C. (E) MCF7 cells were pretreated with a PI3K α inhibitor (Alpelisib, 1 μ M) plus an mTOR inhibitor (Rapamycin, 100 nM), a MEK1/2 inhibitor (Trametinib, 100 nM) and an ERK5 inhibitor (JWG071, 5 μ M) for 2 hours. Later, they were then stimulated with NRG for 24 hours. The expression levels of ER α , PR and several of the downstream proteins involved in NRG receptor signaling were performed by Western blot. (F) The graphics represents the quantitation of ER α levels corresponding to the experiment performed in E.

Supplementary Figure 1



Supplementary Figure 2. (A) Dose-response analyses of the effect of fulvestrant on BT474 and T47D cells. Cells were treated with fulvestrant at the indicated doses and cell proliferation was determined by MTT metabolization 4 days later. The data are plotted as the percentage of MTT metabolization with respect to control. Results are shown as the mean \pm SD of quadruplicates of an experiment repeated three times. (B) Levels of expression of ER α in MCF7 cells treated with NRG (10 nM) plus fulvestrant (1 μ M) for 48 hours. The graphic represents the quantitation of the ER α levels corresponding to the upper panel. (C) Effect of the expression of NRG in MCF7^{TetOff}-NRG α 2c cells treated with fulvestrant. MCF7^{TetOff}-NRG α 2c cells were treated with doxycycline (10 nM) for 72 hours, and later the cells were treated with the indicated doses of fulvestrant. Cell proliferation was determined by MTT metabolization 4 days later. The data are plotted as the percentage of MTT metabolization with respect to control. Results are shown as the mean \pm SD of duplicates of an experiment repeated two times. The expression of proNRG α 2c in MCF7^{TetOff}-NRG α 2c cells treated with doxycycline was analyzed by Western blot.

Supplementary Figure 2



Supplementary Figure 3. (A-B) Effect of NRG in MCF7 or BT474 cells treated with fulvestrant. MCF7 (A) or BT474 (B) cells were stimulated with NRG (10 nM) for 24 hours, and later the cells were treated with fulvestrant (1 μM). Cell proliferation was determined by cell counting 4 days later. The data are plotted as the percentage of the number of cells respect to control. Results are shown as the mean ± SD of triplicates of an experiment repeated two times. (C-D) Effect of NRG in MCF7 or BT474 cells treated with fulvestrant. MCF7 (C) or BT474 (D) cells were stimulated and treated like experiments performed in A and B. Moreover, this experiment was also carried out in the reverse way, (pre-treating 24 hours with fulvestrant, and later stimulating with NRG for 4 days). Cell proliferation was determined by MTT metabolization. The data are plotted as the percentage of MTT metabolization with respect to control. Results are shown as the mean ± SD of quadruplicates of an experiment repeated two times.

Supplementary figure 3

A

	<i>ESR1</i>	<i>PGR</i>
	<i>Mann-Whitney U (p-value)</i>	<i>Mann-Whitney U (p-value)</i>
C vs. 1h	<0.05	<0.05
C vs. 3h	<0.05	<0.05
C vs. 6h	<0.05	<0.05
C vs. 12h	<0.05	<0.05
C vs. 24h	<0.05	<0.05
C vs. 48h	<0.05	>0.05

MCF7**B**

	<i>ESR1</i>	<i>PGR</i>
	<i>Mann-Whitney U (p-value)</i>	<i>Mann-Whitney U (p-value)</i>
C vs. 1h	>0.05	>0.05
C vs. 3h	<0.05	>0.05
C vs. 6h	<0.05	<0.05
C vs. 12h	<0.05	<0.05
C vs. 24h	<0.05	<0.05
C vs. 48h	<0.05	<0.05

T47D**C**

	<i>ESR1</i>	<i>PGR</i>
	<i>Mann-Whitney U (p-value)</i>	<i>Mann-Whitney U (p-value)</i>
C vs. 1h	>0.05	>0.05
C vs. 3h	<0.05	<0.05
C vs. 6h	>0.05	<0.05
C vs. 12h	<0.05	<0.05
C vs. 24h	<0.05	<0.05
C vs. 48h	<0.05	<0.05

BT474**Supplementary Table 1**

A**B****C**

	<i>ESR1</i>	<i>PGR</i>		<i>ESR1</i>	<i>PGR</i>		<i>ESR1</i>	<i>PGR</i>
	<i>Mann-Whitney U (p-value)</i>	<i>Mann-Whitney U (p-value)</i>		<i>Mann-Whitney U (p-value)</i>	<i>Mann-Whitney U (p-value)</i>		<i>Mann-Whitney U (p-value)</i>	<i>Mann-Whitney U (p-value)</i>
C (N) vs. BEZ (N)	<0.05	>0.05	C (N) vs. BEZ (N)	<0.05	>0.05	C (N) vs. BEZ (N)	<0.05	>0.05
C (N) vs. AZD (N)	<0.05	>0.05	C (N) vs. AZD (N)	>0.05	>0.05	C (N) vs. AZD (N)	>0.05	>0.05
C (N) vs. BIX (N)	<0.05	>0.05	C (N) vs. BIX (N)	>0.05	>0.05	C (N) vs. BIX (N)	>0.05	>0.05
C (N) vs. B+A+B (N)	<0.05	>0.05	C (N) vs. B+A+B (N)	>0.05	>0.05	C (N) vs. B+A+B (N)	<0.05	<0.05
MCF7			T47D			BT474		

Supplementary Table 2