Figure S1. Expression of ERα in PNT1A, PC-3 and DU-145 cells. Western blot analysis for the detection of the ERα in PNT1A, DU-145 and PC-3 cells was performed as described in the Materials and methods, using 50 μg of protein/lane and antibody against the carboxy-terminal region of ERα (top panel) or with antibody that recognizes GAPDH (bottom panel). The protein sizes of ERα and GAPDH are shown at the right. The data shown are representative of four independent experiments. Results of the densitometric analysis of the immunoblots were normalized to the respective expression of GAPDH, expressed in arbitrary unit and plotted (mean ± SEM) from four independent experiments (bar graph). $^{\delta}P>0.05$, significantly different from PNT1A cells; $^{\varepsilon}P>0.05$, significantly different from PC-3 cells (determined using ANOVA and the Newman-Keuls test, n=4). ER, estrogen receptor.

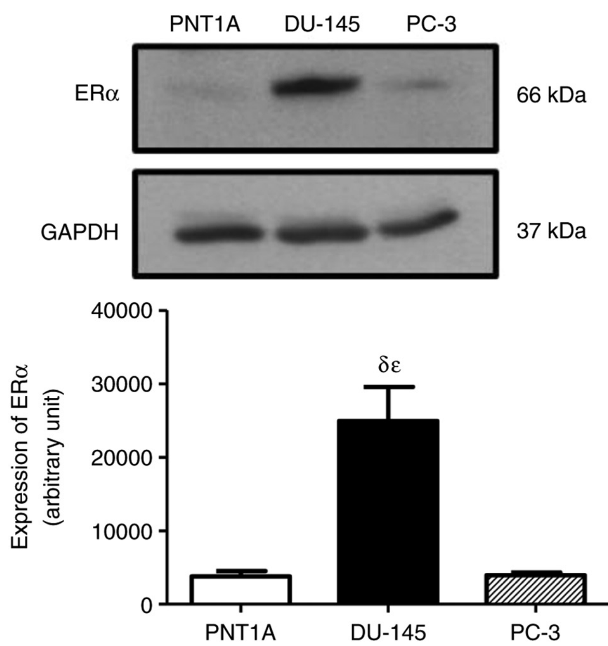

Figure S2. Effects of the ERα-selective agonist, PPT, and the ERβ-selective agonist, DPN, on the invasion of the DU-145 cells. Cells in culture medium without serum were seeded in ThincertR chambers with polyethylene terephthalate membranes pre-coated with phenol red-free Matrigel. These chambers were placed in 24-well plates containing culture medium with 10% FBS in the lower chamber. Cells in upper chambers of the same culture plate were incubated in the absence (C, control) or presence of (A) E2 (10 nM), (B) ERα-selective agonist PPT (10 nM), or (C) ERβ-selective agonist DPN (10 nM) for 24 h at 37˚C. Cells were also untreated or pretreated with both antagonists (MPP 10 nM and PHTPP 10 nM), MPP (10 nM) or PHTPP (10 nM) for 30 min. Incubation was continued in the absence or presence, respectively of (A) E2, (B) PPT or (C) DPN for 24 h at 37˚C. Cell invasion assay was performed as previously in the Materials and methods. Results were expressed in relation to the control (C=1) and plotted (mean ± SEM) from three to four independent experiments, in duplicate (bar graphs). Images (x200 magnification) are representative of three to six independent experiments performed in duplicate. *P<0.05, significantly different from the control; #P<0.05, significantly different from the MPP + PHTPP + E2, MPP + PHTPP, MPP + PPT, MPP, PHTPP + DPN or PHTPP groups (determined using ANOVA and Tukey's post hoc test). E2, 17β-estradiol; ER, estrogen receptor; PPT, 4,4',4"-(4-propyl-(1H)-pyrazole-1,3,5-triyl)trisphenol; DPN, 2,3-bis(4-hydroxyphenyl)-propionitrile; MPP, 1,3-bis(4-hydroxyphenyl)-4-methyl-5-[4-(2-piperidinylethoxy)phenol]-1H-pyrazole dihydrochloride; PHTPP, 4-[2-phenyl-5,7-bis(trifluoromethyl)pyrazolo[1,5-a]pyrimidin-3-yl]phenol.

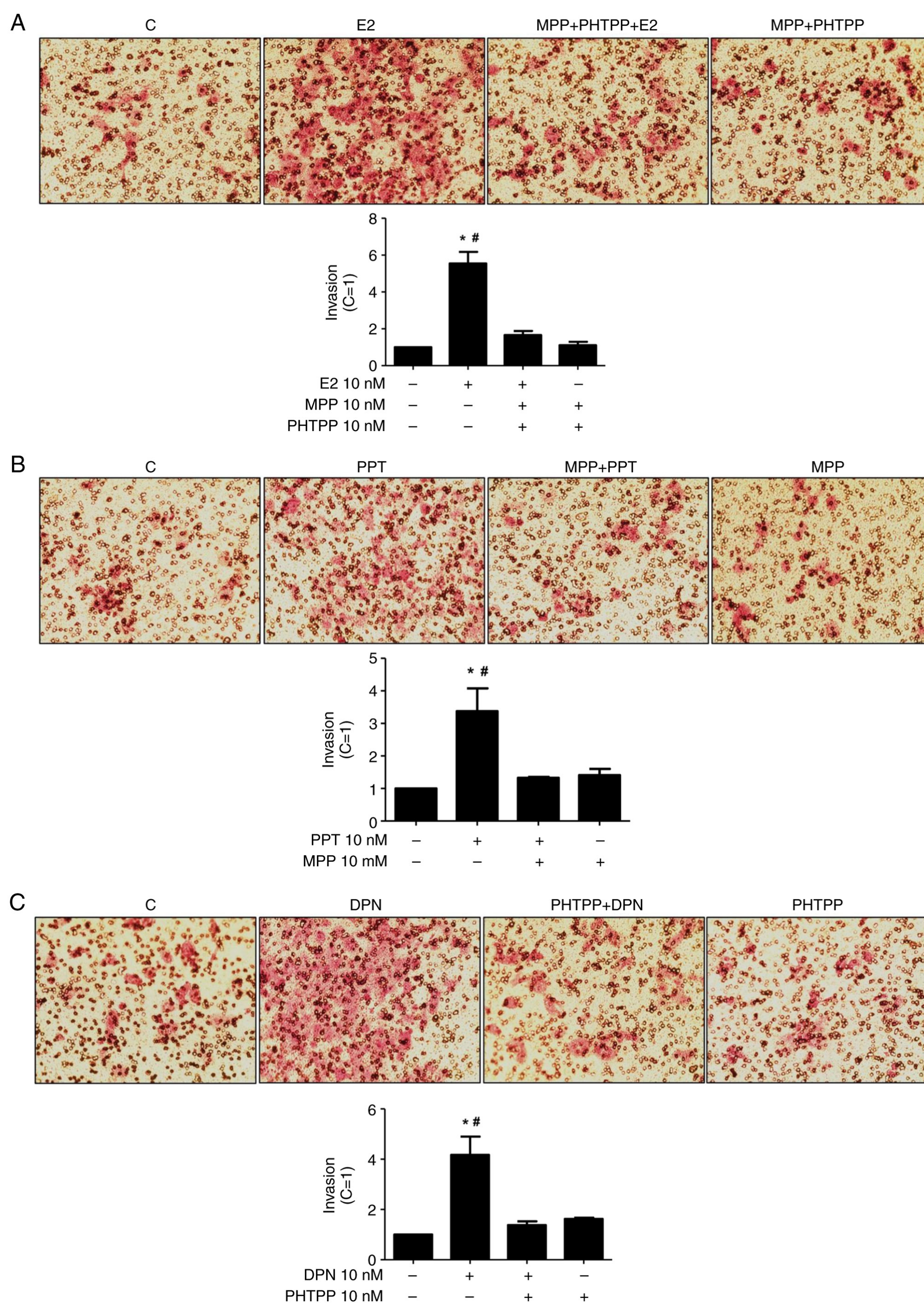

Figure S3. Effects of the ERβ-selective agonist, DPN, for 4 h on the expression and localization of GAL-3 in DU-145 cells. Cells were incubated in the absence (control, C) or presence of the ERβ-selective agonist, DPN (10 nM), for 2 h and 4 h at 37˚C. Immunostaining for GAL-3 (red) was detected as described in the Materials and methods. Nuclei were stained with DAPI (blue). Negative control was performed in the absence of primary antibody (insert). Scale bars, 20 μm. Images shown are representative of three independent experiments. ER, estrogen receptor; GAL-3, galectin-3; DPN, 2,3-bis(4-hydroxyphenyl)-propionitrile.

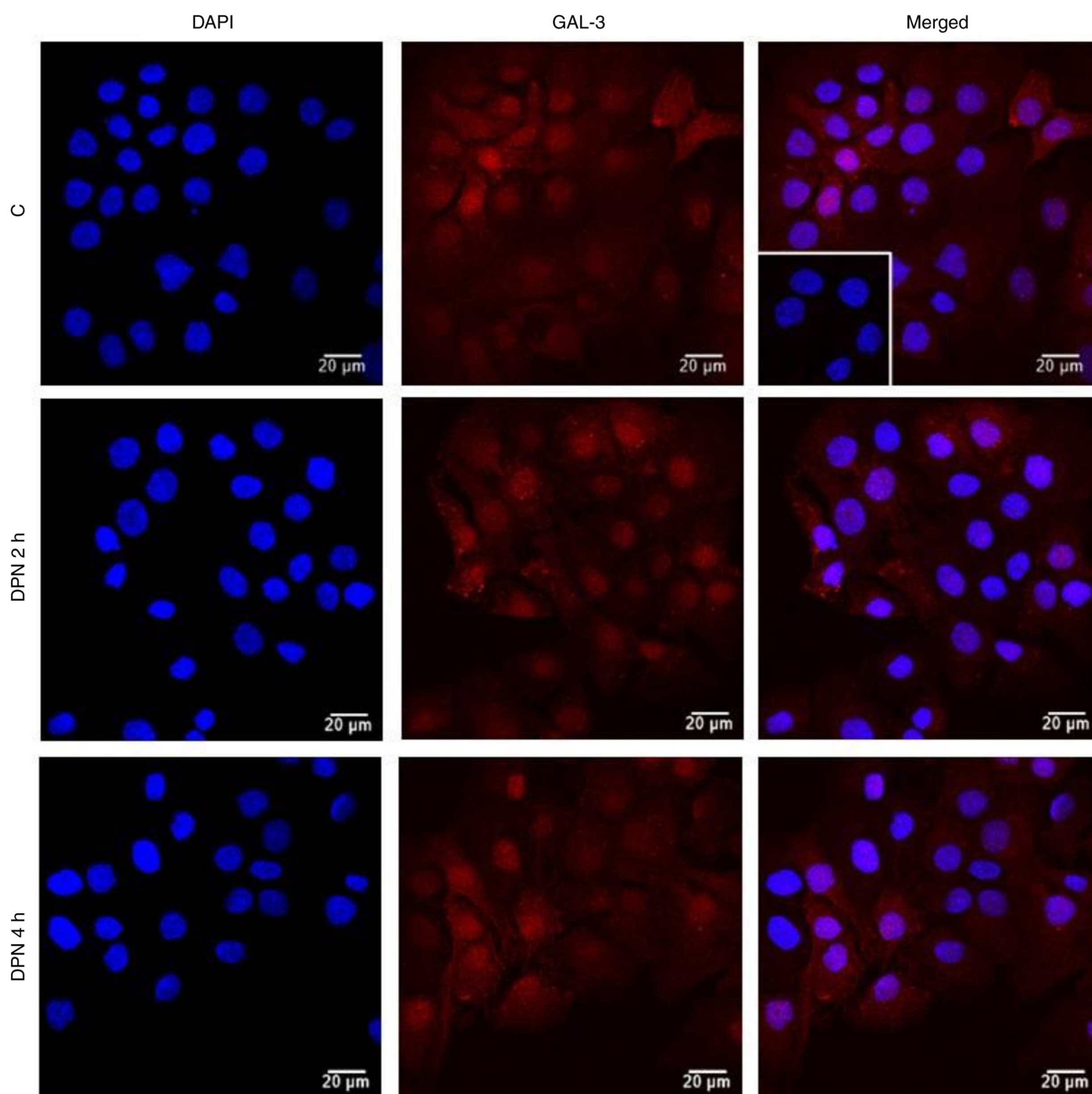

Figure S4. Effects of E2, PPT and DPN on the expression and localization of GAL-3 in DU-145 cells. (A) Cells were incubated in the absence (control, C) or presence of E2 (10 nM) for 4 h at 37˚C. Cells were also untreated or pre-treated with the ERα-selective antagonist ,MPP (10 nM), or the ERβ-selective antagonist, PHTPP (10 nM), for 30 min. Incubation was continued in the absence or presence of E2 (10 nM) for 4 h at 37˚C. (B) Cells were incubated in absence (control, C) or presence of ERα-selective agonist PPT (10 nM) for 2 and 4 h at 37˚C. Cells were also untreated or pretreated with the ERα-selective antagonist, MPP (10 nM), for 30 min. Incubation was continued in the absence or presence of PPT (10 nM) for 4 h at 37˚C. (C) Cells were incubated in the absence (control, C) or presence of E2 (10 nM), ERα-selective agonist PPT (10 nM) or ERβ-selective agonist DPN (10 nM) for 24 h at 37˚C. Fluorescence intensity of GAL-3 in DU-145 cells obtained in Figs. 5, 6 and 7 was plotted in arbitrary units in the bar graphs in panels A, B and C, respectively. E2, 17β-estradiol; ER, estrogen receptor; PPT, 4,4',4''-(4-propyl-(1H)-pyrazole-1,3,5-triyl)trisphenol; DPN, 2,3-bis(4-hydroxyphenyl)-propionitrile; MPP, 1,3-bis(4-hydroxyphenyl)-4-methyl-5-[4-(2-piperidinylethoxy)phenol]-1H-pyrazole dihydrochloride; PHTPP, 4-[2-phenyl-5,7-bis(trifluoromethyl)pyrazolo[1,5-a]pyrimidin-3-yl]phenol.

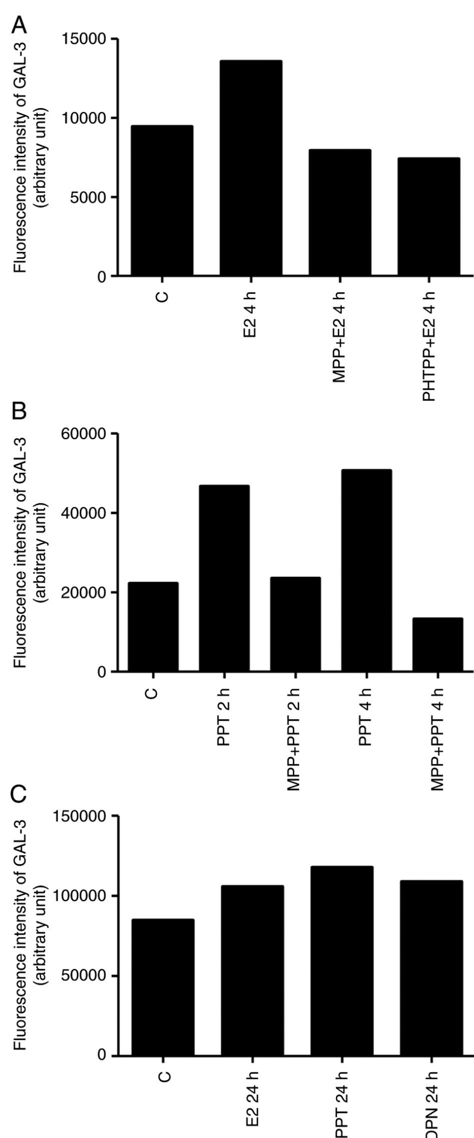

Figure S5. Effects of a specific inhibitor of GAL-3 on the viability of DU-145 cells. Cells were incubated in the absence (control, C) or presence of specific inhibitor of GAL-3 (VA03, 20 or 200 μM) for 24 h at 37˚C. MTT assays was performed in aliquots from triplicate wells of control and treated cells, as described in the Materials and methods. Results were plotted (mean ± SEM) from three independent experiments. No statistically significant differences were observed (P>0.05, determined using ANOVA). GAL-3, galectin-3.