Supplemental Data, Fig. 3

A

2XERE-Luc

Luciferase Activity, Fold Change

V
GFP-V
ERβ
GFP-ERβ

B

MMP1-Luc

RARA-Luc

V
G-V
ERβ
G-β
β_{EBD}
G-β_{EBD}
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.

**Supplemental Data Fig. 3.** Transcriptional responses from reporter systems emulating the ERE-dependent and ERE-independent signaling pathways to ERβ proteins. HeLa cells were transiently transfected with an expression vector bearing none (V) or an ERβ cDNA with or without GFP sequences. Treatment and processing of cells were carried out as described in legend of Fig. 2.

**Supplemental Data, Fig. 4.** The effects of ICI on the nuclear mobility of GFP-ERα_{EBD} in HeLa cells. Transiently transfected cells for 24h were incubated in the presence of 10^{-7} M ICI for 1h. Cells were then subjected to FRAP analysis. Images were obtained before bleaching (pre-bleach, PB), at bleaching for 0.2 second (bleach, B) and at the indicated times in seconds after bleaching. The overlay image (Overlay) was generated with the superimposition of images from differential interference contrast (DIC) and fluorescence (GFP). The time-dependent equilibration of the bleached area (within the white circle) was used to estimate the recovery