Supporting Information

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SI Methods

Cell Culture. MCF7 cells were cultured in MEM supplemented with 10% FBS in a 7% CO_2 humidified incubator. Primary normal human epithelial cells (HMEC) were from Lonza Bioproducts (CC-2651) and cultured using the media and protocol provided by the supplier. Before induction, cells of 60% confluency were hormone-deprived for 4 days in phenol-free media plus charcoal-depleted FBS, synchronized for 2 h by treating cells with 2.5 nM α-amanitin, following washing and recovery in normal stripped media (60 min), cells were then induced with 100 nM 17β-estradiol (E_2) (Sigma) for 60 min.

Single-Cell Microinjection. Single-cell antibody microinjection experiments were performed as described in ref. 27. The antibodies used are listed in Table S2. These siRNAs were purchased from Qiagen, each of which was custom-designed and validated.

Pharmacological Treatment of Cells. Actin depolymerization was induced with latrunculin A (LA), which is known to specifically cap actin monomers, whereas actin stabilization was stimulated by jasplakinolide (JP), which binds F-actin and prevents depolymerization. These drugs (gift of J. Durán and V. Malhotra) were suspended in DMSO as a 1,000× stock and applied to cultured cells at the final concentration of 1 μM as described (1). Nuclear actin was detected by using a monoclonal antibody (2G2) (Progen).

3D Assay. Ligated and unligated DNA after 3C was sonicated as in standard ChIP experiments. The DNA was annealed to a specific biotinylated capture oligonucleotides corresponding to the *TFF1* enhancer (5′-Bio-GAC, AGA, GAC, GAC, ATG, TGG, TGA, GGT, CAT, CTT, GGC, TGA, GGG) together with the oligonucleotide pool corresponding to the tiled paths. After capture, oligonucleotide ligation, selection, amplification, and hybridization were as previously described in the ChIP-DSL assay (2). Doubled blank intensity was first added to raw data to reduce low intensity bias when computing ratios. The percentile rank for each probe was determined within individual experiments and the median percentile- rank (MPR) was calculated for each probe across 4 replicates (3). The data were then smoothed using a sliding window of 10 kb and steps of 500 bp, taking the median MPR value of the probes in each window. A window was assigned a value of zero if it had <5 probes above the background to further minimize stochastic signals. Obviously, this method as designed will miss genuine, highly localized signals in favor of clusters of signals. A P value was calculated for each window by randomly assigning MPR values from a pool of all probes above background 1,000 times and counting the number of times the median value of the randomized window exceeded the experimental value. The negative log P value was plotted at each window position when $P \leq 0.05$.

3C validation was carried out with 0.25, 0.5, and 1 μL of processed DNA under fixed PCR conditions of 34 cycles for short-range interactions, 36 cycles for long-range interactions, and 30 cycles for BAC controls using a ^{32}P-labeled primer for the *TFF1* enhancer in combination with primers targeting individual genomic loci. Six BAC DNA clones covering the genomic regions around the *TFF1* locus were purchased from Invitrogen, amplified, purified, and quantified by qPCR. Equal amount of each BAC DNA was mixed, digested with BamHI and BglII, and ligated in a high volume ($\approx$200 ng/μl in a 20 mL of reaction) to promote intermolecular ligation. The processed BAC DNA was tested by qPCR to determine the linear range and then used to produce reference PCR signals for each primer pair. The products were resolved in a 10% native polyacrylamide gel, and quantified with a PhosphoImager (Molecular Dynamics).

Imaging Acquisition and Processing. The 3D-FISH was performed as described by Cremer *et al.* (4) (PROT23, Epigenome NoE) with some modification. HMECs cultured on sterile glass coverslips were synchronized with 2.5 nM α-amanitin for 2 hours and rinsed with PBS twice to remove α-amanitin. The cells were then maintained in hormone-depleted media for another 2 hours before they were stimulated with 100 nM 17β-estradiol for various durations. The cells were rinsed with PBS twice and fixed with 4% paraformaldehyde at room temperature for 10 min. During the last 3 min of fixation, add a few drops of PBS/0.5% Triton X-100 to the fixative. Then the cells were washed with PBS/0.01% Triton X-100 3 times and permeabilized with PBS/0.5%Triton X-100 for 10 min. After brief rinse in PBS, the cells were kept in PBS/20% glycerol for overnight to increase the membrane permeability. On the next day, repeated freezing in liquid nitrogen was performed. In brief, the coverslips with HMECs were frozen in liquid nitrogen for 20 seconds, allowed to thaw gradually in room temperature and soaked in PBS/20% glycerol. After washing with PBS 3 times, the cells were deproteinized in 0.1 N HCl and in 0.01 N HCl/0.002%pepsin. The cells were then mildly fixed with 1% paraformaldehyde for 3 min and rinsed with 2×SSC. For better preservation of the nuclear architecture, the cells were equilibrated in 2×SSC/50% formamide overnight at room temperature. Before setting up hybridization, the oligonucleotide probes (500 ng of each) were diluted in hybridization buffer, denatured at 80 °C for 3 minutes and kept at 37 °C for 30 min. The cells were denatured in 2×SSC/70% formamide at 73 °C for 3 minutes and immediately snap-cooled on ice. The probes were then applied to the cells and the coverslips were sealed with rubber cement. The hybridization was performed in humid chamber at 37 °C for 2 days. After that, the cells were washed sequentially with 2×SSC/50% formamide, 2×SSC, 2×SSC/0.1% Tween-20 and 2×SSC at 37 °C. To detect the oligonucleotide probes (mildly sonicated) quantum dots conjugated to streptavidin or anti-FITC antibody (Invitrogen) were applied to the cells for 2 h. The cells were then washed with 2×SSC, 2×SSC/0.1% Tween-20 and 2×SSC. To counter stain the nucleus, DRAQ5 (Biostatus) diluted in PBS (5 μM) was applied to the cells for 10 min at room temperature. After brief rinse in PBS, the coverslips were allowed to dry and mounted with Prolong Antifade Mount Medium (Invitrogen). The slides were cured at room temperature overnight before being transferred to 4 °C. The baseline of $\approx$4% is likely to represent at least in part a feature of the Q-dot- based FISH methodology.

2D FISH images were acquired with a Zeiss Axioplan 2e microscope (Carl Zeiss, Inc) and 3D images were obtained with a Nikon TE-200 DeltaVision deconvolution microscope at the UCSD Moores Cancer Center Digital Imaging Facility. The commercial Huygens software package (Scientific Volume Imaging) and the National Institutes of Health Image J package (http://rsb.info.nih.gov/ij/) were used to deconvolve optical sections, which were then merged to produce 2D or 3D pictures.

For colocalization analysis by 2D FISH, individual cells were cropped and a region of interest (ROI) was defined using the software's object analyzer tool and a precise definition of the ROI was obtained for each cell. Colocalization of signals from different channels was determined using the colocalization analyzer tool of Huygens. In each cell, a single value of Pearson's coefficient in the

refined ROI was determined after imposing a threshold value for all channels, each of which was calculated using the automatic thresholding function of the Imaris algorithm in the Huygens package. Nonspecific colocalization was identified from apparently colocalized 2D images by determining Pearson's coefficients of deconvolved stacks after subtracting background signal outside the ROI. Statistical comparison of Pearson's coefficients obtained with individual cells from multiple independent experiments was performed with a 1-tailed 2-sample *t* test using the software SSPS 14.0 for Windows.

1. Bubb MR, Spector I (1998) Use of the F-actin-binding drugs, misakinolide A and swinholide A. *Methods Enzymol* 298:26–32.
2. Kwon YS, *et al.* (2007) Sensitive ChIP-DSL technology reveals an extensive estrogen receptor alpha-binding program on human gene promoters. *Proc Natl Acad Sci, USA* 104:4852–4857.
3. Buck MJ, Lieb JD (2004). ChIP-chip: Considerations for the design, analysis, and application of genome-wide chromatin immunoprecipitation experiments. *Genomics* 83:349–360.
4. Cremer M, Weierich C, Solovei I (2005) Multicolour 3D-FISH in vertebrate cells. *Epigenome NoE* (PROT23).

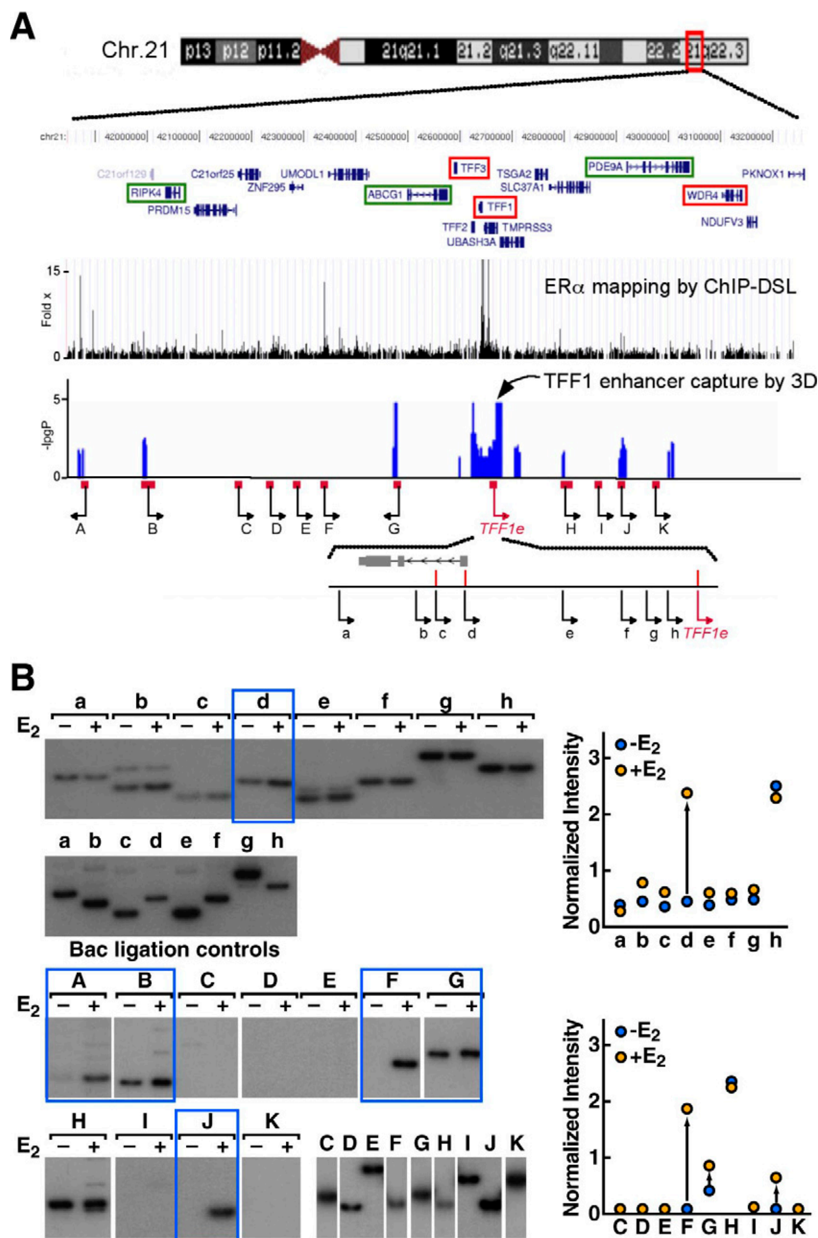

Fig. S1. Identification of long-range, estrogen-induced intrachromosomal interactions by 3D. (*A*) Plot of ERα ChIP (detected by ChIP-DSL) (1) and 3D signals in the 1.4 Mb tiled path around the *TFF1* gene. We repeated the *TFF1* enhancer capture experiments several times and used the median percentage ranking statistics to identify consistently high ranking signals (2). After data smoothing to emphasize signal clusters, we determined the probability that a given signal might be detected by chance via permutating the dataset 1,000 times. The resulting statistically significant signals are shown. (*B*) 3D signals were then validated by individual 3C assays in both mock-treated and E_2-induced MCF7 cells after titrating ligated DNA to ensure that the PCR was operating in a quantitative range (data not shown) and normalizing the 3C signals using randomly ligated BAC controls. We found that 3C validation generally matched the 3D results: Within the ≈150 kb region near the *TFF1* gene (3C probe a to h), we detected both E_2-independent background interactions due to random collisions as previously suggested (3) and the expected, E_2-dependent DNA-DNA interactions (probes b to d as highlighted by a blue box), which is consistent with the proposed looping event between the *TFF1* promoter and enhancer (4). Interestingly, we also detected the long-distance interaction of the *TFF1* enhancer with multiple discrete loci, several of which corresponded to the intergenic ERα binding sites mapped by ChIP-DSL (e.g., 3C probe A, B, H and J). Multiple 3D-negative regions, included as controls (C–E, I, K), gave no 3C signals, whereas the interaction of the *TFF1* enhancer with locus A, B, F, and J (highlighted in blue boxes) was E_2-inducible and that with G and H was either modestly induced by E_2 or ''constitutive.'' Randomly ligated DNA from a pool of 4 BACs corresponding to the tiled region was used to normalize PCR efficiency of individual primer pairs. Note that the BAC control for probe A and B were missing because the region is not present in the 4 BACs.

1. Kwon YS, *et al.* (2007) Sensitive ChIP-DSL technology reveals an extensive estrogen receptor alpha-binding program on human gene promoters. *Proc Natl Acad Sci, USA* 104:4852–4857.
2. Buck MJ, Lieb JD (2004). ChIP-chip: Considerations for the design, analysis, and application of genome-wide chromatin immunoprecipitation experiments. *Genomics* 83:349–360.
3. Dekker J (2006) The three ''C''s of chromosome conformation capture: Controls, controls, controls. *Nat Methods* 3:17–21.
4. Carroll JS, *et al.* (2005) Chromosome-wide mapping of estrogen receptor binding reveals long-range regulation requiring the forkhead protein FoxA1. *Cell* 122:33–43.

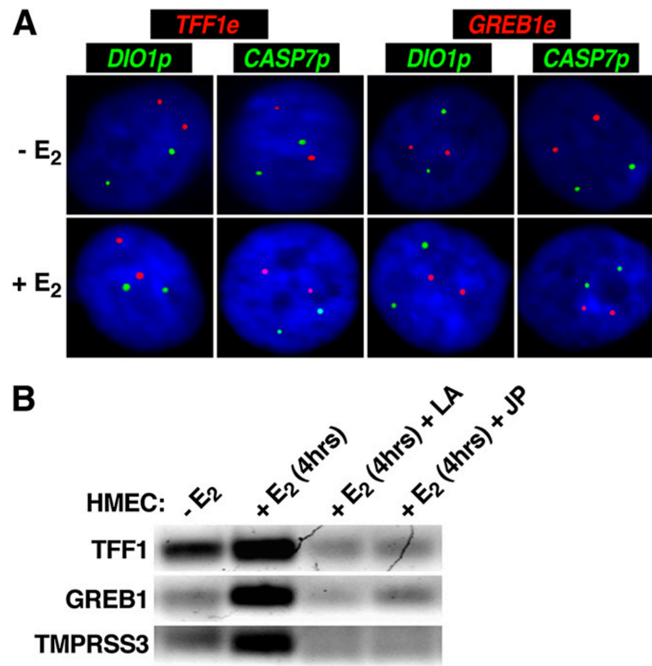

Fig. S2. Selective Chr2:Chr21 interactions in response to E_2. (*A*) Two-color FISH showing the lack of colocalization of both alleles of *DIO1* and *CASP7* with *TFF1* before and after E_2-treatment. (*B*) Chemical disruption and stabilization of actin of E_2-induced (60′) interactions by treatment with latrunculin (LA) and jasplakinolide (JP), respectively. Both drug treatments prevented E_2-induced expression of 3 ERα target genes (*TFF1, GREB1*, *TMPRSS3*), suggesting that dynamics of nuclear actin is required for E_2-induced gene expression.

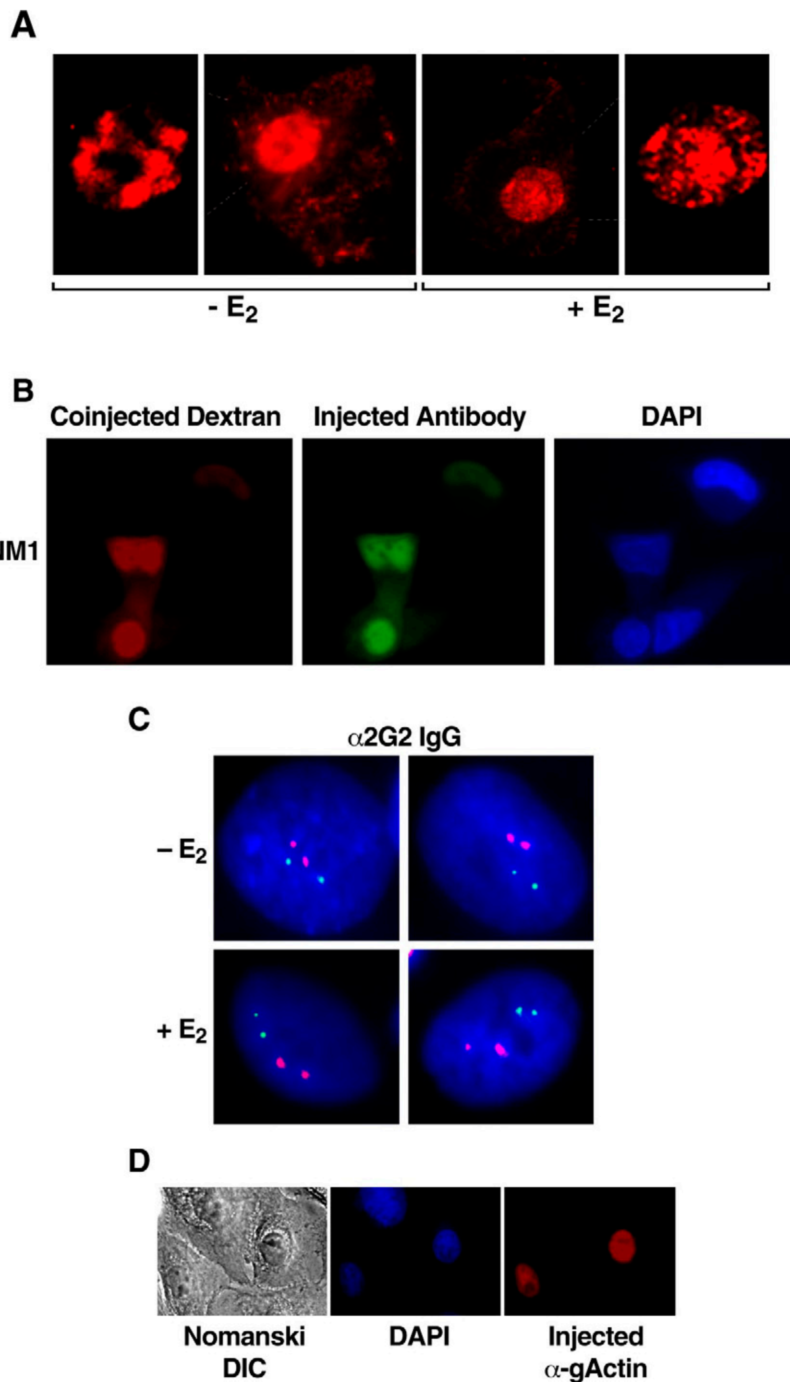

Fig. S3. Nuclear microinjection controls. (*A*) Cells stained with nuclear actin antibody 2G2 before and after E_2 treatment. Each paired panel show higher and lower magnifications to appreciate the prominent nuclear staining, which does not seem to be affected by the hormone treatment. (*B*) Verification of nuclear delivery of antibody by microinjection. The dextran marker was coinjected with the antibody. The first panel shows representation of nuclear staining of a Texas Red-conjugated dextran, while the second panel shows nuclear stain of the injected antibodies (anti-nuclear myosin *I*), which were FITC conjugated. The last panel shows DAPI staining in blue, indicating cells within the field. (*C*) Single cell nuclear microinjection of purified monoclonal 2G2 antibody against g-actin prevented *TFF1*/*GREB1* interchromosomal interactions. These results show that nuclear actin is required for E_2-induced interchromosomal interactions, consistent with the effect of pharmacological disruption by LA and JP. (*D*) Verification of nuclear delivery of 2G2 IgG by microinjection.

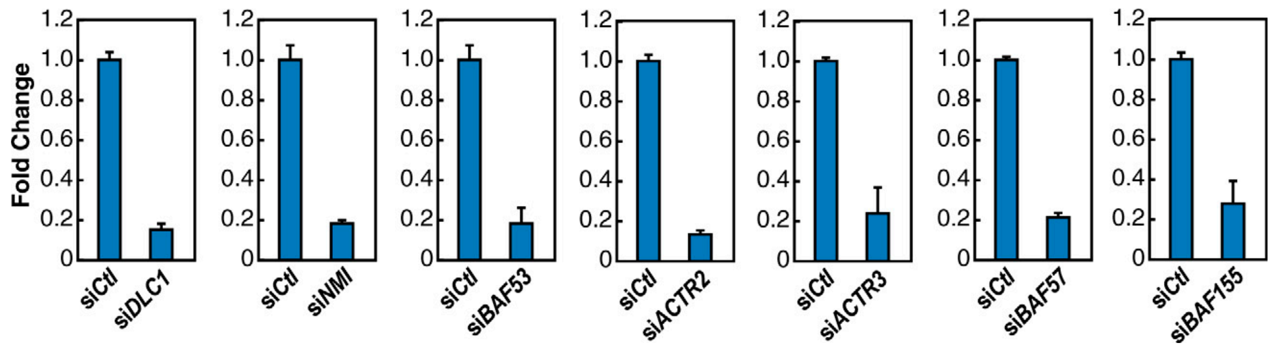

Fig. S4. Validation of siRNA-mediated knockdown. These experiments were performed on E2-induced MCF7 cells transfected with specific siRNAs. Specific transcripts were quantified by real time RT-PCR.

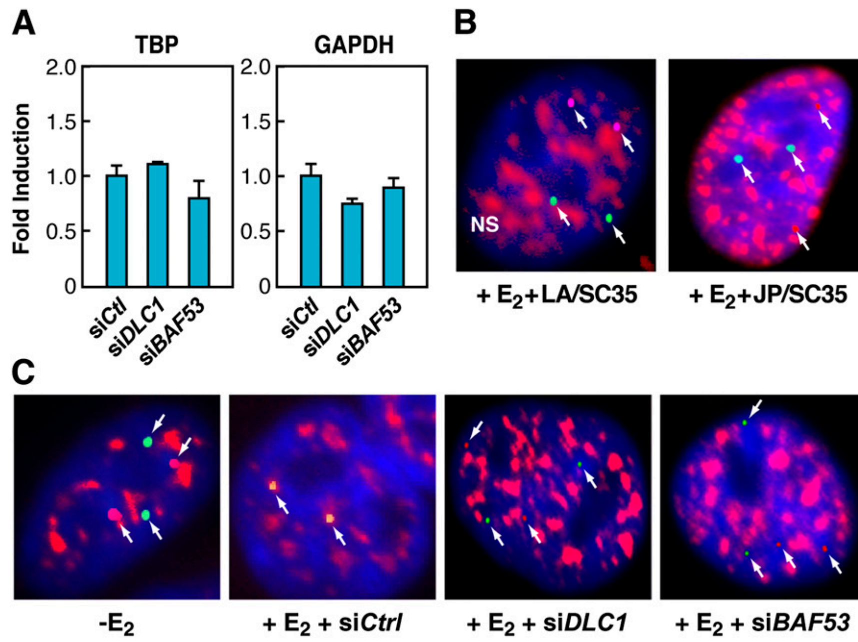

Fig. S5. Role of nucleoskeletal components in E_2-induced interchromosomal interactions and association with interchromatin granules. (*A*) Effect of *DLC1* and *BAF5*3 siRNAs (48h) on *TBP* and *GAPDH* expression in MCF7 cells. (*B*) LA and JP prevented both specific interchromosomal interaction and association with interchromatin granules (IGCs). (*C*) Knockdown of *DLC1 or BAF53* also prevented *TFF1:GREB1* interchromosomal interaction and their association with interchromatin granules.

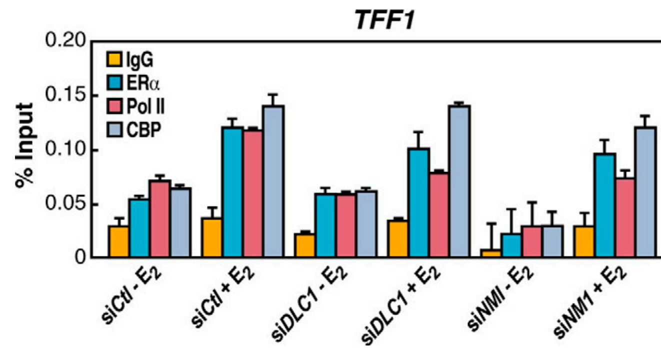

Fig. S6. ChIP analysis of binding of ERα, Pol II, and CBP in MCF7 cells treated for 60 min with E_2 (10^{-7}M) in the presence or absence of control *DLC1* or *NMI* siRNAs (48h). Results are based on independent triplicates. (± SD).

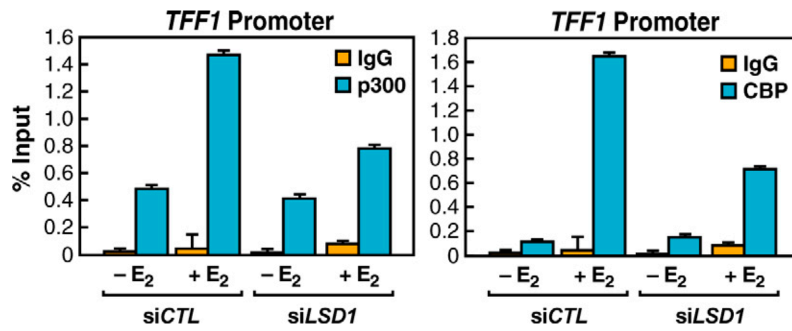

Fig. S7. ChIP assay on the *TFF1* promoter, demonstrating a decreased, but still clearly inducible, recruitment of ERα coactivators CBP/p300 in cells treated with siRNA against *LSD1* (± SD).

Other Supporting Information Files

Table S1
Table S2
Table S3