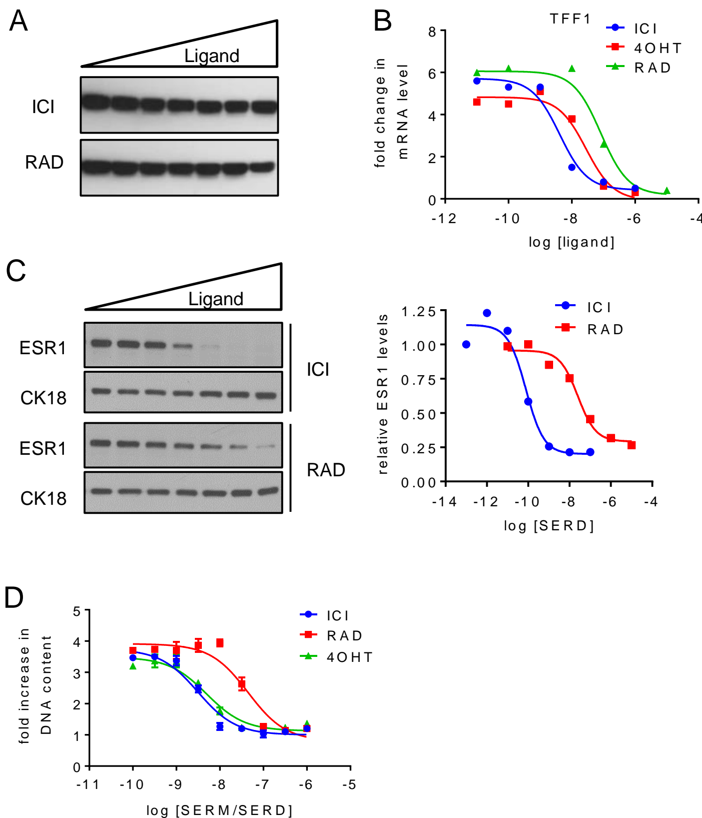

Supplemental Figure 1 The effects of RAD1901 on BT483 breast cancer cells are similar to those observed in MCF7 cells. A) Cytokeratin 18 (CK18) loading control detected for samples illustrated in Fig 2A. B) BT483 cells were plated in phenol red free media supplemented with CFS 48 hours prior to treatment with 10^{-9} M E2 together with ICI 182,780 (ICI), RAD1901 (RAD), GW7604, or 4-hydroxytamoxifen (4OHT) (10^{-11}-10^{-6} M) for 24 hours. mRNA levels of ESR1 target gene trefoil factor 1 (TFF1) were assessed using RT qPCR following RNA isolation. mRNA expression was normalized to the similarly detected 36B4 housekeeping gene, and expression levels are presented as fold change as compared to the vehicle-treated control. C) BT483 cells were treated for 24 hours with ICI (10-13-10-7 M) or RAD (10^{-11}-10^{-5} M). Expression of ESR1 and loading control cytokeratin 18 in whole cell extracts were detected by immunoblot (right). ESR1 levels relative to CK18 were quantitated by densitometry using Adobe Photoshop (left). D) BT483 cells were plated in phenol red free media supplemented with CFS 24 hours prior to treatment, and were treated with 10^{-9} M E2 as well as with the indicated ligands (10^{-11} – 10^{-6} M) on days 1, 4, and 6 of an 8 day proliferation assay. DNA content as assessed by fluorescence was measured as a surrogate for cell proliferation. The relative increase in DNA fluorescence was calculated by normalizing to baseline values detected in a duplicate plate of cells that was harvested on day 1 prior to the initial treatment. Data are representative of at least 3 independent experiments.