

Supplementary Information

Bisphenol-A and diethylstilbestrol exposure induces the expression of breast cancer associated long noncoding RNA HOTAIR *in vitro* and *in vivo*

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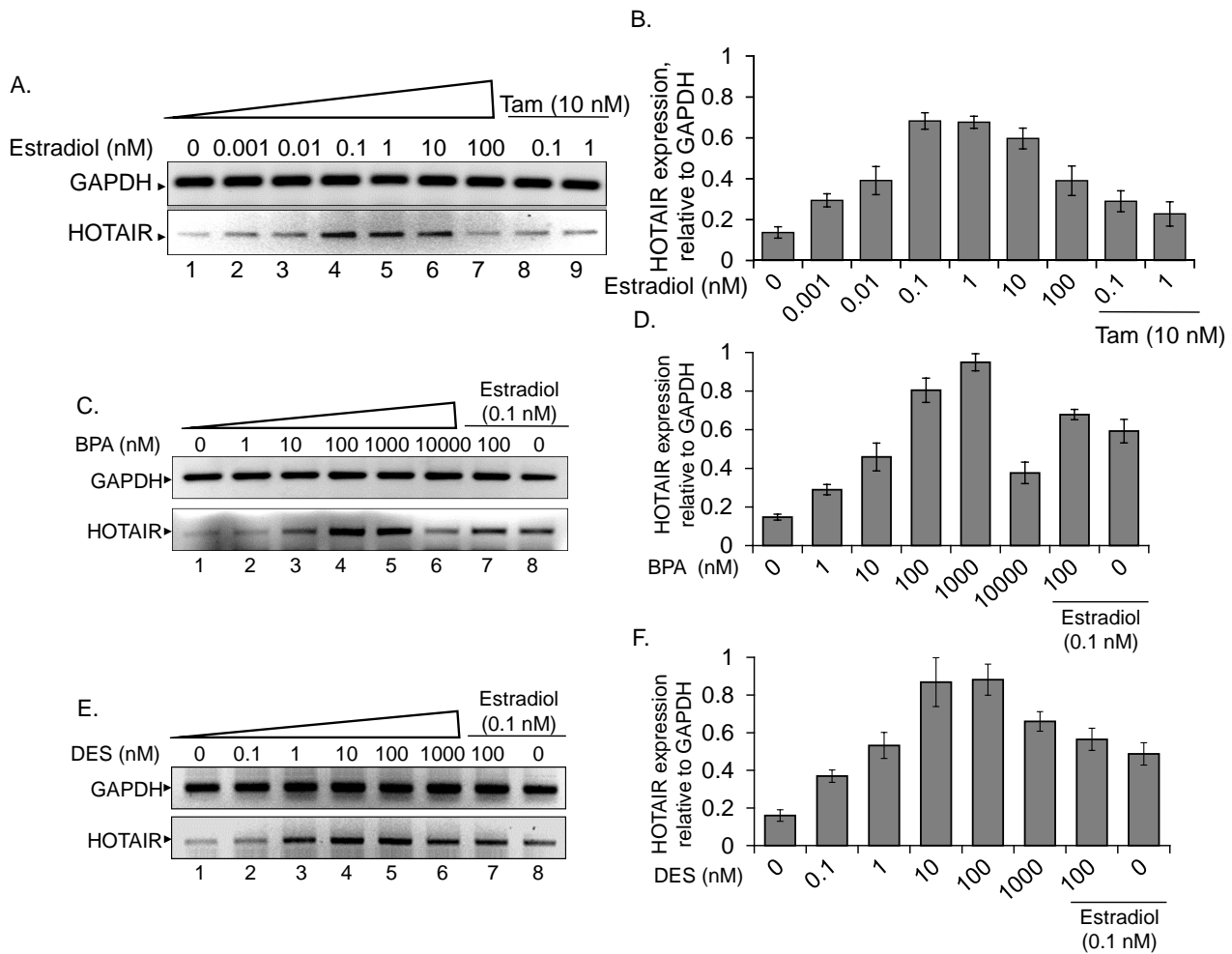


Figure S1. Effect of varying concentration of estradiol, BPA and DES on HOTAIR expression in MCF7 cells: Panels A-B: MCF7 cells were treated with varying concentrations of estradiol in the absence and presence of tamoxifen. Panels A and B show the RT-PCR and qPCR data respectively. Panels C-D: Dose dependent analysis of HOTAIR expression in presence BPA, in the absence and presence of estradiol. Panels C and D show the RT-PCR and qPCR data respectively. Panels E-F: Dose dependent analysis of HOTAIR expression in presence BPA, in the absence and presence of estradiol. Panels E and F: show the RT-PCR and qPCR data respectively. Bars indicate standard errors. Each experiment was repeated for thrice with three parallel replicates.

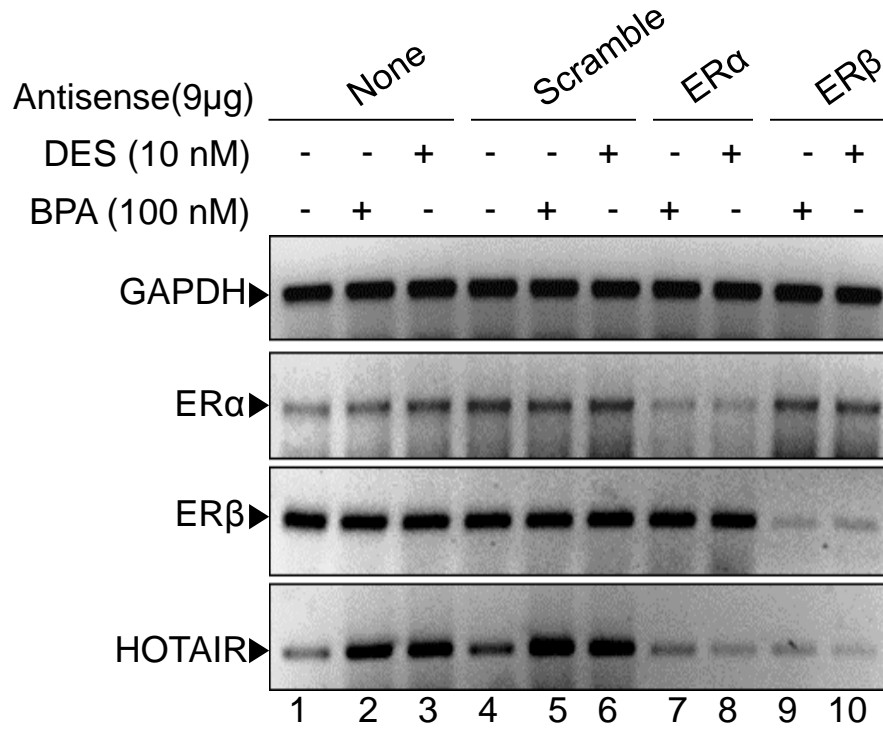


Figure S2. Effect of ERα and ERβ depletion on BPA and DES induced expression of HOTAIR. MCF7 cells (grown in phenol red free media) were treated with ERα, ERβ and scramble antisense (9μg) separately for 48 h, exposed to BPA (100 nM), and DES (10 nM). The RNA was isolated and analyzed by RT-PCR using primers specific to HOTAIR, ERα and ERβ. GAPDH was used as a loading control. The RT-PCR products were analyzed in agarose gel.

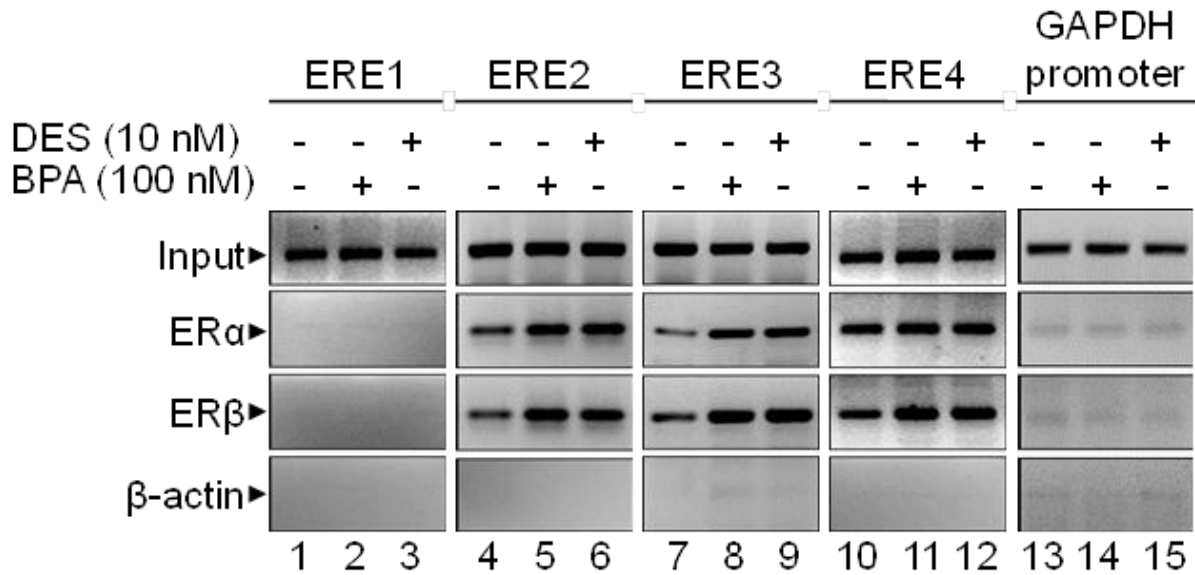


Figure S3. BPA/DES dependent recruitment of ERs in the ERE regions of HOTAIR promoter; MCF7 cells were treated with 100 nM BPA, 10 nM DES for 4 h and subjected to ChIP assay using antibodies specific to ER α , ER β , and β -actin. The immuno-precipitated DNA fragments were PCR amplified using primers specific to ERE1-4 of HOTAIR promoter and GAPDH promoter and analyzed by agarose gel.

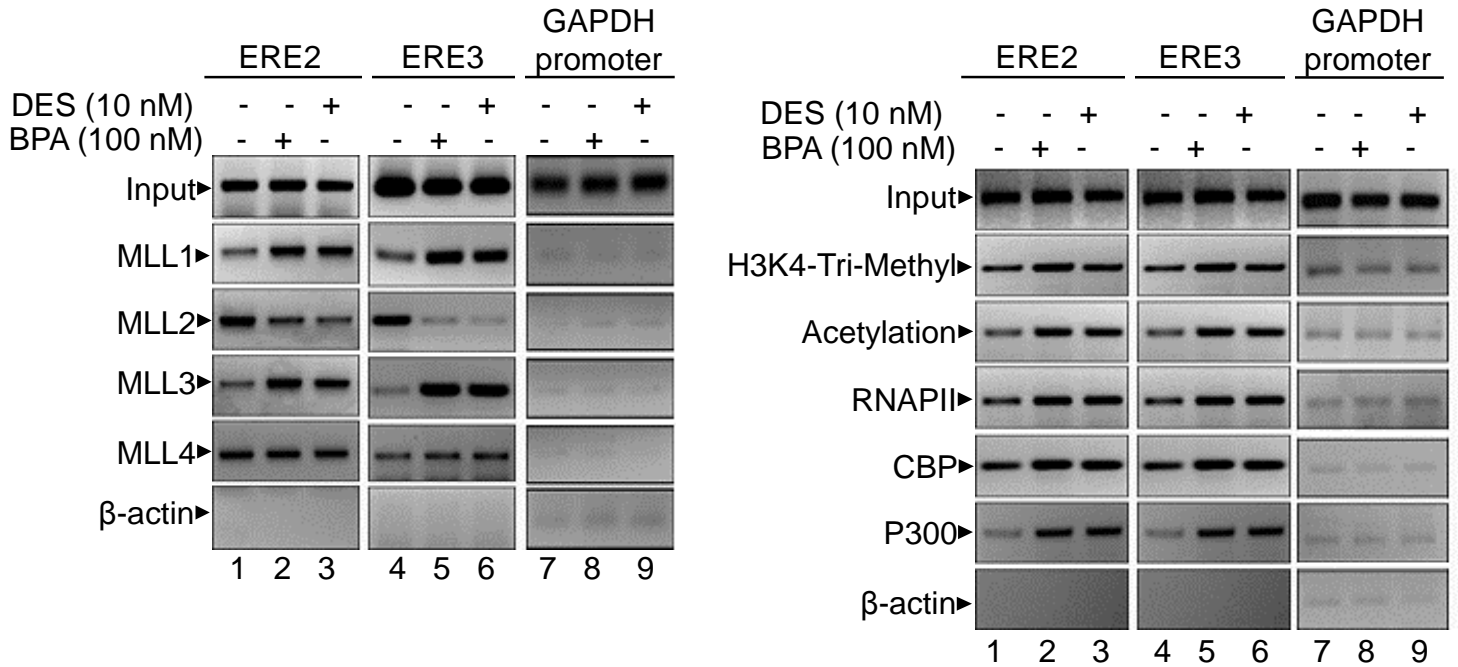


Figure S4. BPA/DES dependent recruitment of ER-coregulators and PIC complex assembly at the HOTAIR promoter. MCF7 cells were treated with 100 nM BPA, and 10 nM DES for 4 h and subjected to ChIP assay using antibodies specific to MLLs (MLL1-4), CBP, p300, RNAPII, and H3K4 trimethylation, histone acetylation. β -actin was used as the control. The ChIP DNA fragments were PCR amplified using primers specific to in the ERE2 and ERE3 regions of HOTAIR promoter and the GAPDH promoter. The RT-PCR products were analyzed in agarose gel.