Supplementary Information

"Distinct Roles for BET Family Members in Estrogen Receptor α Enhancer Function and Gene Regulation in Breast Cancer Cells"

Murakami *et al.* (2019)

This document contains the following supplementary information: Page

1) Supplementary Figures

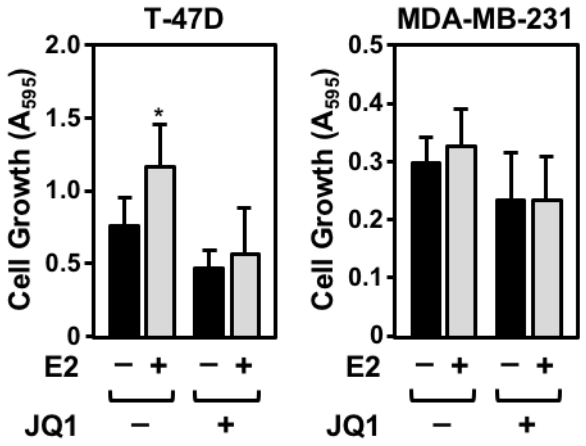

Figure S1. The activity of BET family members is required for the growth of T-47D cells. JQ1 attenuates the E2-depedent growth of T-47D cells *(left)*, but not MDA-MB-231 cells *(right)*. The proliferation of ER-positive T-47D human breast cancer cells and ER-negative MDA-MB-231 human breast cancer cells ± E2 treatment and ± 100 nM JQ1 was determined using an MTT assay. Asterisks indicate significant differences versus corresponding controls ($p < 0.05$; two-way ANOVA).

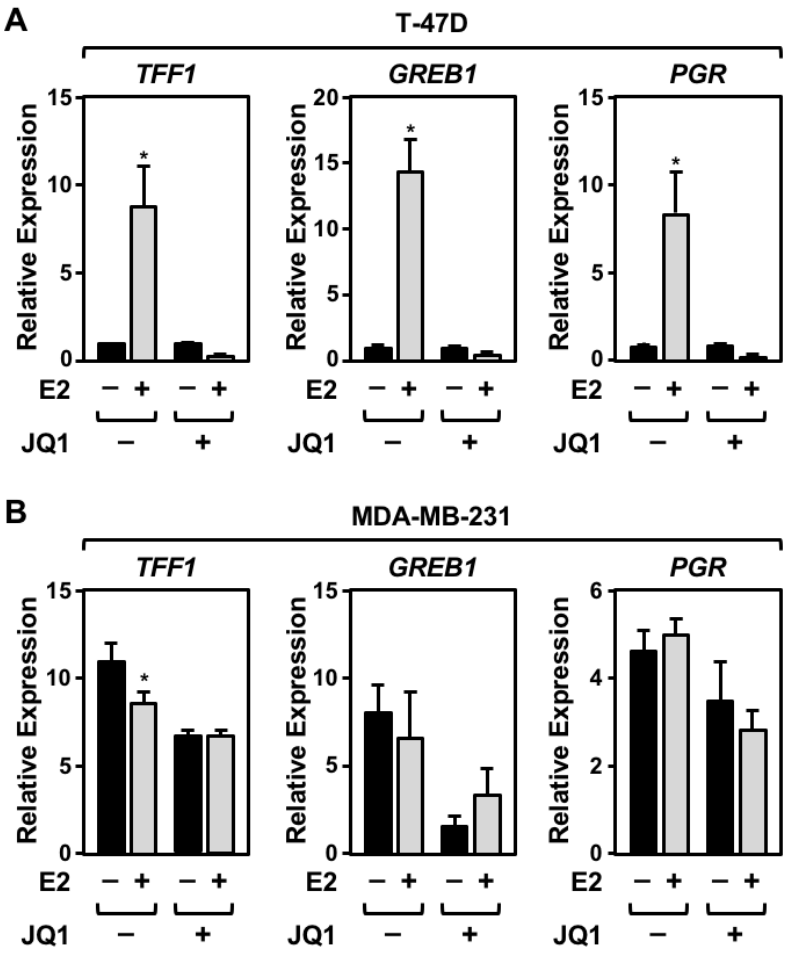

Figure S2. BET proteins are required for E2-dependent gene expression in T-47D cells. JQ1 impairs E2-dependent gene induction in T-47D cells *(top)* but not in MDA-MB-231 cells *(bottom)*. The expression of classical E2-induced genes in the presence of JQ1 were determined by RT-qPCR. Asterisks indicate significant differences ($p < 0.05$; two-way ANOVA).

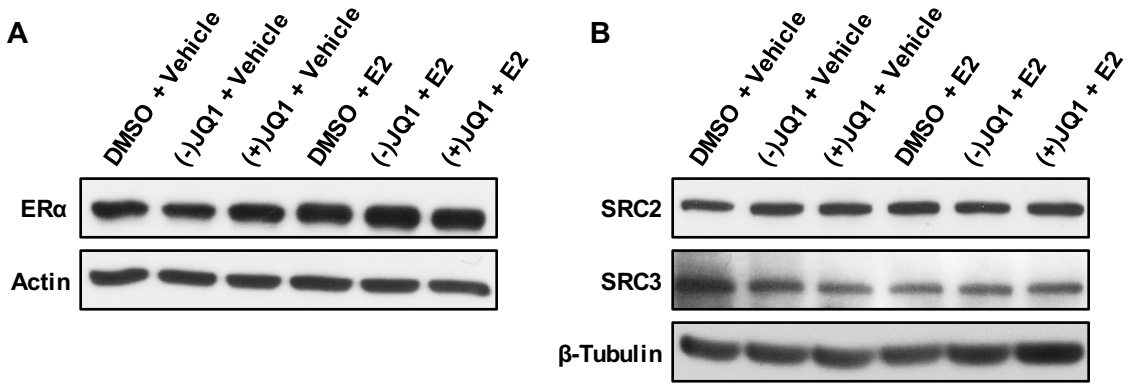

Figure S3. JQ1 does not affect the expression of ERα, SRC2, and SRC3 in MCF-7 cells. Western blot showing the protein levels of ERα, SRC2, and SRC3 in MCF-7 cells treated with E2 for 45 min. in the absence or presence of JQ1.

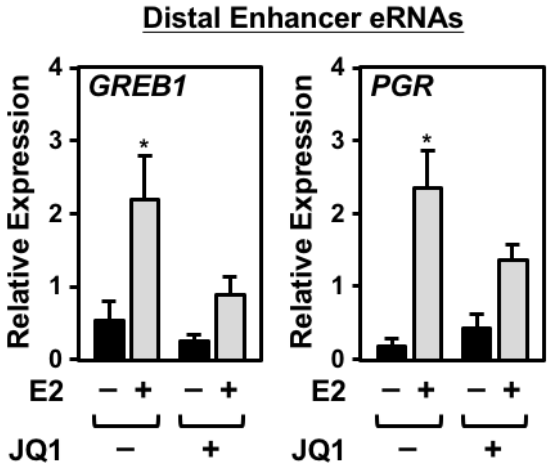

Figure S4. JQ1 inhibits the E2-dependent expression of enhancer RNAs (eRNAs) in MCF-7 cells.

The expression of eRNAs from ERα enhancers ±E2 and ± JQ1 was determined in MCF-7 cells by RT-qPCR. Asterisks indicate significant differences ($p < 0.05$; two-way ANOVA).

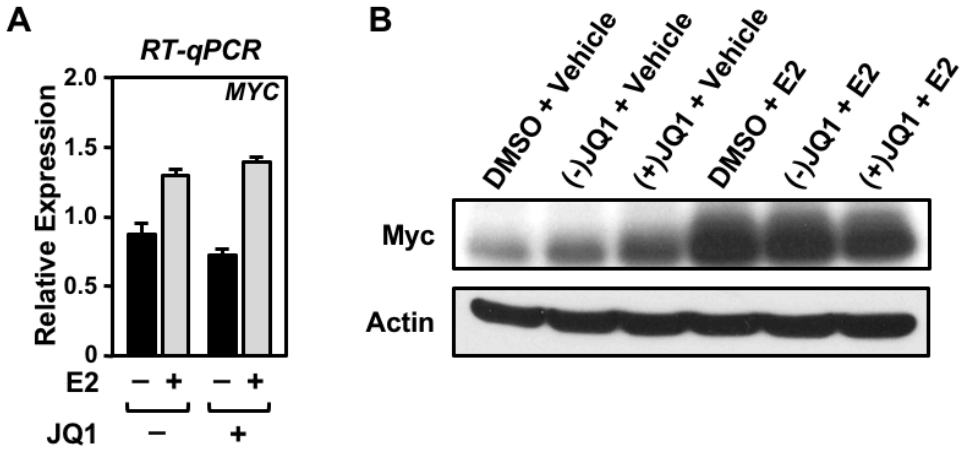

Figure S5. E2-dependent Myc upregulation is not affected by JQ1.
(A) RT-qPCR assays showing *MYC* expression at RNA levels upon E2 treatment in the presence of JQ1.
(B) Western blot showing MYC protein levels in MCF-7 cells treated with ± E2 for 6 hours in the presence of JQ1.

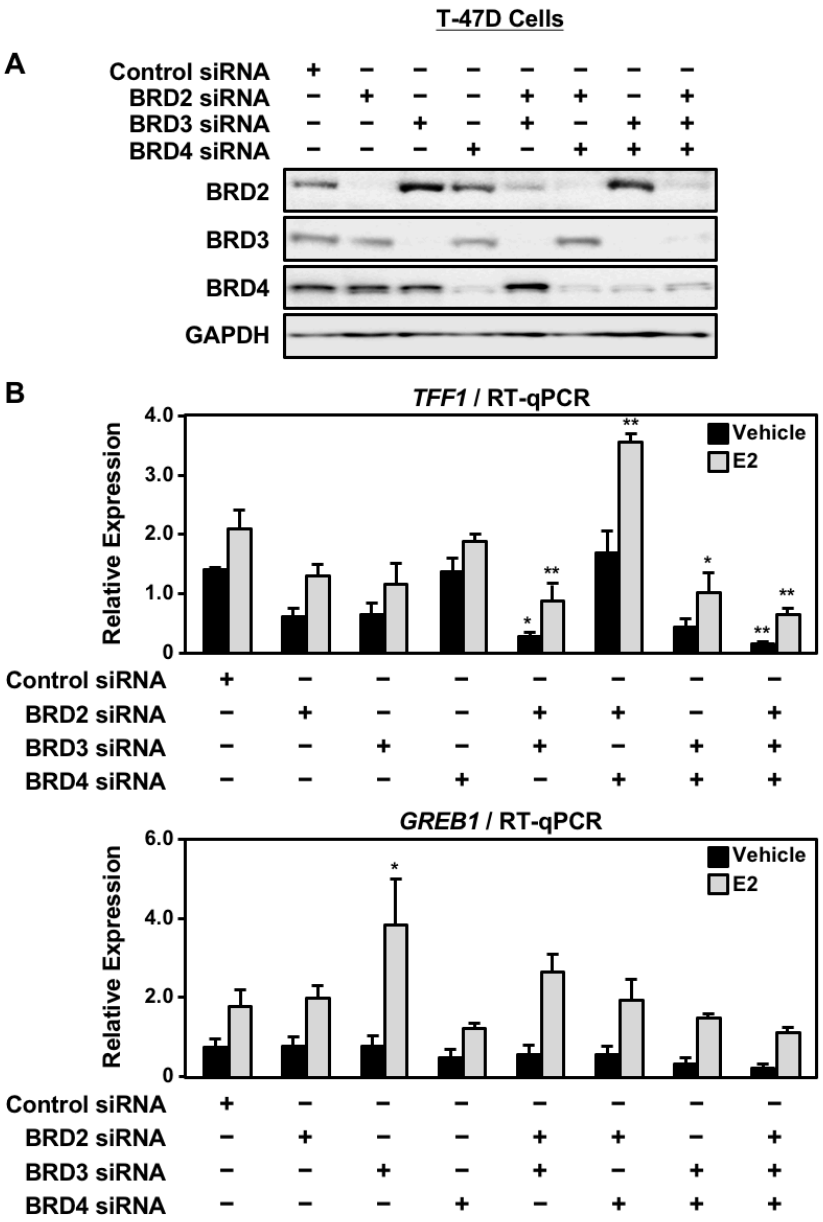

Figure S6. BET proteins are critical regulators of E2-dependent gene expression in T-47D cells.

(A) Western blot showing siRNA-mediated knockdown of BRD2, BRD3, and BRD4 individually or in combination in T-47D cells.

(B) The E2-induced expression of *TFF1*, but not *GREB1*, is attenuated when BRD2 is depleted in combination with BRD3 or both BRD3 and BRD4. E2-dependent gene expression determined by RT-qPCR after depletion of BET family proteins by siRNA individually or in combination, as indicated. Asterisks indicate significant differences compared to the control siRNA under each treatment condition (*, $p < 0.05$; **, $p < 0.005$; two-way ANOVA).

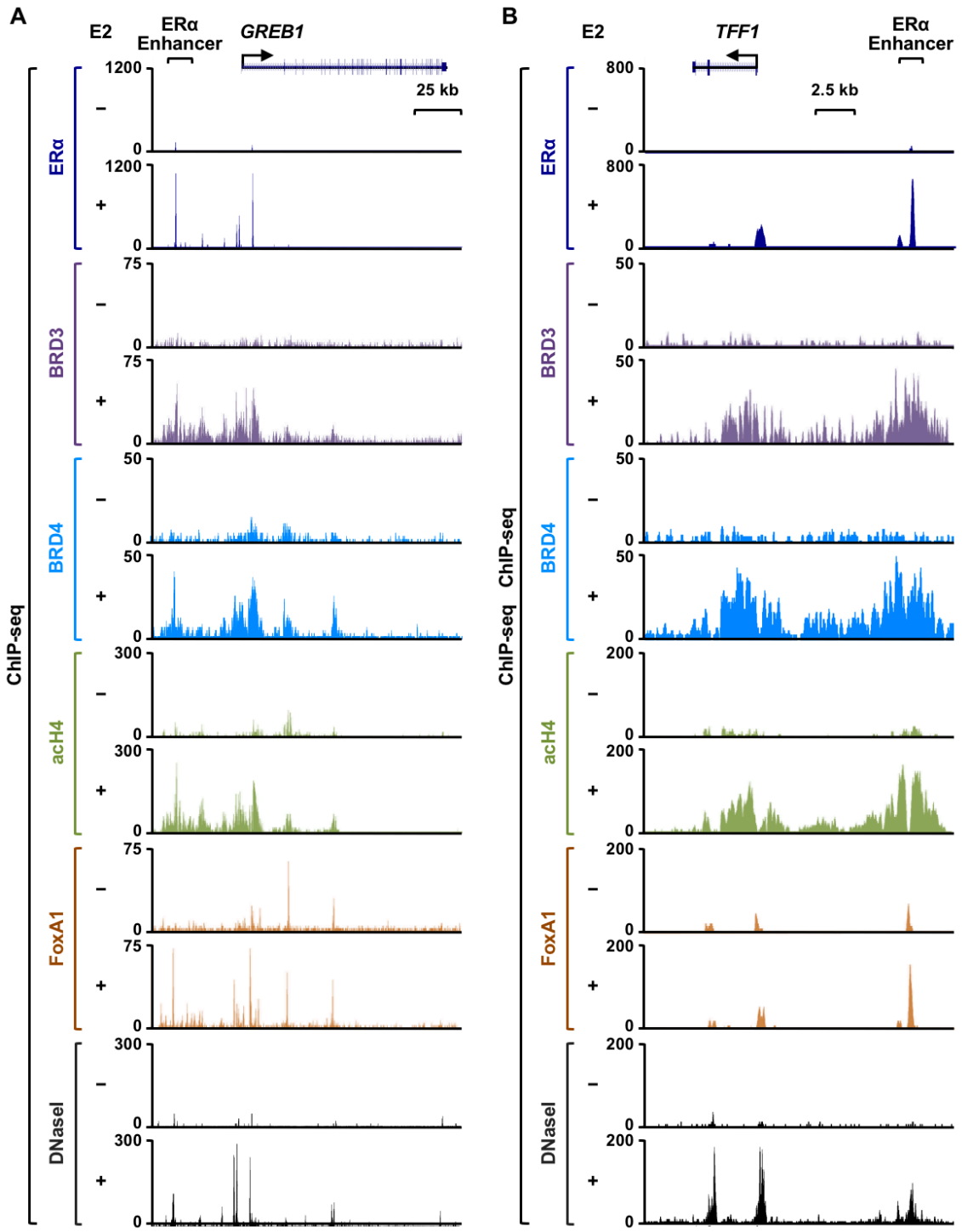

Figure S7. BRD3 is enriched at a subset of ERBSs.
Genome browser tracks of ERα, BRD3, acetyl H4, and FoxA1 ChIP-seq and DNase-seq datasets at *GREB1*(A) and *TFF1* (B) loci representing ERBSs with BRD3 enrichment.

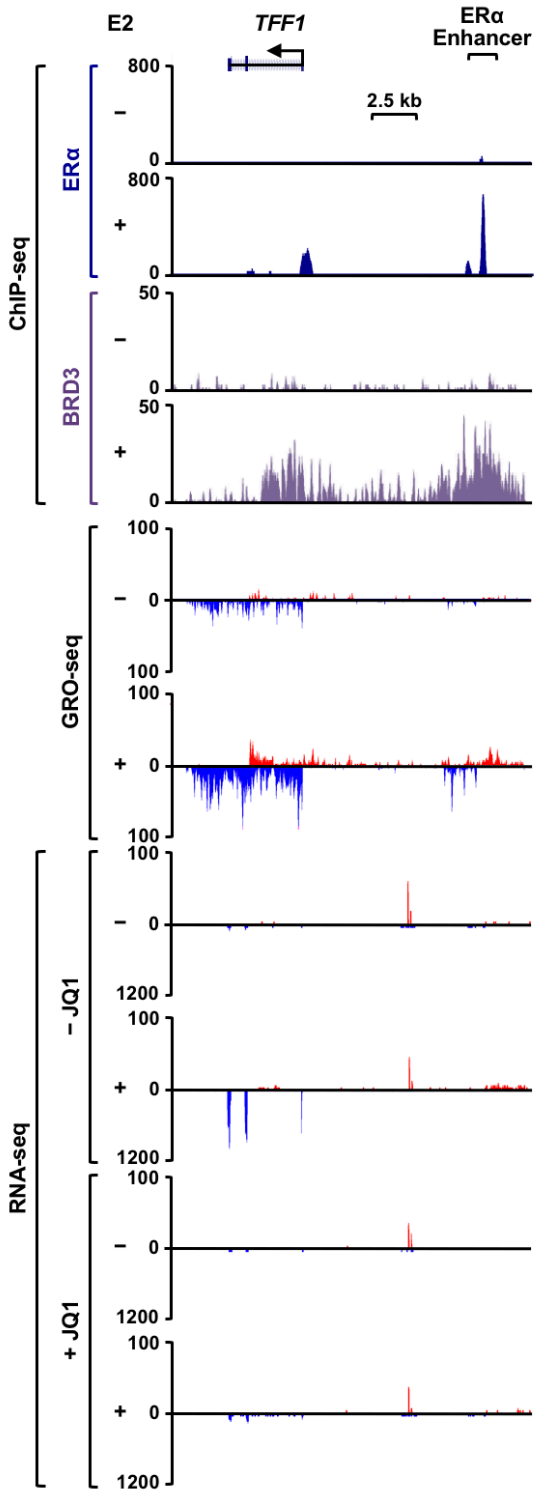

Figure S8. E2-induced genes affected by JQ1 are associated with ERα binding sites enriched with BRD3.
Genome browser tracks of ChIP-seq for ERα and BRD3, GRO-seq, and RNA-seq data at *TFF1* locus representing ERBSs with BRD3 enrichment.

2) Supplementary Table

Table S1. Gene ontologies associated with E2 up-regulated genes repressed by JQ1.*

Category	Term	p-value
GOTERM_BP_FAT	GO:0009719 response to endogenous stimulus	1.2E-5
GOTERM_BP_FAT	GO:0009725 response to hormone	2.5E-5
GOTERM_BP_FAT	GO:0001568 blood vessel development	1.9E-4
GOTERM_BP_FAT	GO:0001944 vasculature development	2.2E-4
GOTERM_MF_FAT	GO:0008092 cytoskeletal protein binding	5.8E-4
GOTERM_BP_FAT	GO:0042445 hormone metabolic process	6.3E-4
GOTERM_BP_FAT	GO:0010033 response to organic substance	2.4E-3
GOTERM_BP_FAT	GO:0048514 blood vessel morphogenesis	2.5E-3
GOTERM_BP_FAT	GO:0010817 regulation of hormone levels	3.0E-3
GOTERM_BP_FAT	GO:0043434 response to peptide hormone	3.3E-3

* Repression >2 fold

Gene ontology analysis of E2-upregulated genes whose expression is repressed by JQ1. E2-induced genes that are sensitive to JQ1 are enriched for terms related to hormone-dependent signaling including hormone response, hormone metabolism, and hormone regulation. GO terms with the 10 smallest p-values are listed.

3) Supplementary Materials and Methods

Materials and Methods

Antibodies

The antibodies used were as follows: ERα (rabbit polyclonal antiserum generated in the Kraus Laboratory against the first 113 amino acids of human ERα (1), BRD2 (Bethyl Laboratories, A302-583A), BRD3 (Bethyl Laboratories, A302-368A), BRD4 (Bethyl Laboratories, A301-985A), pan-acetyl H4 (Millipore, 06-866), Myc (Invitrogen, 13-2500), SRC2 (Santa Cruz Biotechnology, sc-8996), SRC3 (Santa Cruz Biotechnology, sc-7216), SNRP70 (Abcam, ab83306), β-tubulin (Abcam, ab6046).

Cell Culture and Treatments

MCF-7 cells, kindly provided by Dr. Benita Katzenellenbogen (University of Illinois, Urbana-Champaign, IL), were maintained in Minimum Essential Medium (MEM) Eagle with Hank's salts (Sigma, M1018) supplemented with 5% HyClone calf serum (GE Healthcare, SH30072) and 20mM HEPES (ThermoFisher Scientific, BP310). Prior to gene expression and ChIP experiments, the cells were grown for three days in phenol red-free MEM Eagle medium supplemented with 5% charcoal-dextran-treated calf serum. T-47D and MDA-MB-231 cells were purchased from the ATCC. T-47D cells were maintained in RPMI1640 Medium (ThermoFisher, 11875093) supplemented with 10% fetal bovine serum (FBS; Atlanta Biologicals) and 7 μg/mL insulin. MDA-MB-231 cells were maintained in Dulbecco's Modified Eagle Medium (DMEM; ThermoFisher, 11995065) supplemented with 10% FBS. Prior to cell proliferation, knockdown, and gene expression assays, the T-47D and MDA-MB-231 cells were grown for three days in phenol red-free medium supplemented with 10% charcoal-dextran-treated FBS. 293T cells were purchased from the ATCC (CRL-3216) and maintained in high glucose Dulbecco's Modified Eagle's Medium (Sigma, D7777) supplemented with 10% fetal bovine serum. All cells were validated by genotypic and phenotypic (e.g., ERα expression) assays, and were routinely verified as mycoplasma-free using a PCR-based test.

Treatment conditions for the cells were as follows: 17β-estradiol (E2), 100 nM (Sigma, E8875); (+)JQ1 (the active enantiomer of JQ1, referred to herein as JQ1), 500 nM unless otherwise indicated (Cayman Chemical, 11187); and (-)JQ1 (the inactive enantiomer of JQ1), at the same concentrations as (+)JQ1 (Cayman Chemical, 11232). The cells were treated with (+)JQ1 or (-)JQ1 for 3 hours before treatment with E2. For gene expression analyses, the cells were collected after 3 hours of E2 treatment. For ChIP analyses, the cells were collected after 45 minutes of E2 treatment.

Cell Proliferation Assays

MCF-7 cells were plated at a density of 4 x 10^4 cells per well in six well plates in standard grown medium and then switched to phenol red-free MEM Eagle medium supplemented with 5% charcoal-dextran-treated calf serum after attachment. The following day, the cells were treated with ethanol vehicle (Veh), E2 (100 nM), (+)JQ1 (62.5 or 250 nM), or both E2 + (+)JQ1, with fresh treatments added every 2 days. At selected time points over a six-day time course, the cells were fixed with 10% formaldehyde and stained with 0.1% crystal violet and 200 mM phosphoric acid. After washing away unincorporated stain, the crystal violet was extracted using 10% glacial acetic acid and the absorbance was read at 595 nm. The cell

proliferation assays were run a minimum of three times with independent biological samples to ensure reproducibility.

T47D and MDA-MB-231 cells were plated at a density of 2.5 x 10^3 and 1.255 x 10^3 cells per well, respectively, in 96-well plates in standard growth medium and then switched to phenol red-free RPMI1640 medium or DMEM supplemented with 10% charcoal-dextran-treated FBS, respectively, after attachment. The following day, the cells were treated days with ethanol vehicle (Veh), E2 (100 nM), (+)JQ1 (100 nM), or both E2 + (+)JQ1, with fresh treatments added every two days for six days. On day six, the medium was replaced with a 1:1 mix of 5 mg/mL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) in phosphate buffered saline (PBS) and serum-free medium. The cells were then incubated at 37°C at 5% CO_2 for 3 hours. Formazan produced from MTT during the incubation was extracted by addition of MTT solvent (4 mM HCl, 0.1% NP40 in isopropanol) and the absorbance was read at 595 nm. The cell proliferation assays were run a minimum of three times with independent biological samples to ensure reproducibility.

siRNA-mediated Knockdown of BRD2, BRD3, and BRD4

MCF-7 cells were grown to 75% confluence in six well plates. The cells were then transfected with commercially available siRNA oligos directed against BRD2, BRD3 or BRD4 (Sigma) at a final concentration of 5 nM using Lipofectamine RNAiMAX reagent (Invitrogen, 13778150) per the manufacturer's instructions. MISSION universal negative control #2 (Sigma, SIC002) was used as a control siRNA. Treatments with E2 were performed 48 hours after siRNA transfection. The siRNA sequences are as follows:

- siBRD2 5'-GTTACAAGATGTCAGCGGA-3'
- siBRD3 5'-CCAAGGAAATGTCTCGGAT-3'
- siBRD4 5'-CTGGAATGCTCAGGAATGT-3'

Inducible Expression of BRD3

The lentiviral system for inducible expression of BRD3 is based on pINDUCER20 (2), which was kindly provided by Dr. Thomas Westbrook (Baylor College of Medicine, Houston, TX). The "gateway cloning region" of the original pINDUCER20 vector was replaced with a multi-cloning site. The human BRD3 cDNA was cloned by reverse transcription-PCR from MCF-7 cell total RNA and then transferred into the modified pINDUCER20 vector with the addition of a sequence encoding an HA tag at the 3' end of the cDNA (for tagging of BRD3 at the carboxyl terminal end). The BRD3 cDNA was confirmed by sequencing.

The pINDUCER20-BRD3-HA plasmid was co-transfected with pCMV-VSVG, pCMV-GAG-pol-Rev, and pAdVantage (Promega, E1711) into 293T cells using GeneJuice (Millipore, 70967) for recombinant lentivirus production. The supernatant containing the lentiviruses was collected 48 hours after transfection and used to infect MCF-7 cells. The infected MCF-7 cells were selected and maintained in 1 mg/ml Geneticin (Life Technologies, 11811031). For induction of BRD3 expression, doxycycline hyclate (Sigma, D9891) was added to the medium at a final concentration 50 ng/mL. Twenty-four hours later, the cells were collected for Western blotting or RT-qPCR.

Kaplan-Meier Analyses

Kaplan-Meier estimators (3,4) were generated using the Gene Expression-Based Outcome for Breast Cancer Online (GOBO) tool (http://co.bmc.lu.se/gobo/) (5). GOBO is a

multifunctional online tool that "allows a range of different analyses to be performed in an 1,881-sample breast tumor data set, and a 51-sample breast cancer cell line set, both generated using Affymetrix U133A microarrays" (5). In our analyses, we profiled gene expression in 560 ER-positive breast cancer samples. BRD2, BRD3, and BRD4 were provided as the input gene set to assess patient outcomes in ER-positive breast cancers. Patients were stratified into two groups based on the expression levels of BRD2, BRD3, and BRD4 (top half = high expression; bottom half = low expression). The survival outcomes were then determined by Kaplan-Meier estimators within the GOBO tool.

Immunohistochemistry (IHC)

Immunohistochemical staining of patient samples were adapted from the Cancer Atlas of the Human Protein Atlas database, version 15 (www.proteinatlas.org). The antibodies used for IHC were as follows: BRD2 (HPA042816); BRD3 (HPA051830); BRD4 (CAB068177). The direct links to the original datasets are as follows:

BRD2: http://www.proteinatlas.org/ENSG00000204256-BRD2/cancer/tissue/breast+cancer
BRD3: http://www.proteinatlas.org/ENSG00000169925-BRD3/cancer/tissue/breast+cancer
BRD4: http://www.proteinatlas.org/ENSG00000141867-BRD4/cancer/tissue/breast+cancer

Western Blotting

Protein lysates from MCF-7 or T-47D cells were prepared using lysis buffer [20 mM HEPES (pH 7.5), 420 mM NaCl, 1.5 mM $MgCl_2$, 0.2mM EDTA, 25% Glycerol, 0.5% NP-40, 1 mM DTT, 1x complete protease inhibitor cocktail (Roche, 11697498001)], and SDS loading samples were prepared using the normalize amount of total protein. Protein expression was assessed by Western blotting with the antibodies stated above. The signals were developed using a chemilumenescent detection system. (ThermoFisher Scientific, 34080/34095).

mRNA and eRNA Expression Analysis by Quantitative Real-time PCR (RT-qPCR)

Changes in the steady-state levels of target gene mRNAs or enhancer RNAs (eRNAs) were analyzed by RT-qPCR, as described previously (6,7) with a few modifications. MCF-7 cells or T-47D cells were grown in six well plates and treated as described above (± 3 hours with 100 nM E2; ± 3 hour pretreatment with 500 nM JQ1). The cells were collected and total RNA was isolated using TRIzol reagent (Invitrogen, 15596) according to the manufacturer's protocol. Two micrograms of total RNA were reverse-transcribed using oligo(dT) or random hexamers using MMLV reverse transcriptase (Promega, M1701) according to the manufacturer's protocol. The resulting cDNA was analyzed by qPCR using the primer sets listed below with a LightCycler 480 real-time PCR thermocycler (Roche) for 45 cycles. The expression levels were normalized to 18S ribosomal RNA or *ACTB* mRNA as internal standards. All experiments were conducted a minimum of three times with independent RNA isolations to ensure reproducibility.

- *TFF1* mRNA Forward 5'-TTGTGGTTTTCCTGGTGTCA-3'
- *TFF1* mRNA Reverse 5'-GCAGATCCCTGCAGAAGTGT-3'
- *GREB1* mRNA Forward 5'-AAACATCAGCTGCTCGGACT-3'
- *GREB1* mRNA Reverse 5'-CCTGACAGATGACACACAACG-3'
- *GREB1* mRNA Forward.2 5'-CCTATTTTGGAATAAAAACTGACC-3'
- *GREB1* mRNA Reverse.2 5'-GGGGAGAATGACACAAAAGC-3'

• *PGR* mRNA Forward	5'-GACAGAAGCCTCCAGCACAT-3'
• *PGR* mRNA Reverse	5'-GGCATTTGTCCTCTTTGCCC-3'
• *MYC* mRNA Forward	5'-TCGGATTCTCTGCTCTCCTC-3'
• *MYC* mRNA Reverse	5'-CCTGCCTCTTTTCCACAGAA-3'
• *GREB1* eRNA Forward	5'-GGGAATAGAGCCCTGAGCTT-3'
• *GREB1* eRNA Reverse	5'-TTGATCTGCTCTTGCCTGAA-3'
• *PGR* eRNA Forward	5'-ATGCAGAGCCATTGCAAAAT-3'
• *PGR* eRNA Reverse	5'-ATCAGCAAGATGCAAACACG-3'
• 18S ribosomal RNA Forward	5'-TACCACATCCAAGGAAGGCAGCA-3'
• 18S ribosomal RNA Reverse	5'-TGGAATTACCGCGGCTGCTGGCA-3'
• *ACTB* mRNA Forward	5'-AGCTACGAGCTGCCTGAC-3'
• *ACTB* mRNA Rev	5'-AAGGTAGTTTCGTGGATGC-3'

Preparation of polyA+ RNA-seq Libraries

RNA-seq libraries were prepared as described previously (8). Briefly, MCF-7 cells were grown in 10 cm diameter dish to the density of 8.8 x 10^6 cells and treated as described above (± 3 hours with 100 nM E2; ± 3 hour pretreatment with 500 nM JQ1). The cells were collected and total RNA was isolated using the RNeasy Plus kit (Qiagen, 74134). PolyA+ RNA was purified from the total RNA using Dynabeads Oligo(dT)25 (Life Technologies, 61002). Strand-specific libraries were prepared according to the "deoxyuridine triphosphate (dUTP)" method, as described previously (8). After quality control analyses, the libraries were sequenced on an Illumina HiSeq 2000 (single-end sequencing, 50 nt).

Analysis of RNA-seq Data

Quality of RNA-seq reads was assessed using the FastQC tool (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). The RNA-seq reads were aligned using TopHat v2.0.10 (9) on the hg19 reference genome. Using aligned reads as input, we used Cufflinks v.2.1.1 (10) and Cuffdiff v.2.1.1 (11) to assemble the reads into transcripts using RefSeq annotations and to call differentially regulated transcripts, respectively. All programs were run with default parameters. Uniquely mapped reads were visualized on the UCSC genome browser as bigWig files generated using BETtools (12). Expression of differentially expressed genes was visualized as heatmaps using Java Tree View (13). Box plots were generated using the box plot function in R. The read counts +1 to +13 kb from the 5' end of each gene were visualized in box plots as Reads Per Kilobase of gene per Million mapped reads (RPKM). The statistical analysis on the effect of JQ1 upon E2 treatment for all E2-regulated genes was performed by custom R scripts using 100 genes randomly chosen from each category.

Analysis of GRO-seq Data

GRO-seq data was analyzed as previously described (14). The GRO-seq reads surrounding ± 2.5 kb of center of the ERα peaks or surrounding the 5' end of regulated genes nearest to the ERα peaks were visualized in box plots as Reads Per Kilobase of gene per Million mapped reads (RPKM) using box plot function in R.

Gene Ontology (GO) Analyses

Gene ontology analyses were performed using DAVID (Database for Annotation, Visualization, and Integrated Discovery) (15). As input, the list of genes expressed in MCF-7 at

least in one condition tested was used as a background. DAVID returns clusters of related ontological terms that are ranked according to an enrichment score. We listed the top term in each cluster (based on p-value) from the top ten clusters (based on enrichment score). The statistical analysis on E2-dependent gene transcription regulated by ERα enhancer with or without BRD3 enrichment was performed using 100 genes or ERα binding sties for eRNAs randomly chosen from each category.

Chromatin Immunoprecipitation (ChIP)

ChIP was performed as previously described (6,7) with a few modifications. MCF-7 cells were plated at a density of 2.5 x 10^6 cells in a 15 cm diameter dish, grown for three days in estrogen-free medium, and treated as described above (± 45 minutes with 100 nM E2; ± 3 hour pretreatment with 500 nM JQ1). The cells were cross-linked with 1% formaldehyde for 10 min at 37ºC and quenched in 125 mM glycine for 5 min at 4ºC. The cells were then collected and lysed in Farnham lysis buffer [5 mM PIPES pH 8.0, 85 mM KCl, 0.5% NP-40, 1 mM DTT, and 1x complete protease inhibitor cocktail (Roche, 11697498001)]. For acetyl-histone H4 ChIP assays, 10 mM sodium butyrate was added to all buffers to prevent deacetylation of the histones. The crude nuclear pellet was collected by centrifugation, resuspended in lysis buffer [Tris-HCl pH 7.9, 1% SDS, 10 mM EDTA, 50 mM, 1 mM DTT, and 1x complete protease inhibitor cocktail], and incubated on ice for 10 minutes. The chromatin was sheared by sonication at 4°C using a Bioruptor UC200 at the highest setting for five 5-minute cycles of 30 seconds on and 60 seconds off to generate chromatin fragments of ~200-400 bp in length. The soluble chromatin was diluted 1:10 with dilution buffer [20 mM Tris-HCl pH 7.9, 0.5% Triton X-100, 2 mM EDTA, 150 mM NaCl, 1 mM DTT and 1x complete protease inhibitor cocktail] and pre-cleared with protein A agarose beads. Five percent of the material was removed and saved as input, and the rest of the pre-cleared supernatant was incubated overnight at 4ºC with the specified antibodies of interest or without antibody as a control (each 15 cm diameter dish yielded two immunoprecipitations) with continuous mixing.

After the incubation, the immune complexes were collected by adding protein A agarose beads and incubating for 2 hours at 4°C. The immunoprecipitated material was washed once with low salt wash buffer [20 mM Tris-HCl pH 7.9, 2 mM EDTA, 125 mM NaCl, 0.05% SDS, 1% Triton X-100, and 1x complete protease inhibitor cocktail], once with high-salt wash buffer [20 mM Tris-HCl pH 7.9, 2 mM EDTA, 500 mM NaCl, 0.05% SDS, 1% Triton X-100, and 1x complete protease inhibitor cocktail], once with LiCl wash buffer [10 mM Tris-HCl pH 7.9, 1 mM EDTA, 250 mM LiCl, 1% NP-40, 1% sodium deoxycholate, and 1x complete protease inhibitor cocktail], and twice with Tris-EDTA (TE) containing 1x complete protease inhibitor cocktail. The immunoprecipitated material was eluted at room temperature in elution buffer [100 mM $NaHCO_3$, 1% SDS], and the crosslinks were reversed by adding 100 mM NaCl and incubating at 65ºC overnight. The eluted material was then digested with proteinase K and RNase A to remove protein and RNA, respectively, and the enriched genomic DNA was extracted with phenol:chloroform:isoamyl alcohol, followed by isopropanol precipitation. The ChIPed DNA was dissolved in water and analyzed by qPCR using the primer sets listed below using a LightCycler 480 real-time PCR thermocycler (Roche). All experiments were conducted a minimum of three times with independent RNA isolations to ensure reproducibility.

- *TFF1* enhancer Forward 5'-TGGTTGCAGATCTTGTTGGA-3'
- *TFF1* enhancer Reverse 5'-TTCTCACACACATCCCCTCA-3'

• *GREB1* enhancer Forward 5'-GAGCTGACCTTGTGGTAGGC-3'
• *GREB1* enhancer Reverse 5'-CAGGGGCTGACAACTGAAAT-3'

Preparation of ChIP-seq Libraries

ChIP-seq libraries were generated using two biological replicates the ChIPed DNA described above for each condition. The DNA was purified using a MiniElute PCR Purification Kit (Qiagen, 28004). After purification, 50 ng of ChIPed DNA for each condition was used to generate libraries for deep sequencing, as previously described (16,17), with some modifications. Briefly, the DNA was end-repaired and a single "A"-base overhang was added using the Klenow fragment of *E. coli* DNA polymerase. The A-modified DNA was ligated to Illumina sequencing adaptors using the Illumina TruSeq DNA Sample Prep Kit. The ligated DNA (300-500 bp) was size-selected by agarose gel electrophoresis and purified using a QIAquick Gel Extraction kit (Qiagen, 28704). The size-selected fragments were PCR amplified using Illumina TruSeq P5 and P7 PCR primers, and purified using AMPure beads (Beckman Coulter, A63881). After quality control analyses, the libraries were sequenced on an Illumina HiSeq 2000 (single-end sequencing, 50 nt).

Analysis of ChIP-seq Data

Quality Control and Alignment. The quality of ChIP-seq reads was analyzed by FastQC tool (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). ChIP-seq reads were aligned to the hg19 reference genome using Bowtie 2 v2.2.2 using the default parameters (18), and visualized on UCSC genome browser using bigWig files generated by BEDTools (12) and custom R scripts.

Peak Calling and Data Representation. We used an ERα binding site dataset from E2-treated MCF-7 cells from our lab in which peaks were called using the input as a control (17). This study also included FoxA1 ChIP-seq data prepared under the same conditions and in the same MCF-7 cells in which we performed the BRD3 ChIP-seq. To understand the role of BRD3 in ERα enhancer activation, we compared our ERα and BRD3 ChIP-seq datasets with our FoxA1 ChIP-seq data, as well as published BRD4 ChIP-seq data (19) and DNase-seq data (20) from MCF-7 cells treated with E2. Sequencing read densities ± 5 kb around the ERα peaks for BRD3, FoxA1, and DNase1, and ± 10 kb around the ERα peaks for acetyl H4 were calculated using annotatePeaks.pl function in HOMER software (21) and visualized as heatmaps using Java Tree View (13). The read counts within ± 500 bp of top 50% of ERα peaks with BRD3 enrichment and within ± 500 bp of top 10% of ERα without BRD3 enrichment was plotted as box plots using the box plot function in R. The statistical analysis on the recruitment of ERα, BRD3, and FoxA1 and the levels of acH4 and DNaseI hypersensitivity at ERBSs with or without BRD3 enrichment was performed using 100 ERBSs randomly chosen from each category.

Motif Analyses. Directed motif search was performed on ± 5 kb window around the center of all the ERα peaks with and without BRD3 enrichment using the command-line version of FIMO software (22). A p-value of 1e-4 was used to identify the genomic locations with significant half ERE or full ERE in the ERα peaks as mentioned above. As a control, directed motif search was also performed on a set of random sequences of 10 kb length generated using BEDTools v2.16.2. The position weight matrix (PWM) for ESR1 was obtained from JASPAR database (23). The motif logos were generated using WEBLOGO online tool (24).

Genomic Data Set Availability

The following new data sets generated for this study are available from the NCBI's Gene Expression Omnibus (GEO) database (http://www.ncbi.nlm.nih.gov/geo/) using superseries accession number GSE109572.

	Accession Numbers
RNA-seq	GSE109570
• MCF-7 control (reps1, 2)	GSM2946690, GSM2946691
• MCF-7 100 nM E2 for 3 hours (reps1, 2)	GSM2946692, GSM2946693
• MCF-7 500 nM JQ1 pretreatment for 3 hours (reps1, 2)	GSM2946694, GSM2946695
• MCF-7 E2 + JQ1 pretreatment (reps1, 2)	GSM2946696, GSM2946697
ChIP-seq	GSE109571
Input vehicle (reps1, 2)	GSM2946698, GSM2946699
Input E2 (reps1, 2)	GSM2946700, GSM2946701
BRD3 ChIP vehicle (reps1, 2)	GSM2946702, GSM2946703
BRD3 ChIP E2 (reps1, 2)	GSM2946704, GSM2946705

The previously reported data sets used herein include: ERα and FoxA1 ChIP-seq (17), BRD4 ChIP-seq (19), and DNase-seq data (20) from MCF-7 cells treated with or without E2. The publicly available ChIP-seq (ERα ±E2, FoxA1 ±E2) and GRO-seq (±E2) data sets from MCF-7 cells can be accessed from NCBI GEO using the following accession numbers: GSE59532 and GSE27463, respectively.

4) Supplementary References

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