Supplemental Data, Fig. 2

(A) 2XERE-Luc

(B) MMP1-Luc & RARA-Luc
Supplemental Data Fig. 2. Transcriptional responses to ERα proteins from reporter systems emulating the ERE-dependent and ERE-independent signaling pathways. A. Responses from the ERE-dependent signaling pathway. HeLa cells were transiently transfected with an expression vector bearing none (V) or an ER cDNA with or without GFP sequences. Cells were co-transfected with a reporter vector bearing two consensus ERE sequences in tandem located at the upstream of the simple TATA box promoter (2XERE-Luc) or the proximal promoter from the trefoil factor 1 gene (TFF1-Luc) that contain a variant ERE. Promoters drive the expression of the Firefly luciferase cDNA as the reporter enzyme. Cells were also transfected with a reporter vector bearing the Renilla luciferase cDNA for transfection efficiency. Four-hours after transfections, cells were incubated in a fresh medium containing vehicle or $10^{-9}$ M E2, $10^{-7}$ M 4HT and/or ICI for 24h. Insets show cells treated without or with $10^{-9}$ M E2 in the absence (NL, E2) or presence of $10^{-7}$ M 4HT or ICI (E2+4HT or E2+ICI). The cell extracts were assayed for luciferase activities. The normalized (Firefly/Renilla luciferase) luciferase activities are presented as fold change compared to those observed in cells transfected with the parent vector (V) without GFP in the absence of ligand, a value that was set to one. Shown are the mean ± SEM of three independent experiments performed in duplicate. B. Transcriptional responses from the ERE-independent signaling pathway. HeLa cells were transiently transfected with an expression vector bearing none (V) or an ER cDNA with or without GFP sequences together with reporter vector bearing a fragment of the proximal promoter of the MMP1 (MMP1-Luc) or RARA (RARA-Luc) gene that drive the expression of the Firefly luciferase enzyme cDNA as the reporter. The MMP1 promoter contains one Activating Protein 1 (AP1) response element, while the proximal promoter of the RARA gene has two Stimulatory Protein 1 (SP1) response elements, or GC-boxes. Cells were also co-transfected with a reporter vector bearing the Renilla luciferase cDNA for transfection efficiency. Four-hour after transfection, cells were treated with fresh medium with or without $10^{-9}$ M E2, $10^{-7}$ M 4HT or ICI for 24h. Cell extracts were assayed for luciferase activities. The normalized luciferase values are presented as fold changes compared to those observed in cells transfected with the parent vector (V) without GFP in the absence of ligand, which was set to one. Shown are the mean ± SEM of three independent experiments performed in triplicate.