

Supplementary Fig. 1. (A) mRNA expression of TFF1, cathepsin D and PR in BCAHC-1 and MCF-7 cells treated for 24 h with vehicle (-) and the indicated concentrations of E2, as evaluated by real-time PCR. Values are normalized to the actin beta (ACTB) expression and shown as fold changes of mRNA expression induced by treatments compared to vehicle (-). (B) Densitometric analysis of the blots shown in panels A-D of figure 3 normalized to IR, which served as loading control. (C) Densitometric analysis of the blots shown in panels E-H of figure 3 normalized to ERK2, which served as loading control. (D) Densitometric analysis of the blots shown in panels I-J of figure 3 normalized to IR, which served as loading control. (E) Densitometric analysis of the blots shown in panels K-L of figure 3, which were normalized to IR or ERa, as indicated. (F) Total lysates (input), in reference to co-immunoprecipitation assays shown in panels K-L of figure 3, were evaluated as control. In control samples, nonspecific IgG was used instead of the primary antibody. (G-H) mRNA expression of c-Fos (G) and Cyclin D1 (H) in BCAHC-1 cells treated with vehicle (-) and 100nM E2 or 10nM insulin (Ins) for the indicated times, as evaluated by real-time PCR. Values are normalized to the actin beta (ACTB) expression and shown as fold changes of mRNA expression induced by treatments compared to vehicle (-). (I) Densitometric analysis of the blots shown respectively in panels M-N of figure 3, normalized to β-actin. (J) Densitometric analysis of the blots shown respectively in panels O-P of figure 3, normalized to β-actin. (K) Densitometric analysis of the blots shown respectively in panels Q-R of figure 3, normalized to βactin. (L) Densitometric analysis of the blots shown in panels S-T of figure 3 normalized to β-actin. (M) Representative immunoblots of the efficacy of ERa or IR silencing. Side panel shows densitometric analysis of the blots normalized to β -actin. Values represent the mean \pm SD of three independent experiments performed in triplicate. (*), (■) indicate p < 0.05 for cells exposed to treatments versus vehicle (-).