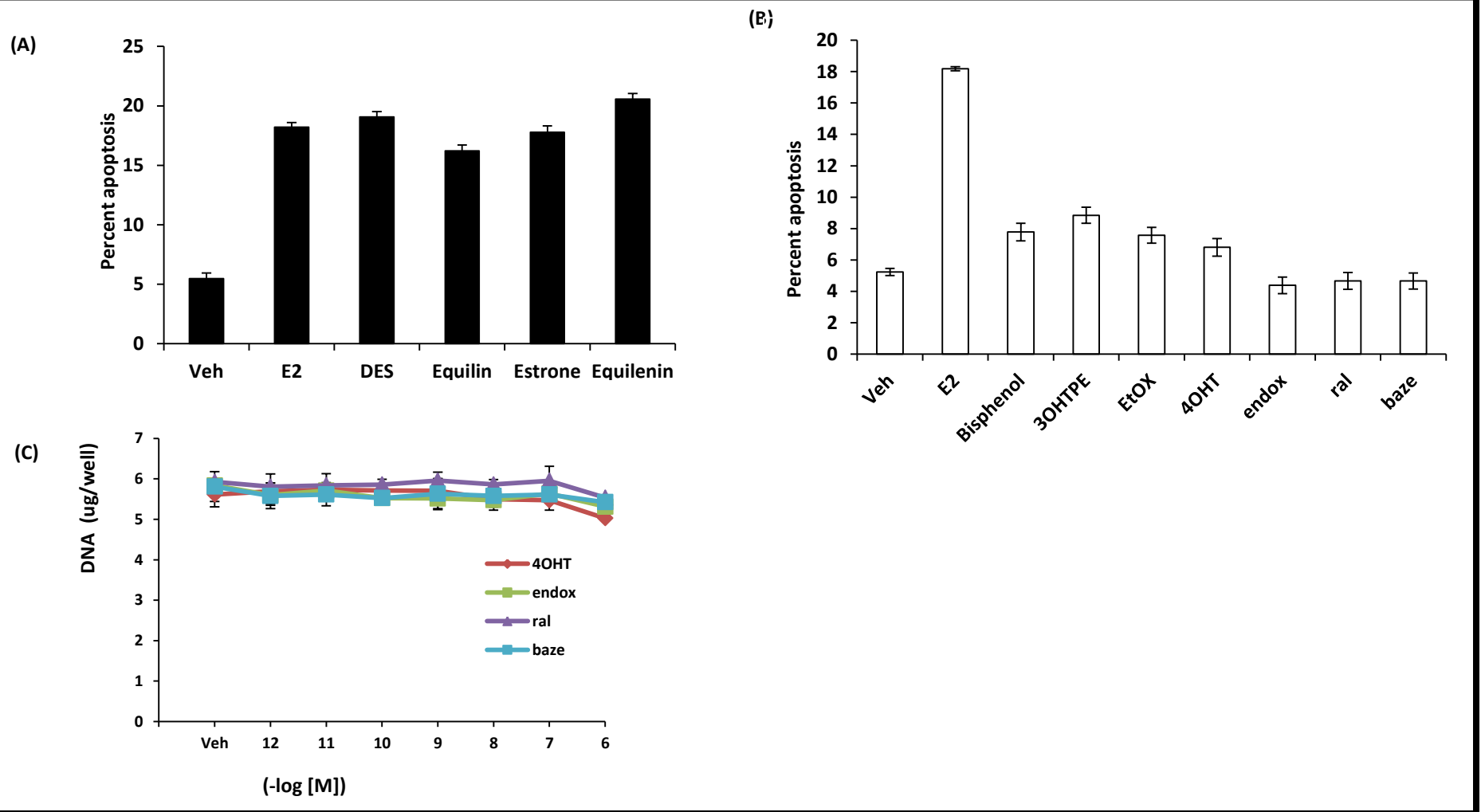


Article: Defining the conformation of the ER complex that controls estrogen induced apoptosis in breast cancer.

Authors: Ifeyinwa Obiorah , Surojeet Sengupta , Ramona Curpan and V. Craig Jordan

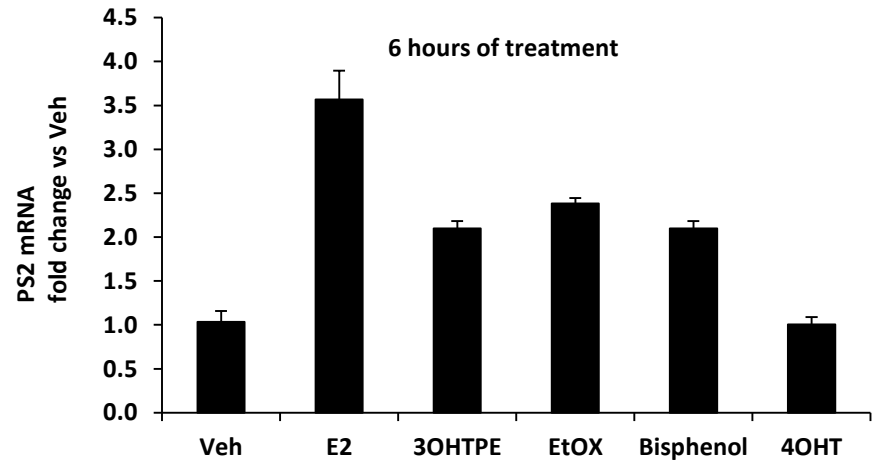
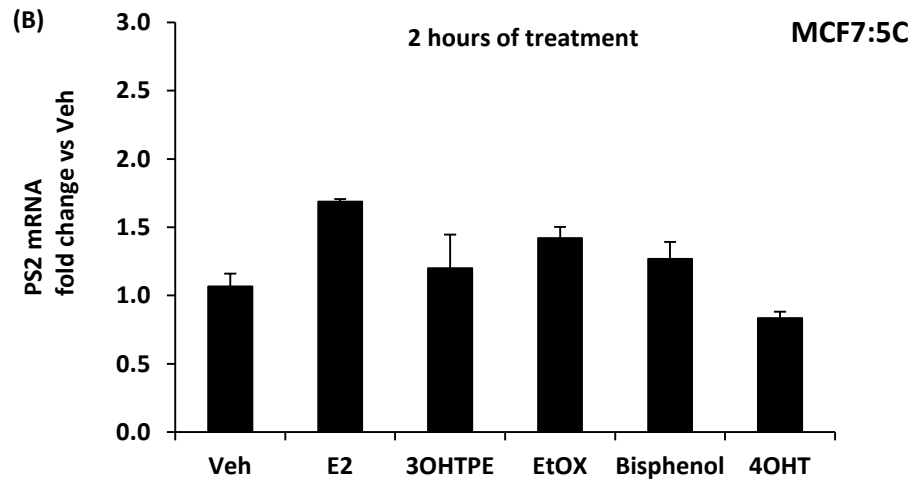
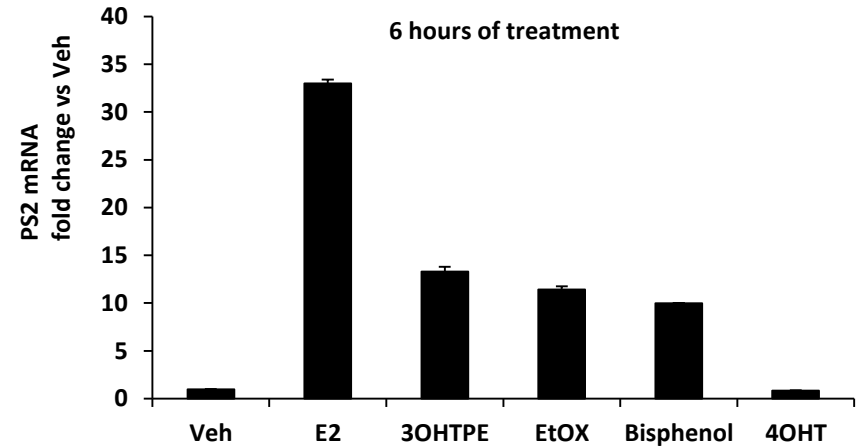
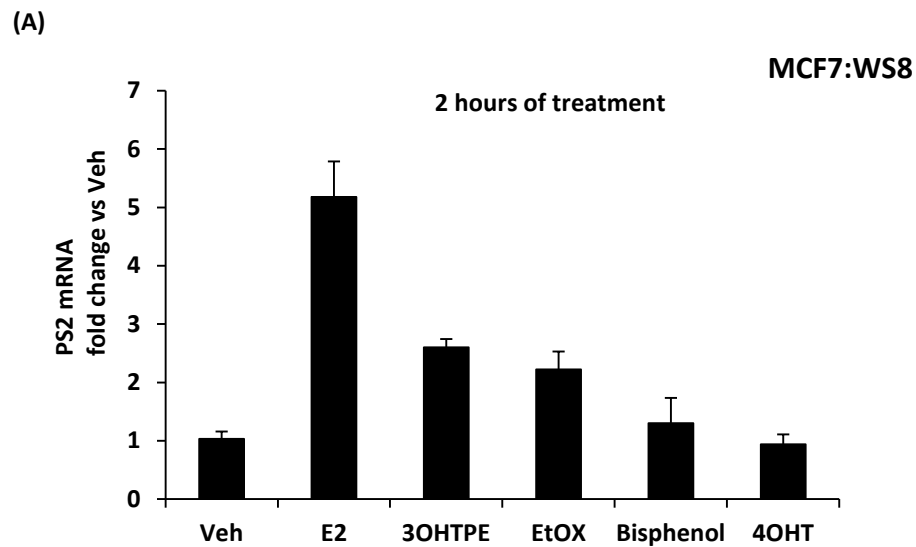
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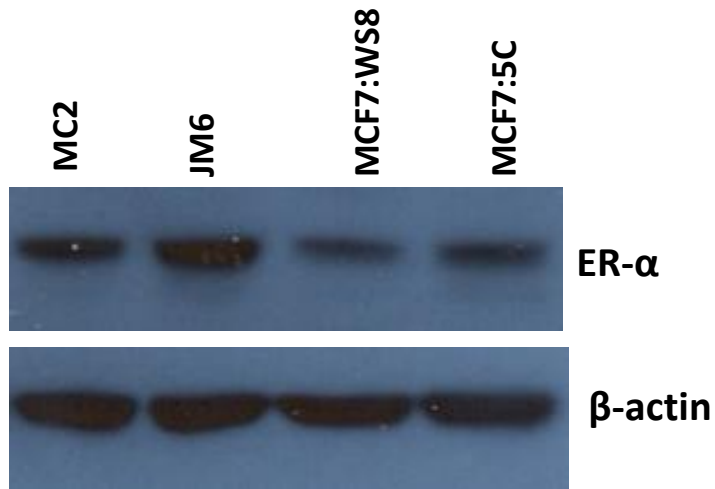
Supplemental Figure 1. Differential effect of planar and non planar estrogens and SERMs on apoptosis.

Annexin V staining for apoptosis . MCF7:5C cells were treated with (A) ethanol Vehicle (Veh), planar estrogens (1 nM) (B) TPE (1 μ M) and SERMS(1 μ M) for 72h, and then cells were stained with FITC – annexin V and propidium iodide (PI) and analyzed by flow cytometry.(C) MCF7:5C cells treated with either Veh or 1μM SERMS for 7 days and apoptosis was quantified by measuring DNA content using a fluorescent DNA quantitation kit .



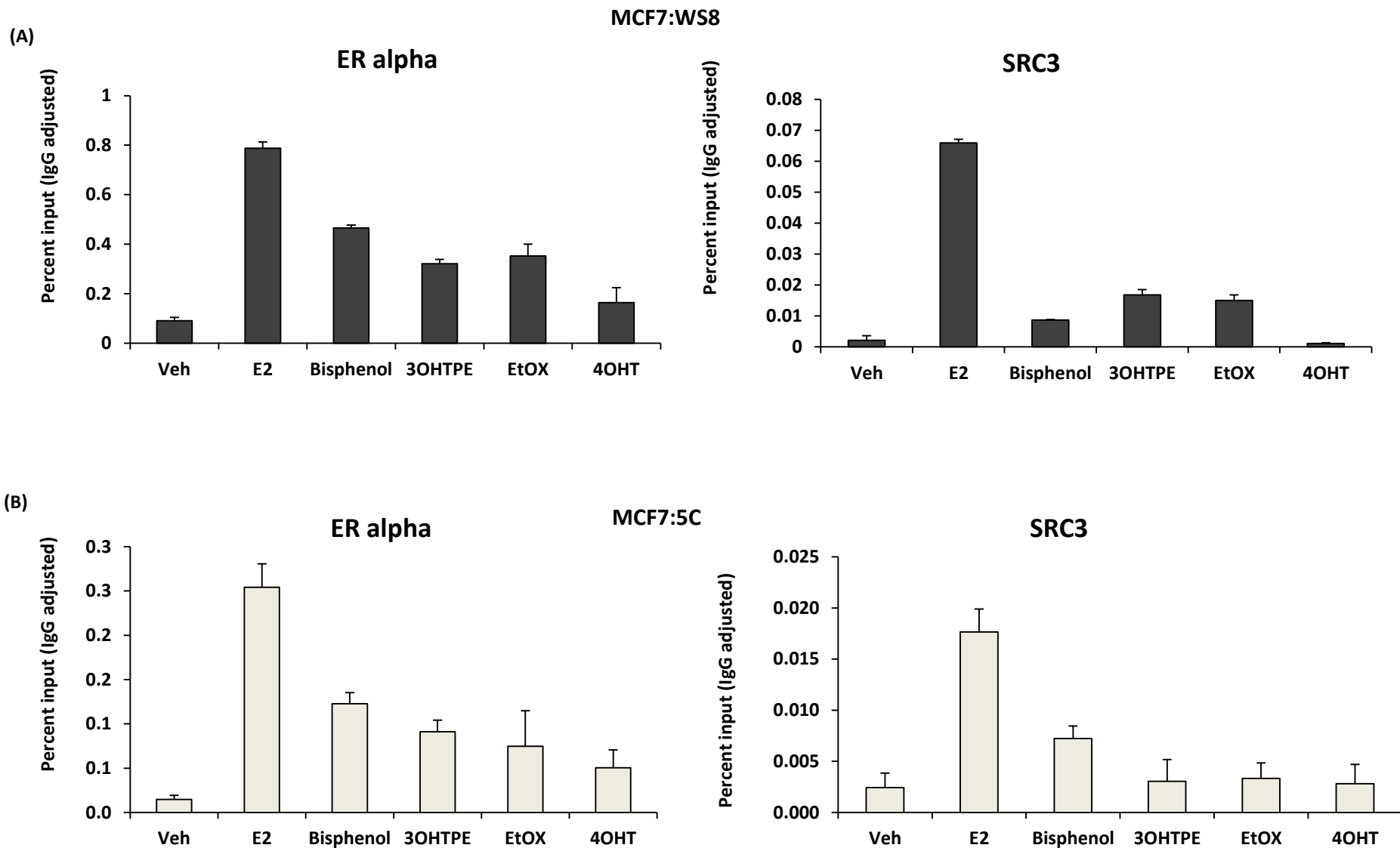
Supplemental Figure 2. Regulation of PS2 (TFF1) gene by triphenylethylenes, E₂ and 4OHT in MCF7:WS8 and MCF7:5C cells.

(A) MCF7:WS8 and (B) MCF7:5C cells were treated E₂ (1 nM), 3OHTPE (1 μM), EtOX (1 μM), bisphenol (1 μM) and 4OHT (1 μM) for 2 h or 6 h. Cells were harvested and RNA extracted. Expression level of PS2 (TFF1) was assessed using RT-PCR. Data is represented as fold difference versus vehicle (veh) treated cells. All data points are average of three replicates ± SD.

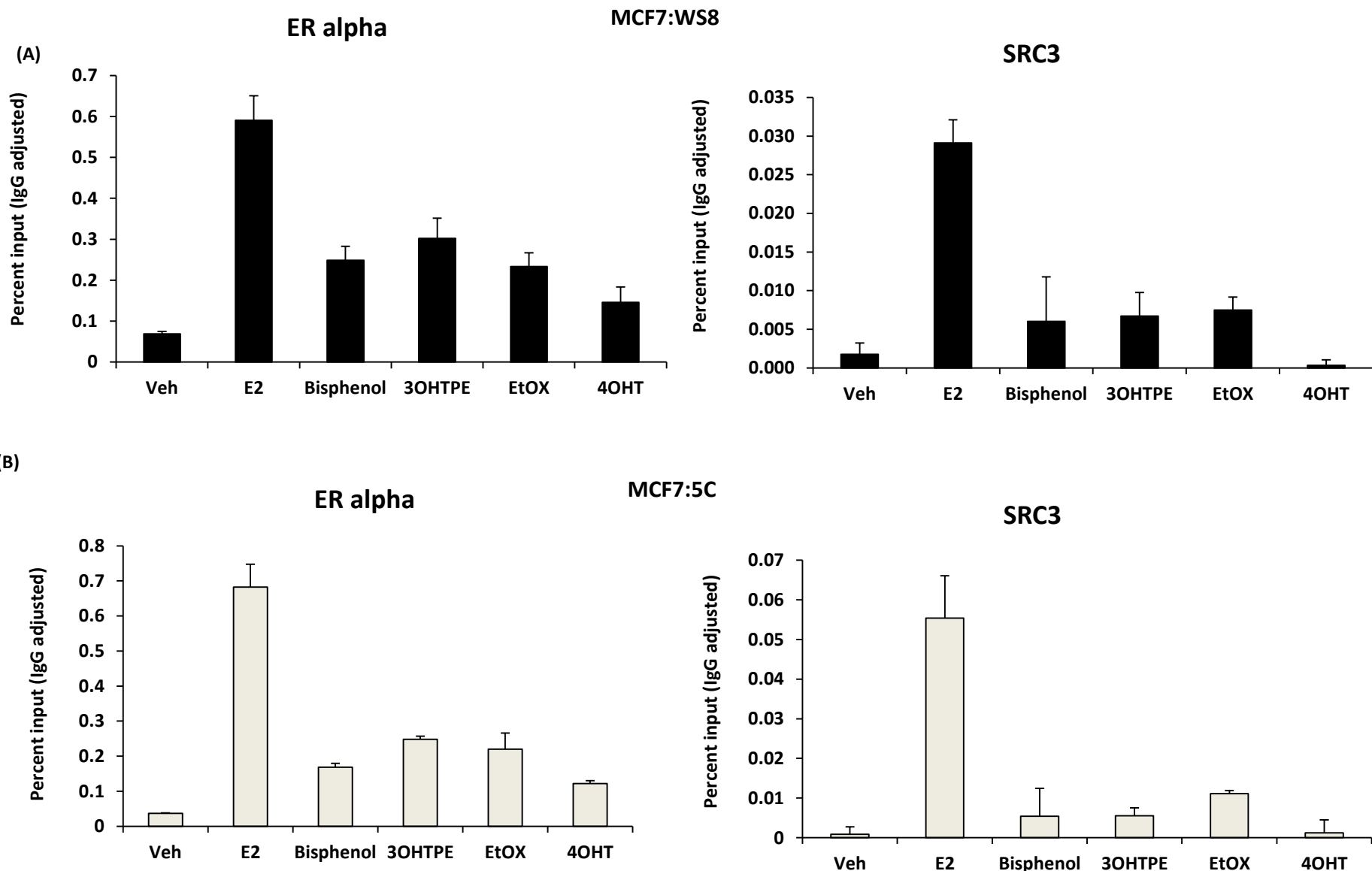


Supplemental Figure 3. ER α expression levels in breast cancer cell lines used in the study.

MC2, JM6, MCF7:WS8 and MCF7:5C cells were treated with control (0.1% ethanol) for 24h and lysates were prepared and analysed by immunoblotting for ER α protein levels. β actin was used as a loading control.



Supplemental Figure 4. Recruitment of ER alpha and SRC3 (AIB1) at PS2 proximal promoter region containing ERE in MCF7:WS8 and MCF7:5C cells. (A) MCF7:WS8 cells treated for 45 minutes with E₂ (1nM), 3OHTPE (1μM), EtOX (1μM), bisphenol (1μM) and 4OHT (1μM) and CHIP assay was performed as described in materials and methods. (B) MCF7:5C cells were treated identically as mentioned above and CHIP assay was performed under identical conditions. Data is represented as percent input of the starting chromatin used for the CHIP



Supplemental Figure 5. Recruitment of ER alpha and SRC3 (AIB1) at PS2 proximal promoter region containing ERE in MCF7:WS8 and MCF7:5C cells. (A) MCF7:WS8 cells treated for 45 minutes with E₂ (1nM), 3OHTPE (1μM), EtOX (1μM), bisphenol (1μM) and 4OHT (1μM) and CHIP assay was performed as described in materials and methods. (B) MCF7:5C cells were treated identically as mentioned above and CHIP assay was performed under identical conditions. Data is represented as percent input of the starting chromatin used for the CHIP