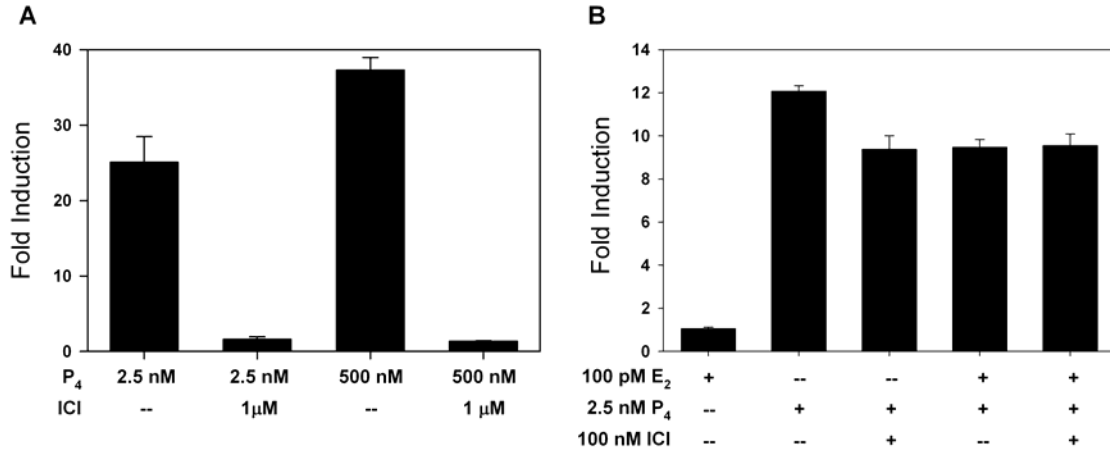


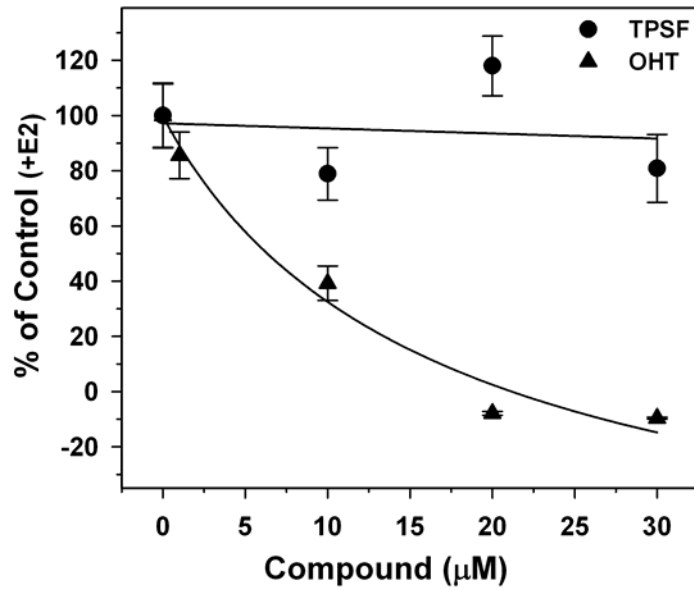
## SUPPLEMENTAL FIGURES

### Supplemental Figure 1



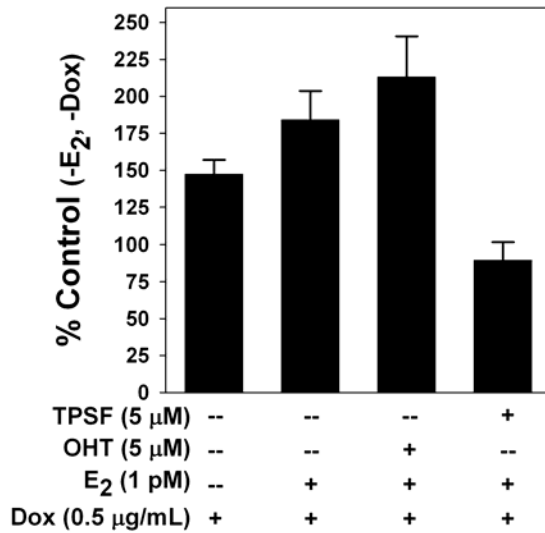
**Fig. S1. The ER inhibitor ICI 182,780, decreases PR-mediated gene expression in T47D cells.** In panel *A* before plating the cells were maintained in full serum (5% FBS) that contains some estrogen. In panel *B* the cells were depleted of estrogen by 4 days in medium containing 5% 1X CD-FBS. *A*. T47D cells were maintained in MEM containing 10 mM HEPES pH 7.4 and 5% FBS. 10,000 cells/well were plated in 96 well plates in 100  $\mu$ l of medium containing 5% CD-FBS. *B*. The cells were maintained for 4 days in medium containing 5% 1X CD-FBS before plating. *A*. After 24 h 2.5 nM or 500 nM progesterone (P<sub>4</sub>) was added with or without 1  $\mu$ M ICI 182,780. 500nM progesterone was used to be certain that ICI 182,780 was not competing with P<sub>4</sub> for binding to PR. *B*. After 24 h cells were maintained in medium with or without 5 nM progesterone (P<sub>4</sub>) and 1  $\mu$ M ICI 182,780. Alkaline phosphatase assay. After 24 h in the indicated treatment, the cells were washed once with PBS, lysed in 100  $\mu$ l of buffer (20 mM potassium phosphate, pH 7.8, 5 mM MgCl<sub>2</sub>, 0.5% Triton X-100, frozen. Lysate was stored at -70°. 5  $\mu$ l of supernatant was removed and assayed in a 96 well plate in 25  $\mu$ l of assay buffer (100 mM diethanolamine, pH 9.5, 1 mM MgCl<sub>2</sub>, 0.4 mM CSPD (substrate, Applied Biosystems/Tropix) and 1X Emerald II enhancer (Applied Biosystems/Tropix). After 1 h at room temp., luminescence in the visible (emission max. 542 nm) was read for 1 sec. Data were the average of 3 experiments  $\pm$  SEM

## Supplemental Figure 2



**Fig. S2. TPSF does not exhibit nonspecific toxic effects in ER $\alpha$  negative MDA-MB-231 cells.** Cell maintenance, growth and plating were as described in the Fig. 4 legend. The indicated concentrations of OHT (filled triangles) and TPSF (filled circles) and 1 pM E<sub>2</sub> were added and cell number was determined using MTS and a standard curve (Fig. 4 legend). Cell number in the absence of TPSF or OHT was set as 100%. Data were the average of 3 experiments  $\pm$  SEM.

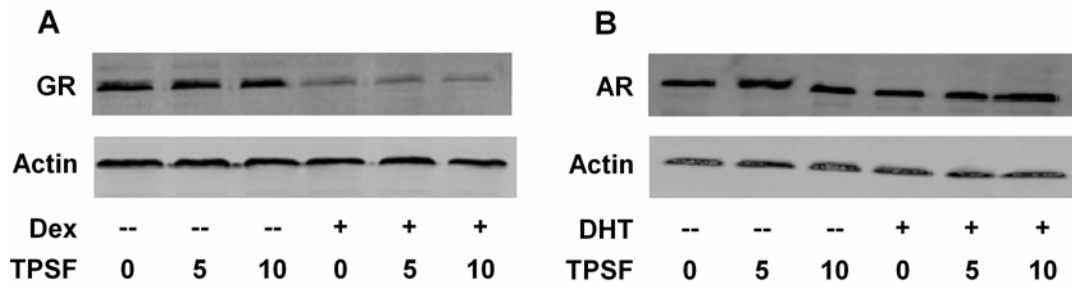
### Supplemental Figure 3



**Fig S3. TPSF but not OHT inhibits E<sub>2</sub>-ER $\alpha$ -dependent growth of MCF7ER $\alpha$ HA cells.**

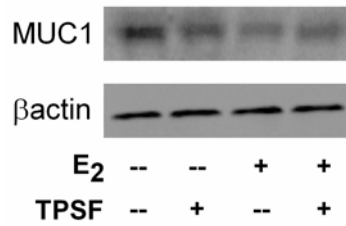
MCF7ER $\alpha$ HA cells were maintained for 4 days in 6x CD-FBS (22,37), seeded into 12 well plates with or without 0.5  $\mu$ g/ml Dox , 1 pM E<sub>2</sub>, 5  $\mu$ M OHT and 5  $\mu$ M TPSF, grown for 4 days and assayed using MTS and a standard curve for cell number (see Fig. 4 legend). Cells in ethanol and DMSO vehicle alone were set to 100%. Data represent the average of 3 experiments  $\pm$  SEM. The difference between the cells incubated with TPSF + E<sub>2</sub> + Dox and cells in OHT + E<sub>2</sub> + DOX was significant (P <0.05 using Student's t test).

### Supplemental Figure 4



**Fig. S4. TPSF has little or no effect on levels of AR and GR.** To test the selectivity of TPSF for down-regulation of ER $\alpha$ , we evaluated the effect of 5  $\mu$ M and 10  $\mu$ M TPSF on the levels of AR and GR. The cell lines used and cell growth conditions were those employed in the dose-response studies evaluating effects of TPSF on gene expression (Fig. 2D). Cells were maintained in medium containing or lacking dexamethasone (panel A) or DHT (panel B) and 0, 5 or 10  $\mu$ M TPSF for 24 hours. Extracts were prepared and analyzed on separate gels for AR and GR levels by western blotting. Total protein (12  $\mu$ g for AR and 40  $\mu$ g for GR) was fractionated on 10% polyacrylamide gels and AR and GR levels determined by western blot. The antibody used to detect AR was AR441 monoclonal antibody used at 1:1000 dilution (NeoMarkers, Fremont, CA). The GR antibody was MA1-510 monoclonal antibody (Thermo Scientific, Rockford, IL) used at 1:1000 dilution. Data is representative of at least 2 gels for each sample.

## Supplemental Figure 5



**Fig. S5. TPSF does not down-regulate the level of Muc1.** MCF-7 cells were maintained and plated as described in Materials and Methods. After 4 days in MEM + 5% 1X CD-FBS, the medium was replaced with medium with or without 10 nM  $E_2$  and with or without 10  $\mu$ M TPSF. Cells were harvested at 24 h extracts prepared and Muc1 detected using Muc1 specific antibody (60). The actin samples were run on a separate gel.