Supplementary figure legends

Figure S1. Evaluation of ERβ1 expression in NSCLC cell lines.

(A) Immunoblot of ERβ1 in lysates from control, ERβ1 or ERβ2-expressing H1299 cells (clones #1 and #2) following treatment with 10 nM E2 or 10 nM 3β-Adiol. Membrane was probed with a rabbit polyclonal antibody against the C-terminus of ERβ (Invitrogen) that recognizes only the ERβ1 isoform and not the splice variant ERβ2 that has a unique C-terminal amino acid sequence. Recombinant full-length ERβ1 protein (rERβ1) was loaded as positive control. **(B)** Immunoblot of ERβ1 in lysates from control and ERβ1-expressing H1299 cells using a rabbit monoclonal antibody against the N-terminus of ERβ (clone 68-4, Millipore). The bands in two vertically sliced images correspond to protein samples run in the same gel. **(C)** ERβ1 and ERβ2 levels in control, ERβ1 or ERβ2-expressing H1299 cells following treatment with 10 nM E2. Membrane was probed with an antibody against the N-terminus of ERβ (14C8, GeneTex) that recognizes both ERβ1 and ERβ2. Notice in A and C that only ERβ1 that contains a fully functional ligand binding domain is downregulated in the presence of E2 in contrast to ERβ2 that is known to bind E2 with much lower affinity than ERβ1 (1). **(D)** Immunoblot of ERβ1 in nuclear (n) and cytoplasmic (c) extracts from control and ERβ1-expressing H1299 cells following treatment with 10 nM E2. p84 was used as a loading control for the nuclear fraction. Recombinant full-length ERβ1 protein (rERβ1) was loaded as positive control.

Figure S2. ERβ1 reduces cell proliferation in NSCLC cells.

Cell proliferation was monitored in control and ERβ1-expressing H1299 (upper panel) and H661 (bottom panel) cells following treatment with or without 10 nM E2 for the indicated times.

Figure S3. Expression of Cyclin D2 in NSCLC cells.

Immunoblot (uncropped gel image) of Cyclin D2 in lysates from control and ERβ1-expressing H1299 cells following treatment with 10 nM E2 or 10 nM 3β-Adiol.

Supplementary references

1. Hanstein B, Liu H, Yancisin MC, Brown M. Functional analysis of a novel estrogen receptor-beta isoform. Molecular endocrinology. 1999;13:129-37.