Legends for Supplementary Figures and Tables

Supplementary Figure S1: Recombinant COMT (10 µg/mL) was incubated with S-(5'-adenosyl)-L-methionine (500 nM) and raloxifene, DMA, or bazadoxifene (1 µM) at 37^0 C for 5 minutes prior to the addition of 6,7-dihydroxycoumarin (5 µM). The reactions were then monitored at 37^0 C (λ_{ex} = 355nm, λ_{em} = 460 nm) for 10 min. The data is reported as the change in fluorescence after 10 min.

Supplementary Figure S2: Chromatograms showing (A) OHE_2 / E_1 metabolites and (B) $MeOE_2$ / E_1 metabolites in E_2 (1 µM) treated MCF-10A cell samples. Relatively higher amounts of E_2 metabolites were detected compared to E_1 metabolites in E_2 treated cell samples.

Supplementary Figure S3: Effect of SERMs on CYP450 1B1 and 1A1 enzyme expression. MCF-10A cells were treated with E_2 (1 µM) in the presence and absence of SERMs (1 µM). Protein lysates were prepared and expression of CYP450 1B1 and 1A1 was analyzed using western blotting. Protein concentrations were determined by BCA assay and 30 µg of total protein was loaded into each lane.

Supplementary Figure S4: Inhibition of CYP450 1B1 by SERMs. Recombinant CYP450 1B1 was incubated in potassium phosphate buffer (50 mM, pH = 7.4) in the presence of NADPH (1 mM) and different concentrations of SERMs (0, 10, 20, 30, 40, 50, 60 µM). Relative EROD activity was measured after incubation at 37 °C for 10 min for each sample and each data point represents the average EROD activity of duplicates or triplicates ± SEM.

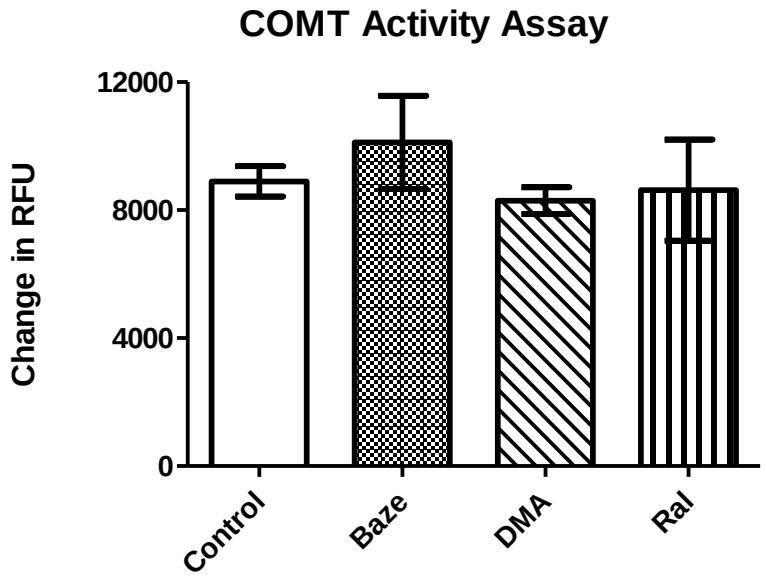
Supplementary Figure S5: Representative western blot images showing the (A) effect of SERMs on GST-P1,catechol-O-methyl transferase (COMT) and (B) SULT 1A1, SULT1E1 and NQO1 in MCF-10A cells. Cells were treated with E_2 (1 µM) in the presence and absence of SERMs (1 µM) for 6 days. Protein lysates were prepared and expressions of phase 2

detoxification enzymes were analyzed using western blotting. Protein concentrations were determined by BCA assay and 30 µg of total protein was loaded into each lane.

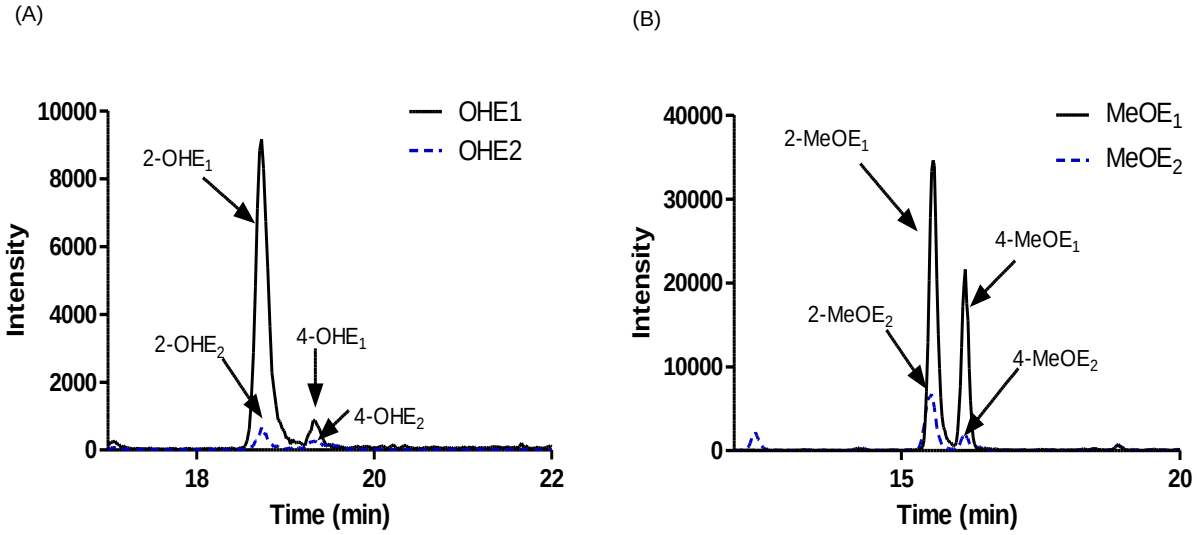
LOWER: Gene transcription of SULT 1A1 was not significantly affected by any of the treatments. Gene transcription was measured by qPCR after isolating RNA from 24 h treated MCF-10A cells. Each point represents an average of at least two independent experiments ± SD. Treatment groups, other than control, were not significantly different by ANOVA.

Supplementary Figure S6: MCF-7 cells were were plated (4 x 10^5 cells) in 12 well plates. Cells were transfected with 3µg of the pERE-luciferase plasmid, which contains three copies of the Xenopus laevis vitellogenin A2 ERE upstream of firefly luciferase. To normalize for cell viability and transfection efficiency, 1µg pRL-TK plasmid (Promega, Madison, WI) containing a cDNA encoding Renilla luciferase was co-transfected along with ERE plasmid. Transfection was performed overnight using Lipofectamine 2000 transfection reagent (Invitrogen), in Opti-MEM media according to the manufacturer's instructions. Cells were treated with test compounds for 24 h and the luciferase activity was measured in cell lysates using the Dual Luciferase assay system (Promega) with FLUOstar OPTIMA (BMG LABTECH, Durham, NC). The above procedure was repeated in MDA-MB231:β41 cells (kindly provided by Dr. D. Tonetti) to test for ERβ activity.

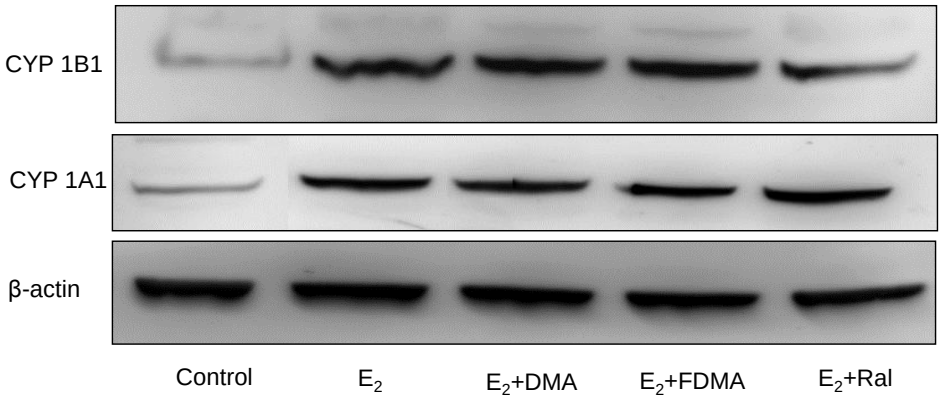
Supplementary Figure S1



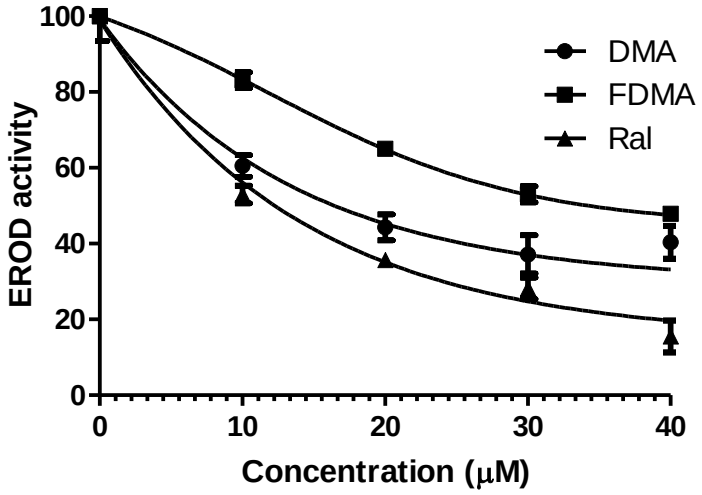
Supplementary Figure S2



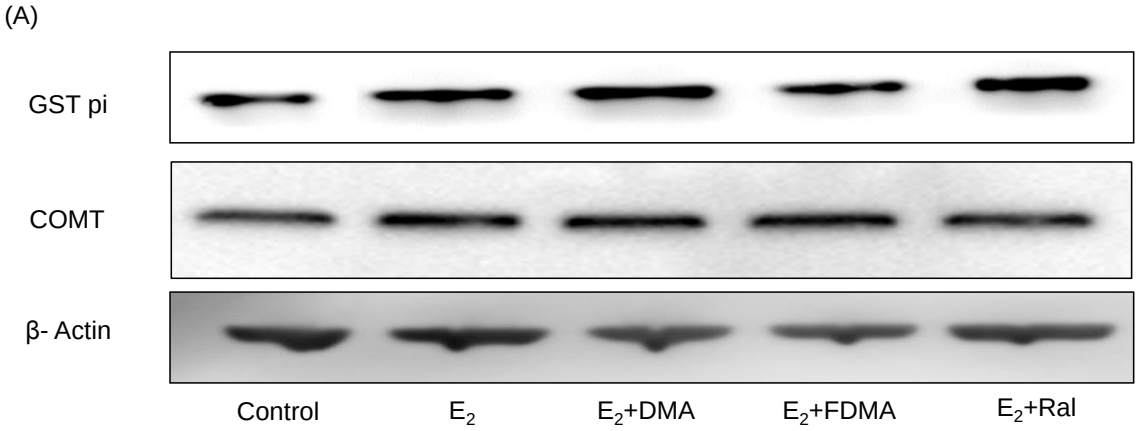
Supplementary Figure S3

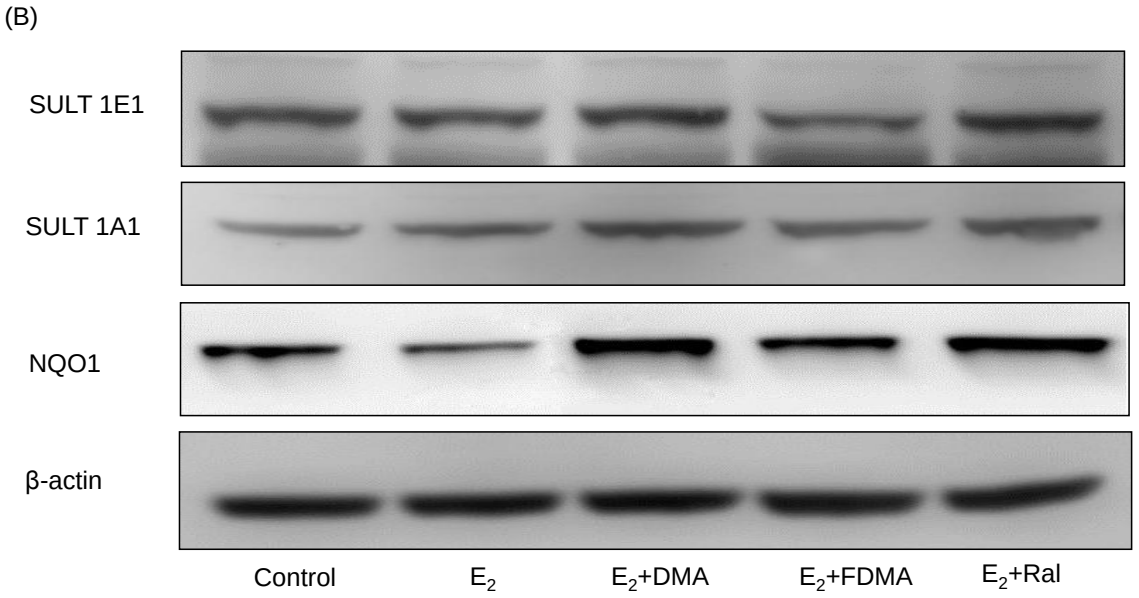


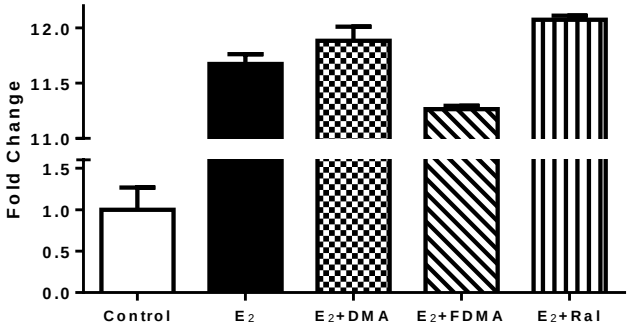
Supplementary Figure S4



Supplementary Figure S5







Supplementary Figure S6