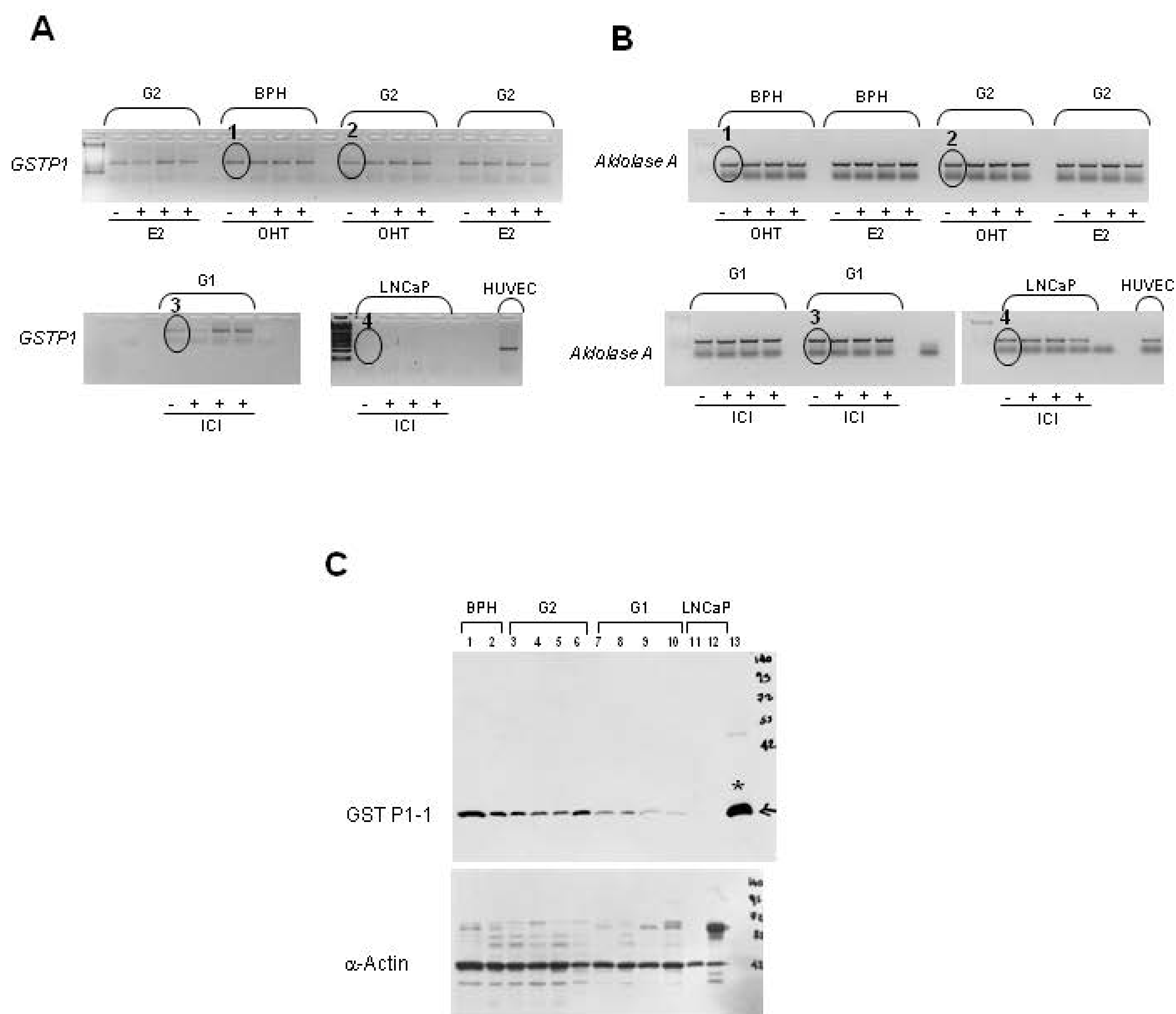
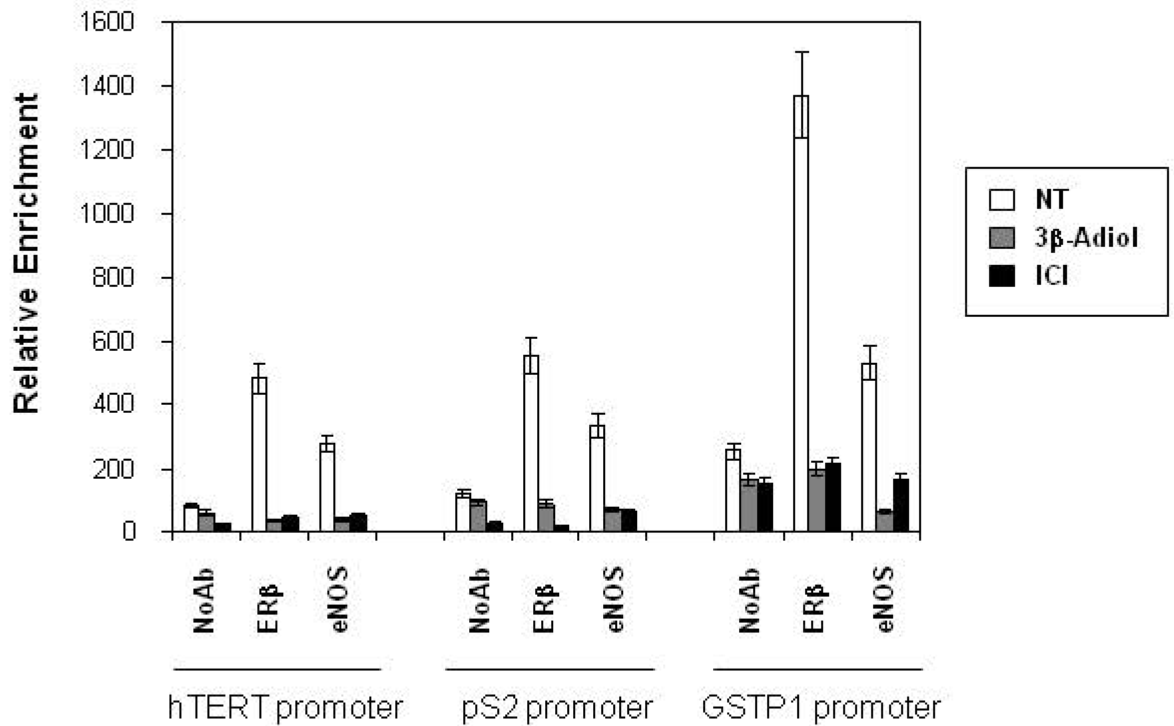
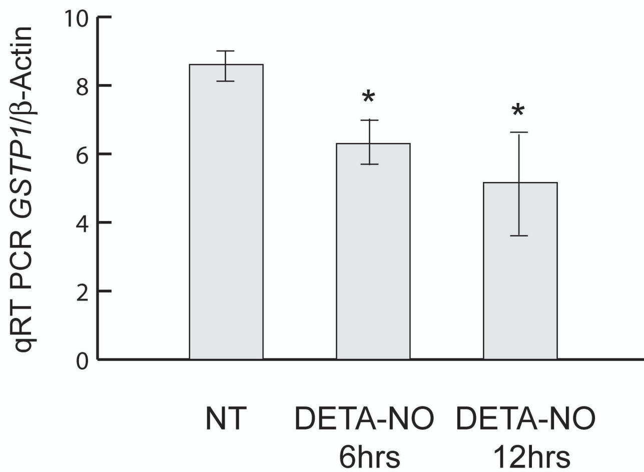
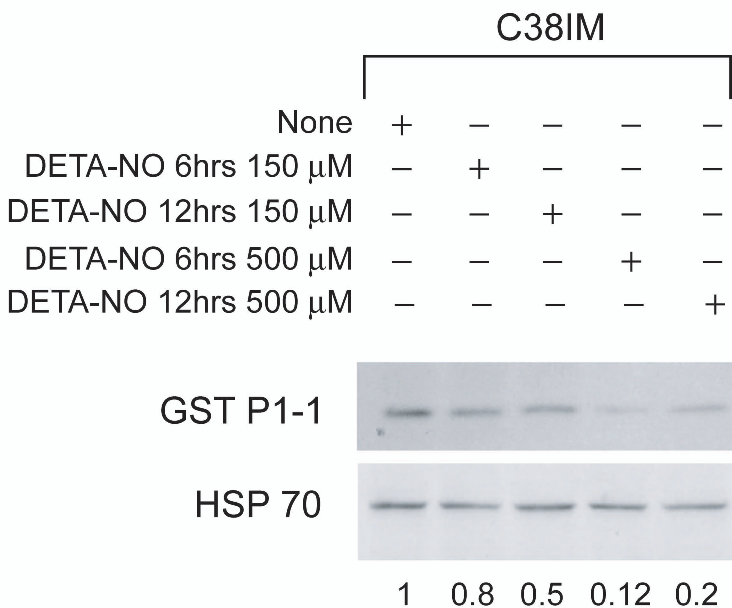


Supplemental Figure 1. Original scans of Figure 2, A-B. A-B, Semiquantitative RT-PCR analysis of *GSTP1* (A) and housekeeping gene *Aldolase A* (B) mRNAs in immortalized cells derived from Benign Prostate Hyperplasia (BPH), PCa cells of the G1 and G2 groups and LNCaP cells after treatment with E_2 or OHT or ICI. Circles and relative numbers indicate bands showed in Figure 2A right Panel, corresponding exactly to BPH (first lane), G2 (second lane), G1 (third lane) and LNCaP cells (fourth lane). C, Western Blots for GST P1-1 (*upper panel*) and loading control α-Actin (*lower panel*) performed in BPH, G2, G1 and LNCaP cells. Gels were first blotted and stained with anti GST P1-1 antibody, then were washed and re-stained with antibody to α-Actin. Recombinant GST P1-1 served as positive control (*). Bands 1, 3, 7 and 12 are displayed in Figure 2B right Panel, corresponding exactly to BPH, G2, G1 and LNCaP cells.



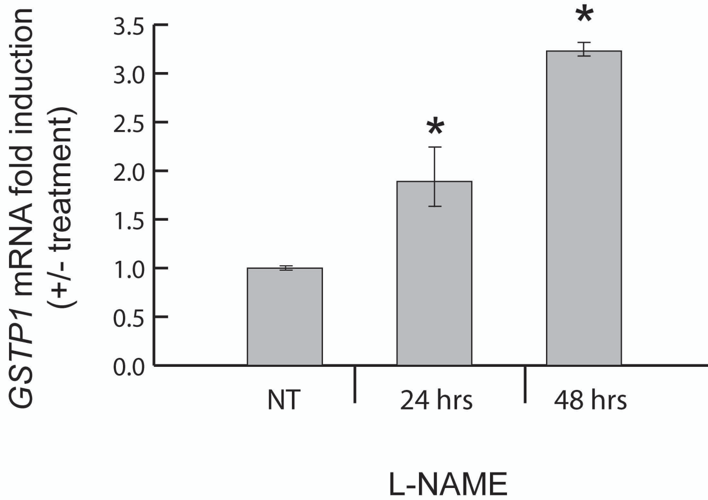
Supplemental Figure 5. Effect of 3β-Adiol and ICI on the *in vivo* ERβ and eNOS recruitment onto positive and negative estrogen target genes. After formaldehyde crosslinking and chromatin precipitation with ERβ, and eNOS antibodies or in the absence of antibody (NoAb), the DNA was subjected to quantitive RT-PCR amplification using primers for the *GSTP1*, *hTERT* or *pS2* promoter encompassing the well characterized ERE sites. Analyses were in duplicate, and values were normalized to the corresponding DNA input. Results are expressed as relative enrichment.



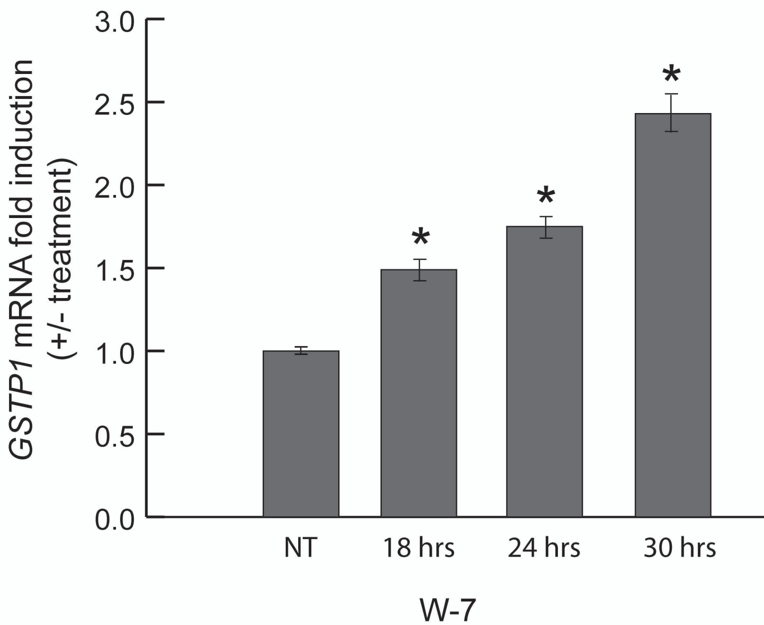


Supplemental Figure 4. GSTP1 mRNA and protein expression in G2 cells after treatment with DETA-NO. A, G2 cells (C38IM) were treated with DETA-NO for 6 and 12 hours and *GSTP1* mRNA was evaluated by qRT-PCR. The data are expressed after normalization to β-Actin and represent the mean ± SEM of 4 experiments (* $P<0.05$ vs NT). B, Western blot of GST P1-1 in G2 cells (C38IM) in basal conditions and after treatment with DETA-NO for 6 and 12 hours at 150 µM and 500 µM. HSP70 was the loading control. Ratio (+/- treatment), after normalization to the HSP70 signal by densitometric analysis, is indicated in the bottom row.

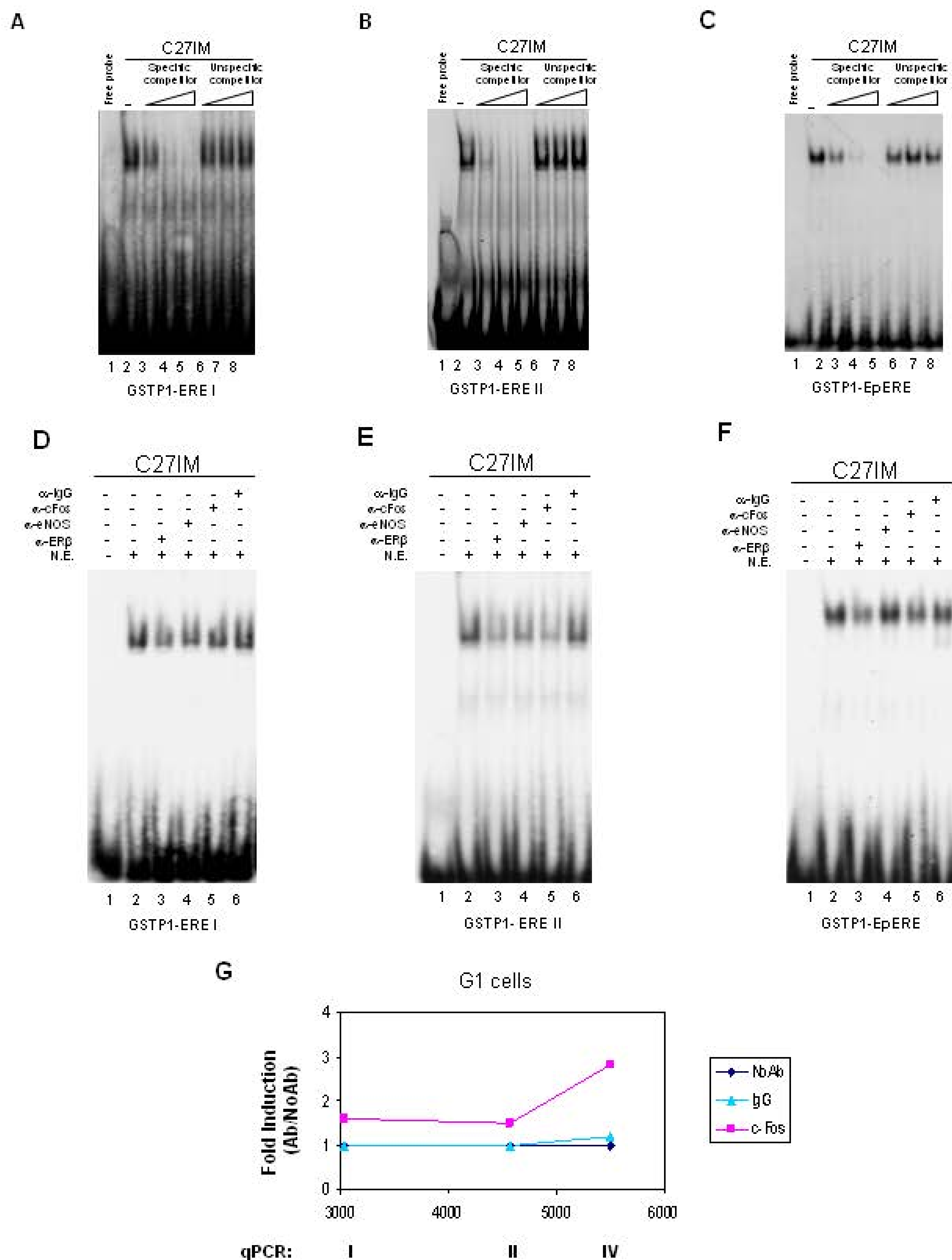
A



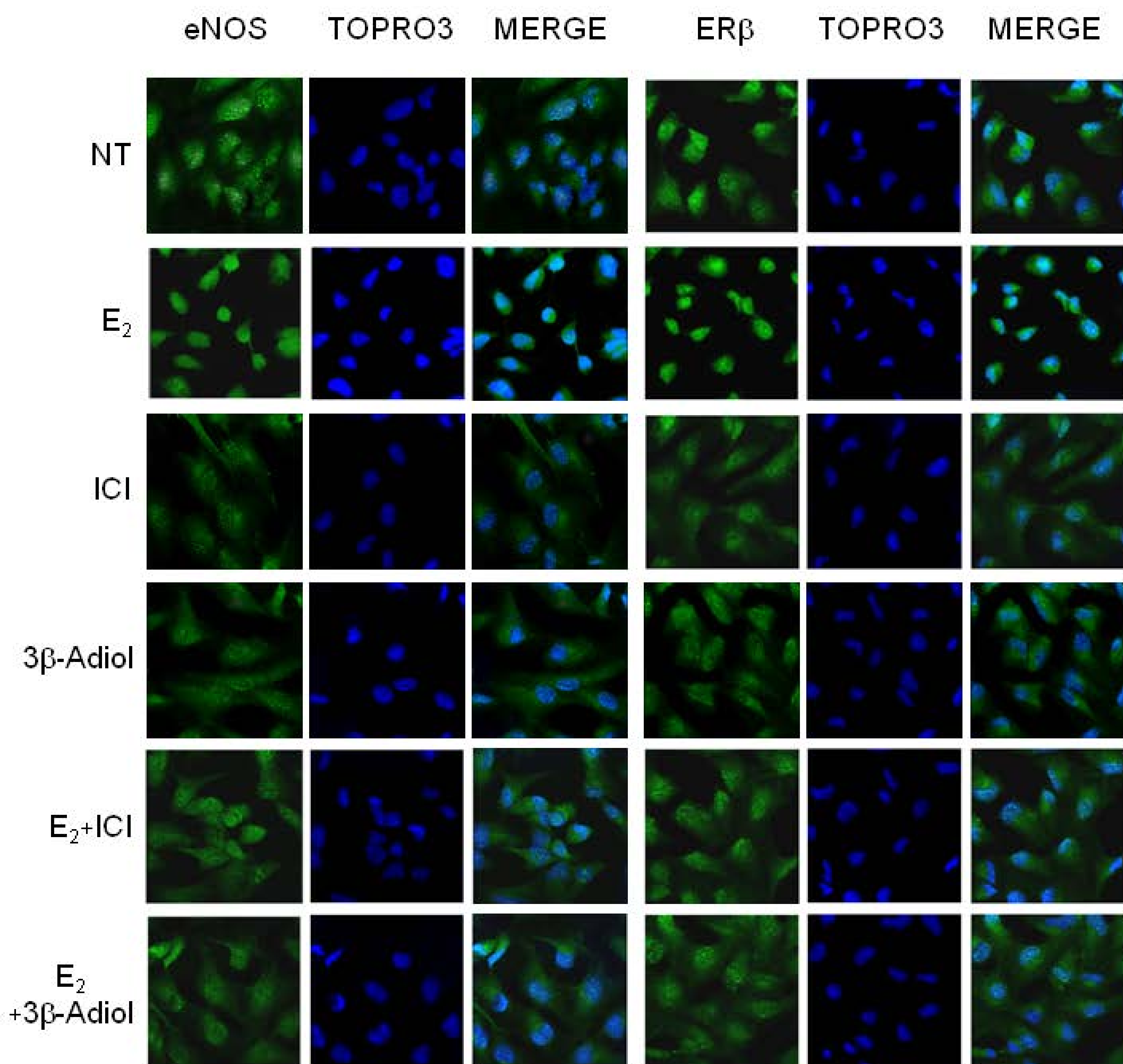
B



Supplemental Figure 3.GSTP1 expression upon treatment with eNOS and Calmodulin inhibitors. GSTP1 mRNA levels were assessed by qRT-PCR in G1 cells (n = 2) (A) upon treatment with 5 mM L-NAME (Papapetropoulos et al., J. Clin. Invest. 1997, 100:3131-3139; Grasselli et al., Circ. Res. 2008,103(1):34-42) and (B) with 25 μM W-7 (Sivanandam A. et al., J Cell Physiol 2010, doi 10.1002/jcp. 22516) for the indicated times. The data represent the mean ± SEM of 4 experiments performed in duplicate (* P<0.05 vs NT or empty vector).



Supplemental Figure 2. Analysis of PCa nuclear complex binding to the ERE and EpERE sites of *GSTP1* promoter by EMSA and c-Fos recruitment by ChIP. A-C, A ^{32}P-labeled double-stranded oligonucleotide containing the GSTP1-ERE I (A) GSTP1-ERE II (B) or EpERE (C) sequence was incubated with extracts of G1 PCa cells alone (lane 2 in each panel). Five- 50- and 200-fold molar excess of unlabeled oligonucleotides for ERE I (A) or ERE II (B) or EpERE (C) sequences were used as competitors (lanes 3, 4, 5, respectively). An unrelated DNA sequence was used as a non specific competitor (lanes 6-8). D-F, ERβ, eNOS and cFos binding to *GSTP1* promoter. A ^{32}P-labeled double-stranded oligonucleotides containing the GSTP1-ERE I (D) or GSTP1-ERE II (E) or EpERE sequence (F) were incubated with nuclear extracts of G1 cells alone (lane 2) or in the presence of antibody anti ERβ (lane 3) or anti eNOS (lanes 4) or anti cFos (lanes 5) or with unrelated antibody IgG as control (lane 6). G, After formaldehyde crosslinking and chromatin precipitation in the absence of antibody (NoAb) or with antibody to c-Fos or unrelated IgG, the DNA was subjected to quantitive RT-PCR amplification using specific primers for *GSTP1* promoter.



Supplemental Figure 6. Localization of ERβ and eNOS in the presence or absence of estradiol, with or without ICI and 3β-Adiol treatment. G1 cells were incubated in presence of estradiol (E_2, 10^{-7}M) or vehicole alone (NT) for 135 minutes with or without a pre-treatment with ICI (10^{-7}M) or 3β-Adiol (10^{-6}M). Cells were fixed, stained with anti-eNOS or anti-ERβ antibodies and examined by confocal microscopy. Nuclei were stained with TOPRO 3. Individual and merged fluorescence signals are shown. Original magnification x80. Images are from a typical experiment of 2 performed.