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Supplementary Information for
The SET Oncoprotein Promotes Estrogen-Induced Transcription by Facilitating Establishment of Active Chromatin

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SI References

Reports is limited to extended methods only.

Supplementary Information Text

Methods.

ChIP and ChIP-seq

Briefly, ~2-4 X10^6 MCF7 cells treated with E2 for the indicated times were fixed with ChIP Cross-link Gold (Diagenode) according to the manufacturer's instructions. Nuclei were purified and fragmented using a nuclear Co-IP kit (Active Motif). After digestion, chromatin was sonicated to an average size of 250 bp. The fragmented chromatin was immunoprecipitated overnight by using the corresponding antibodies. DNA enrichment was measured by quantitative PCR using SYBR green. For ChIP-seq, the NEBNext Ultra II DNA Library Prep Kit was used to produce high yield libraries from a small amount of purified DNA. Sequences were generated using the Illumina HiSeq 2000 genome analyzer (using 50 bp reads) and aligned to the Human Reference Genome using Bowtie 1.0. The MACS peak caller was used to identify enriched regions of the genome by comparison to an input or control sample. Primers used for ChIP assays are listed in Table S2.

Fastq from previously published ChIP-seq data were downloaded from GEO and analyzed in the same way as our ChIP-seq data. Reads were aligned to the Human Reference Genome hg19 (GRCh37/hg19) using Bowtie 2.3.4 with default parameters. Duplicated reads and reads with a MAPQ < 10 were discarded using macs2 filterdup and samtools v1.7, respectively. The remaining alignments were used as input for MACS 2.1.1 for peak calling, using their respective input as control with an FDR of 5e-4, except for SET, for which an FDR of 5e-2 was used. Peaks falling into blacklisted regions from the ENCODE project were discarded and bigwig files were generated for visualization from MACS2 with a window size of 150 bp. Heatmaps were generated with deeptools 3.02 and statistical analyses were performed with R 3.3.3. Table S3 contains the performance of the ChIP-seq analyses as well as the sources for the analyzed data.

CRISPRi, RNAi, RNA-seq and qRT-PCR

For CRISPRi, the gene coding Cas9 in plasmid lentiCRISPRv2 was mutated and fused with transcriptional repressor KRAB, generating lentiCRISPRv2-dCas9-KRAB. Two sgRNAs were designed to target the individual promoter region of human SETα or SETβ. dCas9-KRAB and sgRNAs were introduced into cells by lentiviral infection. After 4-day incubation with puromycin (2 μg/mL), cells were harvested for further analysis.

For siRNA mediated SET knockdown (KD), MCF7 cells were transfected with siGENOME siRNA SMARTpool (Dharmacon) using the DhamaFECT transfection reagent (GE Dharmacon). The following siRNAs were used for this study: Human siGENOME SMARTpool against SET (M-019586-01-0005); H2A.Z (M-011683-02-0005), and H2A.X (M-011682-02-0005). siGENOME Non-Targeting siRNA #4 was used as control. Knockdown efficiency was determined by real-time qPCR of SET using gene specific primers.

Total RNA was extracted with TRIzol (Life Technologies) as described by the manufacturer. RNA-seq was carried out and analyzed by Novogene Biology Co., Ltd. 500 ng of total RNA was reverse transcribed using the SuperScript One-Step RT-PCR System (Life Technologies) and random hexamers following the manufacturer's instructions. Real-time qPCR was performed using primers listed in Table S4.

Coimmunoprecipitation assay

MCF7 cells were incubated with or without 100 nM E2 for indicated times. The nuclear Co-IP Kit (Active Motif) was used to prepare nuclear extracts. 2-4 μg of antibodies were added to equal amounts of nuclear extracts and incubated overnight at 4℃. Then, the protein complexes were collected using protein G-coated magnetic beads (Dynabeads G) for 2 h at 4℃ with rotation. Antibody-protein-bead complexes were washed following the manufacturer's instructions and boiled for immunoblot analysis.

In vitro competitive binding assays

10 nmol of purified recombinant FLAG-tagged SET was incubated with 12 nmol of purified H2A (NEB) at 37℃ for 20 min. The reaction solution was then immunoprecipitated using anti-FLAG antibody M2-coated magnetic beads (protein G Dynabeads). The H2A-SET-antibody-bead complex was equally aliquoted into 5 tubes and indicated amounts of purified H2A.Z protein (Merck)

were added to each tube. After 20 min incubation, the supernatants and wash fractions containing unbound histones were collected and combined. SET, together with associated histones, was then eluted from the beads by adding SDS-PAGE sample buffer. Histone H2A.Z and H2A levels were analyzed by immunoblot.

***In vitro* H2A.Z replacement assay**
Recombinant SET protein was purified from HEK-293T transiently expressing FLAG-tagged SET. The SWR1 complex was purified from recombinant S. cerevisiae stably expressing SWR1-FLAG fusion protein. Recombinant H2A.Z and histone octamer were purchased from BD Bioscience and Epigenome, respectively. A 280 bp biotin-labeled probe containing one ERE site amplified from the *TFF1* gene locus was assembled into mononucleosomes. In vitro Histone H2A.Z replacement by the SWR1 complex was performed as previously described (1).

Antibodies
Antibodies used in this study were: SET (ab85389, ab181990), ERα (ab108398, ab32063), KDM3A (ab91252), H3K9me2 (ab1220), H2A.Z (ab4174), γH2A.X (ab11175), NCAPH2 (ab200659), and NAPL1 (ab178687) from Abcam; H2A (12349), H2B (12364), H3 (4620), Lamin A/C (4777) and pERα (2511) from CST; H3K4me1 (61634), H3K4me2 (39914), H3K9me2 (39754) from Active Motif; SETα (MABN1863, Merck Millipore), ERα (49-1002, ThermoFisher Scientific), MLL1 (A300-086A, Bethyl Laboratories), NCAPG (67655-1-Ig, Proteintech), H2A.Z (17-10048, Merck Millipore), Flag (F3165, SigmaAldrich), α-tubulin (T9026, SigmaAldrich), and H2A.C (NBP1-45619, Novus Biologicals). A purified rabbit anti-N-terminal SETβ antibody was produced in the lab.

CUT&Tag assay
CUT&Tag was performed according to the protocol of Hyperactive Universal CUT & Tag Assay Kit for Illumina (Vazyme, TD903). In brief, approximately 200,000 cells were harvested by centrifugation at 600 g for 5 min in low-retention tubes. To obtain nuclei each sample was incubated with 100 μL of precooled NE Buffer on ice for 10 min, followed by centrifugation at 600 g for 5 min. Nuclei were incubated in diluted antibody (1:50 dilution in Antibody Buffer) overnight. Permeabilized nuclei were then incubated with anti-mouse or rabbit IgG (1:100 dilution) in 50 μL of Dig-Wash buffer with rotation at room temperature for 1 h. After washing twice with Dig-Wash buffer, nuclei were incubated with pA/G-Tnp (1:50 dilution in Dig-300 buffer) for 1 h at room temperature with rotation. Nuclei were washed three times with Dig-300 buffer and resuspended in 50 μL of fragmentation buffer and incubated at 37°C for 1 h. 5 μL Proteinase K, 100 μL Buffer L/B and 20 μL DNA Extract Beads were added to the fragmented samples, which were then incubated at 55 °C for 10 min. After washing with Buffer WA and Buffer WB, beads with DNA were magnetically collected, and 22 μL sterilized water was added at room temperature for 5 min to elute DNA. TruePrep® Index Kit V2 for Illumina (Vazyme, TD202) was used for library amplification. PCR reaction products were purified using VAHTS DNA Clean Beads (Vazyme) according to the manufacturer's protocol. After quality control, libraries were quantified and pooled for sequencing by Novogene.

High quality and adaptor-removed clean data were analyzed by Bowtie2 (v2.25) for alignment using hg19 assembly. Mapped reads contain abundant redundant duplicates were tagged by Picard tools (v2.27.1) and then indexed by Samtools (v1.6). To obtain enriched peaks over the background, MACS2 software (v2.2.6) was used with arguments "-f BEMPE -q 0.01" for narrow peaks and "-f BEMPE --broad --broad-cutoff 0.01" for broad peaks. The "--min-length" parameter was configurated depending on the average peak length. MACS2-generated bedgraph files were normalized using the MACS2 default argument and transformed into bigwig files using bedGraphToBigWig tools (v1.04). Bigwig data was visualized using the IGV browser (v2.12.3). Deeptools (v3.5.1) was used to plot heatmaps, profiles and correlations.

Capture C assay
3C library preparation with high-resolution was carried out using restriction enzyme Nla III which recognizes 4 bases as described previously (2). After measuring DNA concentration using a Quant-iT PicoGreen (Life Technologies), 40 μg of 3C DNA was sheared to an average size of 300 bp

(Diagenode), end repaired, adenine tailed and ligated to paired-end adaptors (NEB). Capture C of cis-acting regulatory elements was carried out with SureSelect target enrichment, using a custom-designed biotinylated RNA bait library according to the manufacturer's instructions (Agilent Technologies). Biotinylated 120-mer RNA baits were designed to target 8 specific gene loci that cover enhancer, promoters and partial coding regions (Table S1). After library enrichment, a post-capture PCR amplification step was carried out. Capture-C libraries were sequenced on the Illumina HiSeq 2000 platform and sequences were aligned to the Human Reference Genome using Bowtie 2.0 with the local alignment mode (version: 2.3.5). The alignment output files from sam format to bam format was converted using sam tools. HiC Explorer (version: 3.2) was used to build the interaction matrix and heatmap visualization. Once the reads had been mapped, the HiC matrix was built using hicBuildMatrix. The Hi-C matrix was corrected by using hicCorrectMatrix to remove GC, open chromatin biases and, most importantly, to normalize the number of restriction sites per bin. Next, using hicNormalize, the given matrices were normalized to a 0-1 range. For each single example, a heatmap with the region of interest was directly plotted from the corrected matrix using hicPlotMatrix.

According to the distance between two genomic locations that interact with each other captured by Capture-C, long range interactions were distinguished from local interactions by a specified distance, the window size. Contacts whose distance was greater or equal to the indicated window size were defined as long range chromatin interactions and counted and compared to the total contacts of each gene locus. To test whether the mean of E2-induced long range interaction frequencies of 6 estrogen-responsive gene loci in the SET-depleted MCF7 cells (siSET) significantly differ from that in control (siNT) cells, we normalized the number of contacts from each gene locus to that in the estrogen-unresponsive *ACTB* locus as control using the same window size. The normalization formula is $x = \left(\frac{N_C}{N_T} w_s\right)_{GENE} / \left(\frac{N_C}{N_T} w_s\right)_{ACTB}$ where N_C: the number of long-range contacts; N_T: total number of contacts; w_s: the indicated window size. A paired two sample Wilcoxon test was then used to analyze the difference of long-range interaction frequencies between siSET and siNT samples.

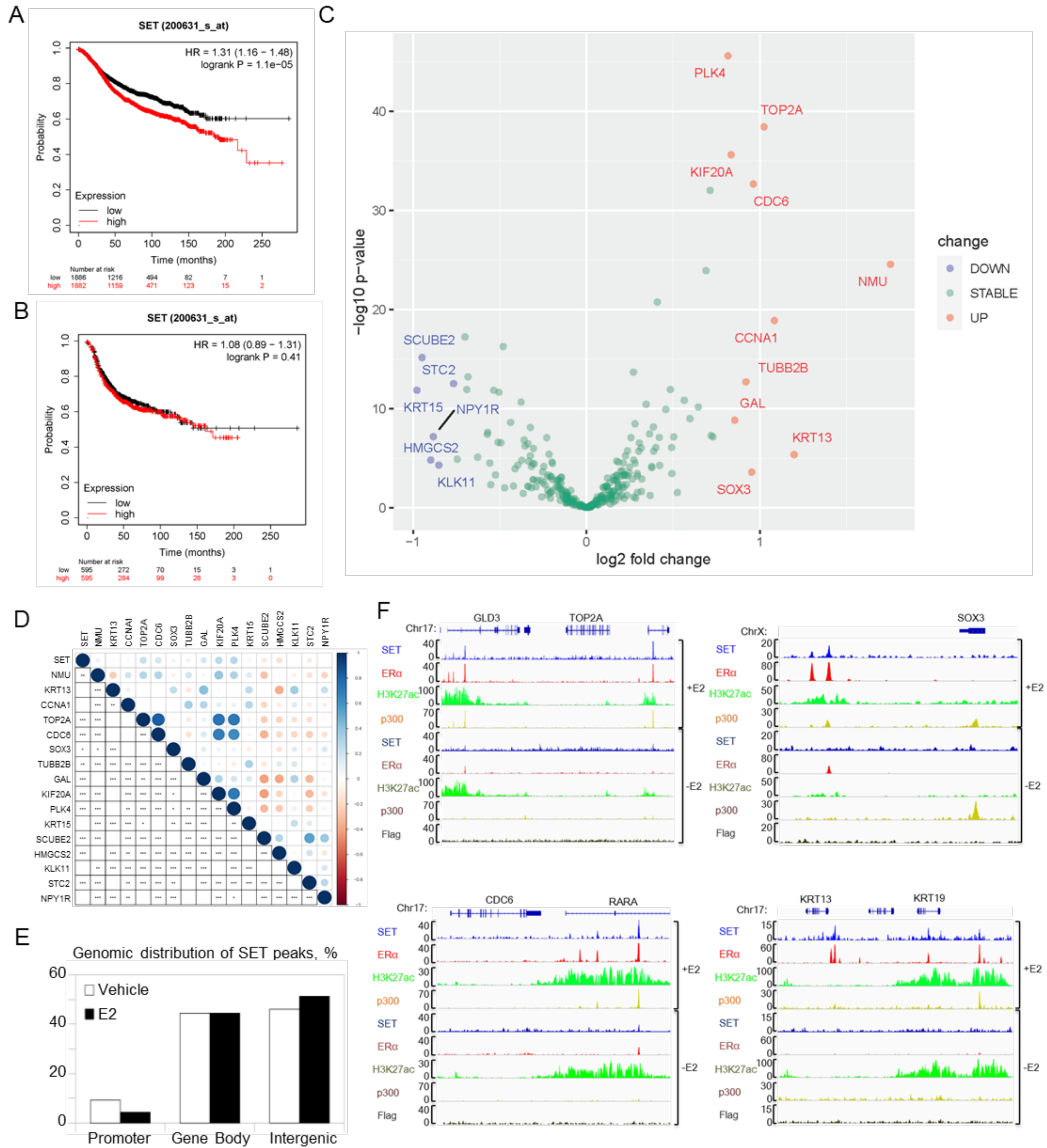

Fig. S1. SET closely associates with ERα in breast cancer. (*A*) Kaplan-Meier plots of breast cancer survival for ERα$^+$ breast cancer patients from sources for the databases include GEO, EGA, and TCGA, according to SET expression. (*B*) Kaplan-Meier plots of breast cancer survival for ERα$^-$ breast cancer patients from sources for the databases include GEO, EGA, and TCGA, according to SET expression. (*C*) Volcano plots showing the differentially expressed estrogen-responsive genes in ERα$^+$ breast cancer patients with distinct levels of SET expression. More than 300 estrogen-responsive genes were analyzed in BRCA based on the TCGA database. The logFC cutoff for SET gene expression fold changes is 0.75. Among them, 10 genes were significantly up-regulated and 6 genes were significantly down-regulated. The probability of having 16 differentially expressed genes (DEG) by chance from a pool of 300 genes was p<1.53e-15 by hypergeometric test. With our p-value cutoff, one would expect 0.015 significant DEGs by chance. (*D*) Spearman correlation of those top 16 estrogen-responsive genes and SET analyzed in BRCA. (*E*) Distribution of SET binding sites in the whole genome of MCF7 cells with or without adding E2. (*F*) Integrative

Genomics Viewer (IGV) snapshots show the distribution of normalized reads of SET, ERα, H3K27ac, p300 and FLAG at the *TOP2A*, *SOX3*, *CDC6*, *KRT19* gene loci with and without E2 treatment.

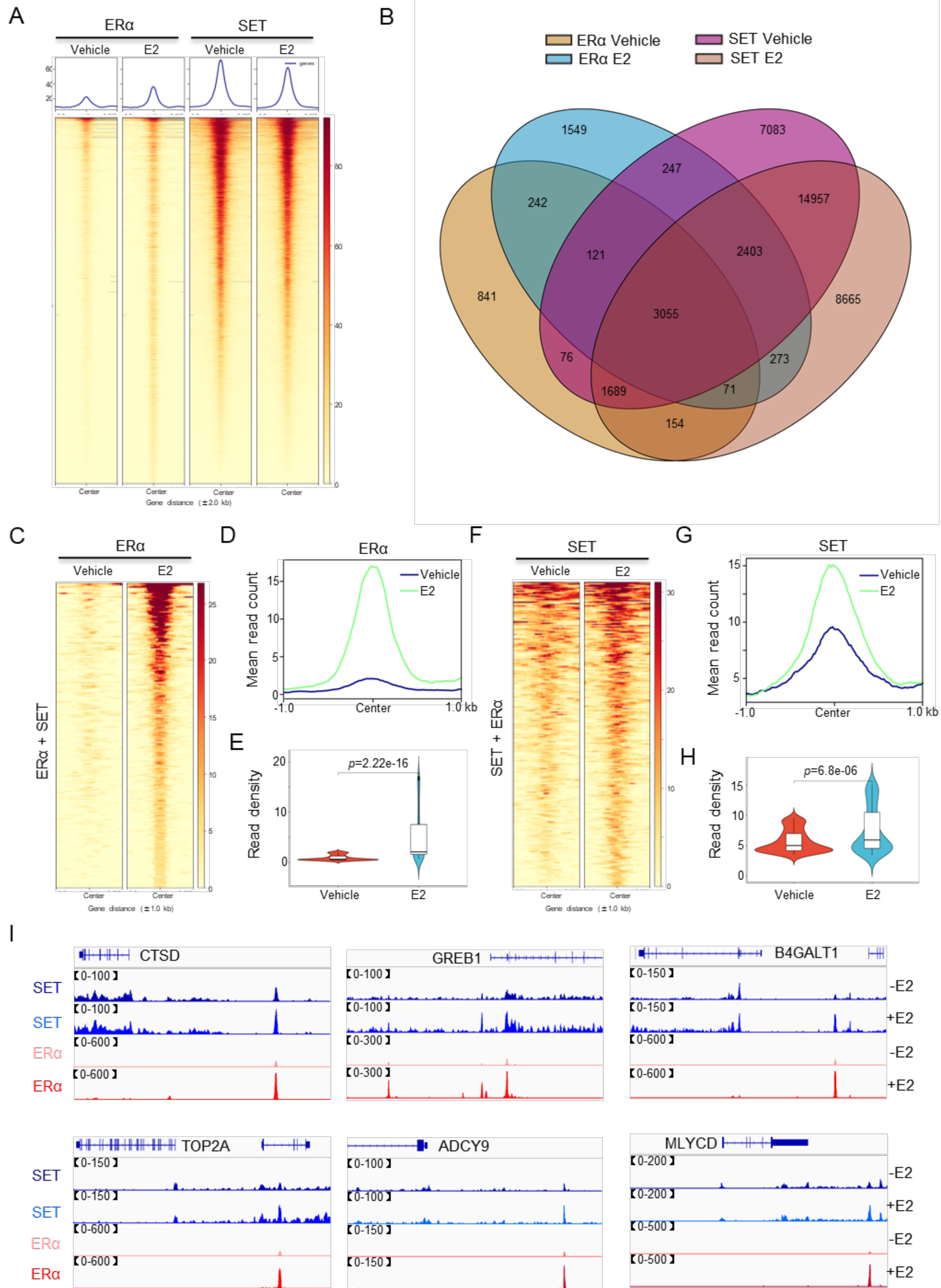

Fig. S2. The association of ERα and SET across the T47D genome. *(A)* Genome-wide profile of ERα or SET binding in T47D cells treated or not with E2. Profile plots (top panel) display average read counts of ERα or SET detected by CUT&Tag. Heatmaps (bottom panel) show global read density of ERα or SET around detected peak centers. For ERα CUT&Tag with or without E2

treatment, *p*-value is 3e-8. For SET CUT&Tag with or without E2 treatment, *p*-value is 2.22e-16, Mann-Whitney test. *(B)* Venn diagram of numbers of SET and ERα peaks detected with or without E2 treatment. SET overlap probability in E2-treated cells according to hypergeometric test is *p*=2.38e-20. SET-ERα overlap significance without and with E2 are *p*=2.22e-14 and *p*=0.0015, respectively, according to the hypergeometric test. *(C)* E2-induced upregulation of ERα recruitment to ERα-SET co-bound sites. Heatmaps show read density of ERα at ERα-SET co-bound sites with or without E2 treatment. *(D)* Profile plots show average read counts of ERα at ERα-SET co-bound sites. *(E)* Comparison of read density of ERα at ERα-SET co-bound sites with or without E2 treatment. *(F)* E2-induced SET recruitment to ERα-SET co-bound sites. Heatmaps show read density of SET at ERα-SET co-bound sites with or without E2 treatment. *(G)* Profile plots show average read counts of SET at ERα-SET co-bound sites. *(H)* Comparison of read density of SET at ERα-SET co-bound sites with or without E2 treatment. *(I)* Screen shots of E2-induced occupancy of SET and ERα in estrogen-responsive gene loci, *CTSD*, *GREB1*, *B4GALT1*, *TOP2A*, *ADCY9* and *MLYCD*.

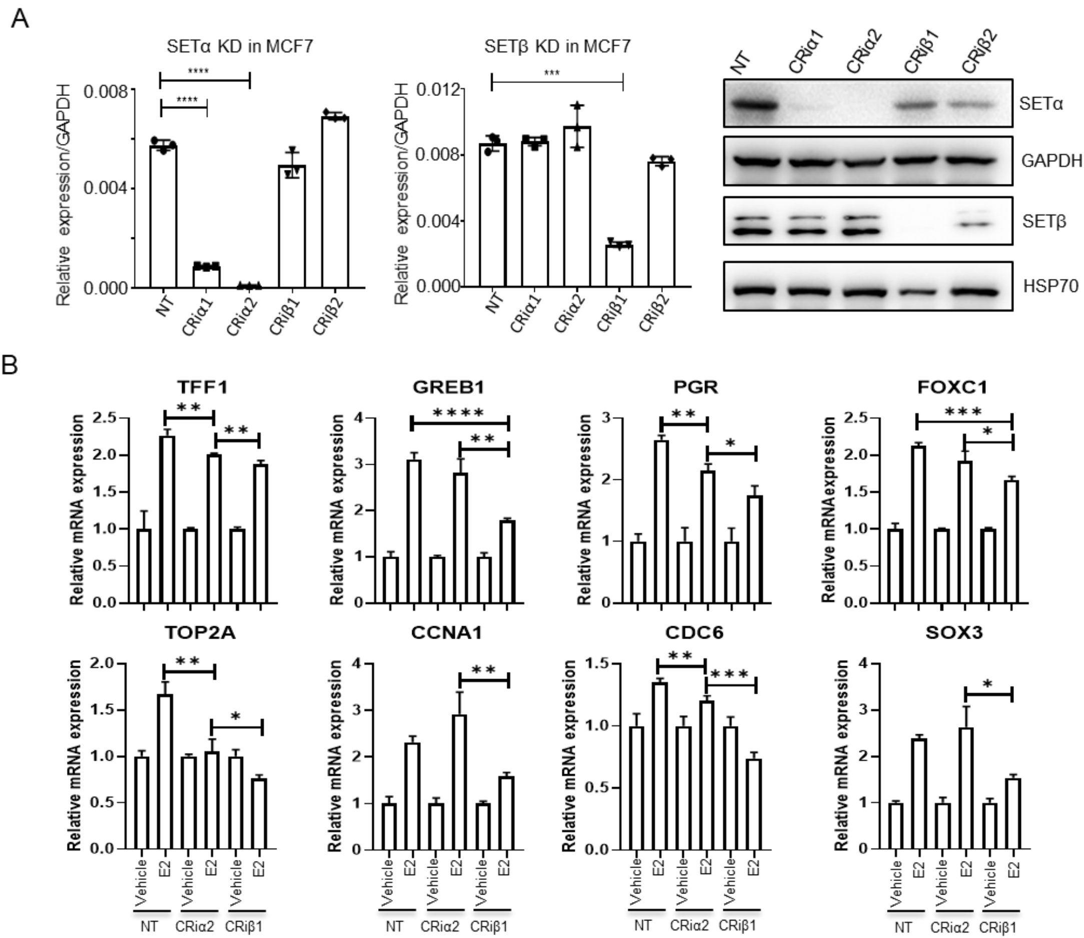

Fig. S3. Estrogen signaling in SET isoform-specific knockdown MCF7 cells. (*A*) Detection of SETα and SETβ mRNA expression in breast cancer MCF7 cells after SETα or SETβ knockdown by CRISPRi. SETα and SETβ mRNA levels in SETα knockdown MCF cells (left panel), and SETβ knockdown MCF cells (middle panel). Data are the mean ± SD of three independent replicates. Student's *t*-test. $*p < 0.05$; $**p < 0.01$, and $***p < 0.001$. Representative immunoblots showing the reduction of SETα and SETβ protein after SETα or SETβ knockdown (right panel). (*B*) Effect of SETα or SETβ knockdown on E2-induced gene expression. qPCR data show mean ± SD of three biological replicates, Student's *t*-test. $*p < 0.05$; $**p < 0.01$, and $***p < 0.001$.

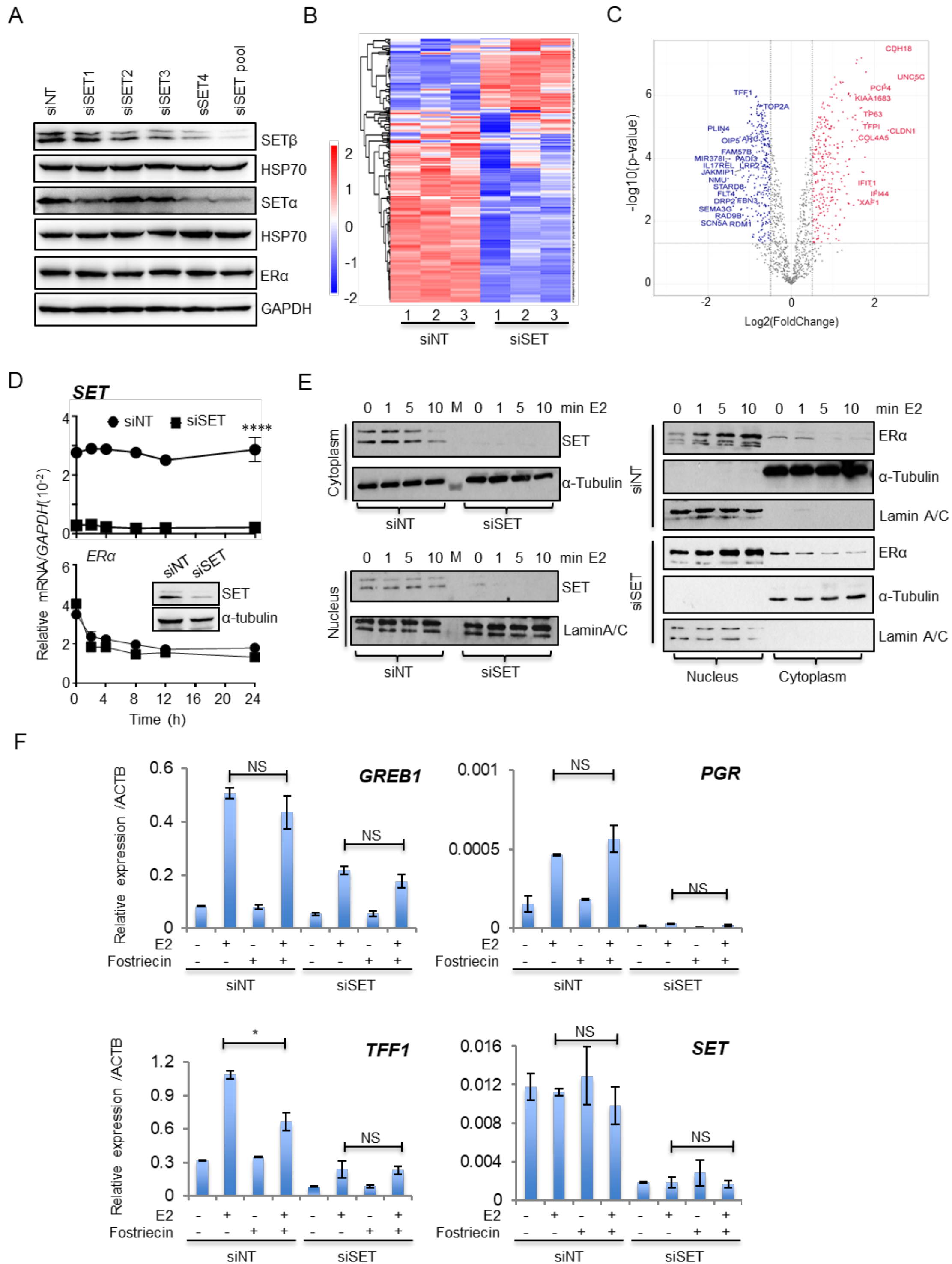

Fig. S4. RNAi-mediated *SET* knockdown (SET KD) in MCF7. (*A*) The protein levels of specific SET isoforms and ERα in MCF7 cells after SET KD using 4 individual SET siRNAs and an siRNA pool assessed by immunoblotting. (*B*) RNA-seq analysis of known estrogen response genes in control (siNT) and SET-depleted (siSET) MCF7 cells treated with E2 for 24 hours. Gene coregulation was

determined by hierarchical clustering. (*C*) Volcano plot showing the differentially expressed estrogen-responsive genes in WT and SET KD cells. The cutoff used for logFC was 0.5. (*D*) mRNA and protein levels of ERα and SET in control (siNT) and SET-depleted MCF7 cells treated with E2 for the indicated times. Data show mean ± SD of triplicate experiments. Student's *t*-test. *$p < 0.05$; **$p < 0.01$, and ***$p < 0.001$. (*E*) ERα translocation in control (siNT) and SET-depleted MCF7 cells in response to E2. Cell fractions were prepared from control (siNT) and *SET* knockdown (siSET) MCF-7 after E2 treatment at the times indicated. The knockdown efficiency and subcellular location of SET (left) and effect of SET knockdown on ERα (right) are shown. (*F*) A specific PP2A inhibitor did not rescue estrogen response in SET depleted MCF7 cells. Relative mRNA levels of estrogen-responsive gene were measured in control (siNT) and SET depleted (siSET) MCF-7 in the absence or presence of fostriecin. Data show mean ± SD of triplicate experiments. NS, not significant, Student's *t*-test. *$p < 0.05$; **$p < 0.01$, and ***$p < 0.001$.

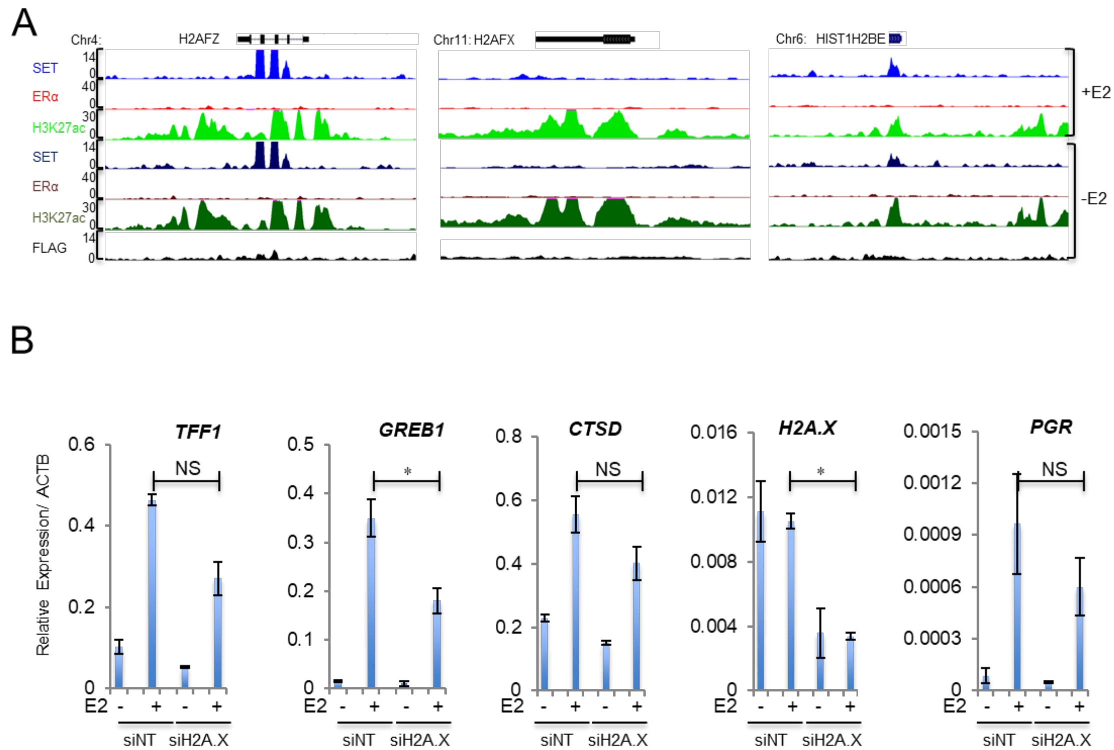

Fig. S5. RNAi-mediated H2A.Z and H2A.X knockdown in MCF-7 cells. (*A*) UCSC browser snapshot showing the distribution of normalized peaks of SET, ERα, H3K27ac and FLAG at *H2AFZ*, *H2AFX* and *HIST1H2BE* loci treated or not with E2. (*B*) Effect of *H2AFX* depletion on E2-induced gene expression. qPCR data show mean ± SD of three biological replicates; Student's *t*-test. $^*p < 0.05$; $^{**}p < 0.01$, and $^{***}p < 0.001$.

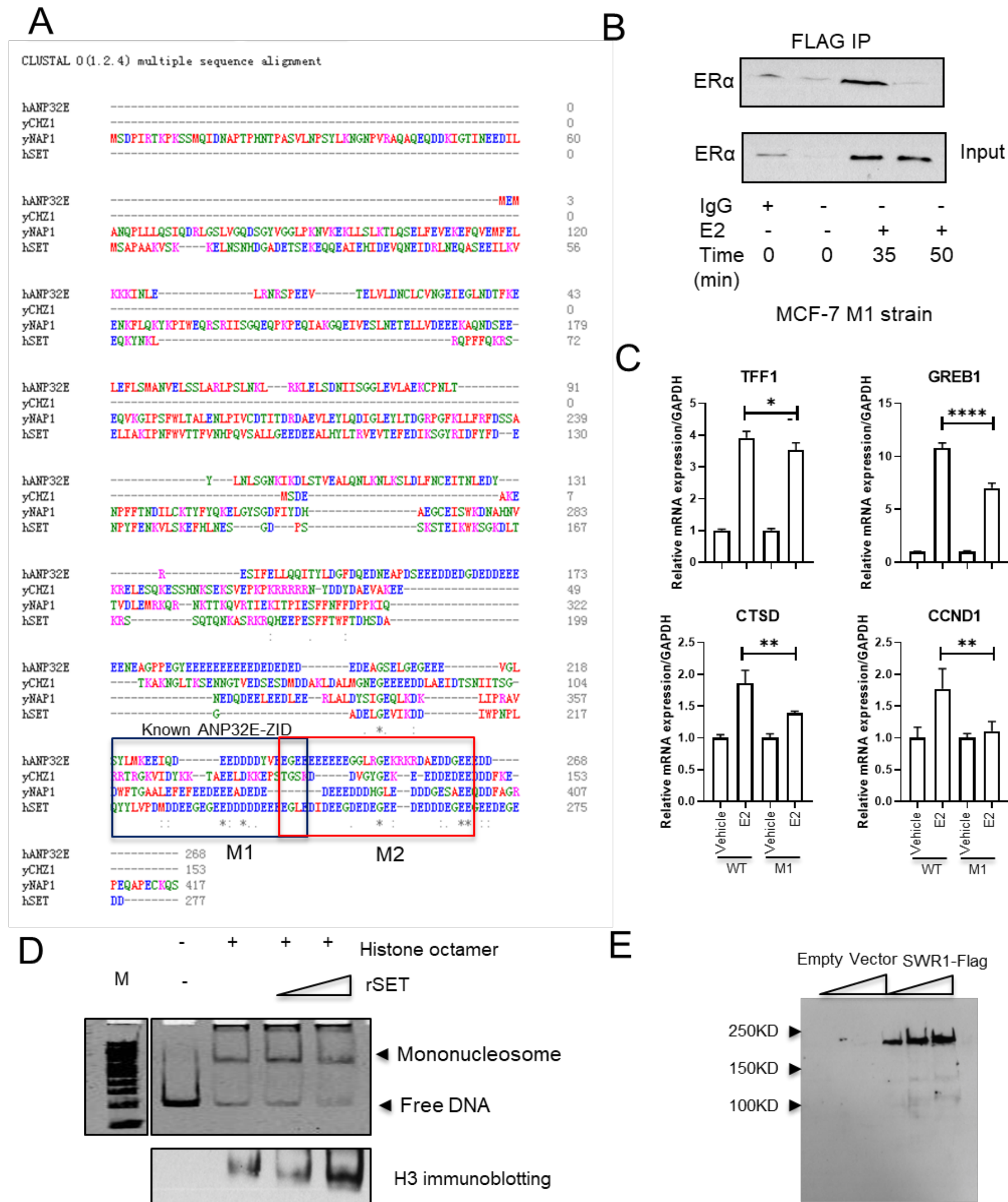

Fig. S6. SET acts as a histone chaperone to facilitate histone H2A.Z replacement. (*A*) Protein sequence alignment of SET with known H2A.Z histone chaperones including human ANP32E, and yeast Chz1 and NAP1 using the Clustal Omega online tool. (*B*) Association of truncated M1 with ERα shown by Co-IP using FLAG antibody treated with E2 for the indicated time. (*C*) Effect of overexpression of SET mutant M1 on E2-induced gene expression. qPCR data show mean ± SD of three biological replicates; Student's *t*-test. **p* < 0.05; ***p*< 0.01, and ****p* < 0.001. (*D*) Mononucleosome assembly in the presence of recombinant SET purified from 293T cells. Mononucleosome waere detected on acrylamide gels stained with Ethidium bromide and by immunoblot probed for H3. (*E*) Purified SWR1 complex from yeast lysate using anti-FLAG M2 affinity gel. SWR1 was FLAG-tagged and overexpressed in *S.cerevisiae*.

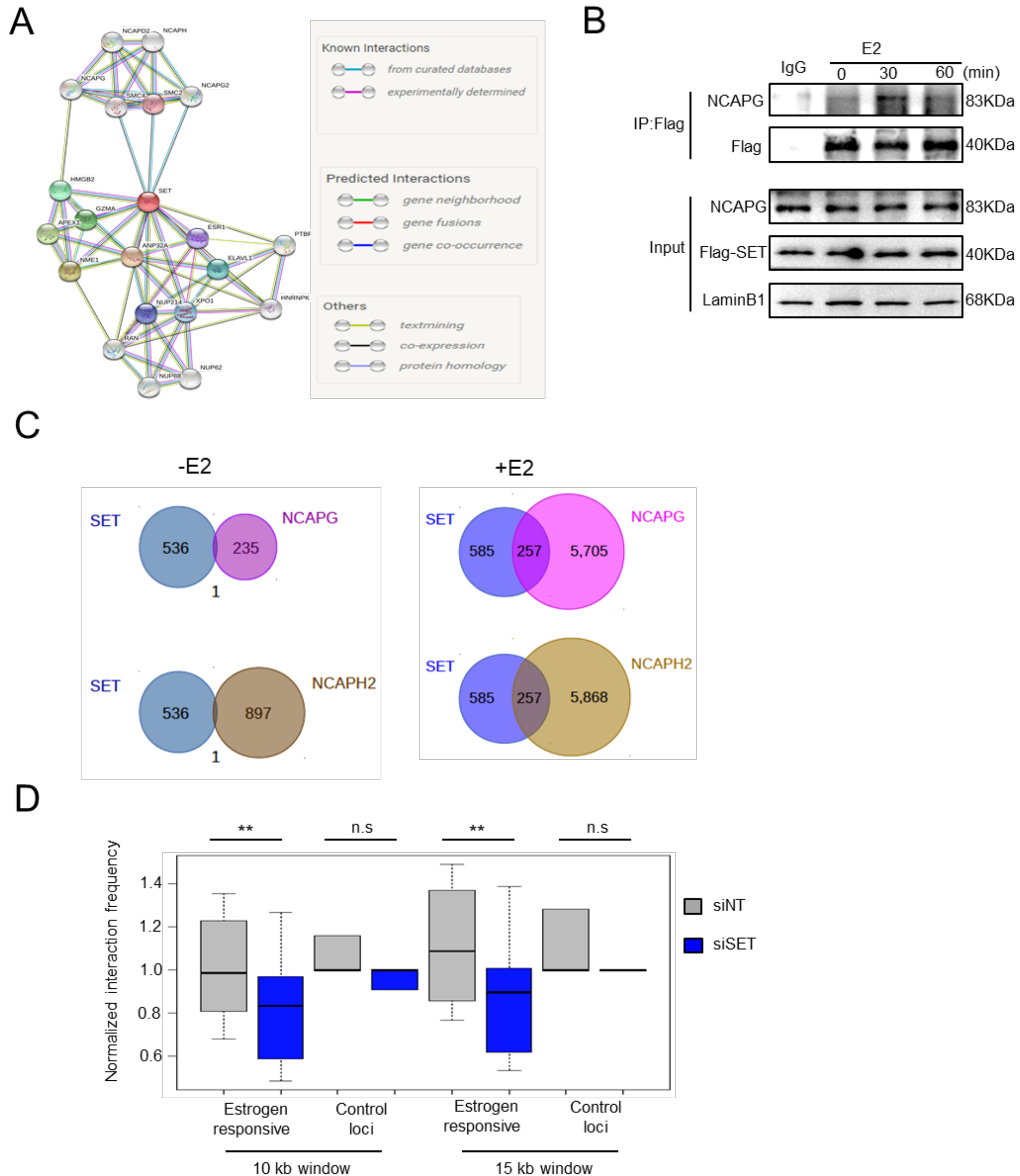

Fig. S7. SET is involved in chromatin conformation formation. (*A*) Known and predicted protein-protein interactions involving SET (obtained using STRING, a database of known and predicted protein interactions). (*B*) Association of SET with condensin component NCAPG shown by Co-IP using FLAG antibody in MCF7 cells expressing FLAG-tagged SET that were treated with E2 for the indicated times. *(C)* Venn diagram showing the genome-wide ChIP peak numbers of SET and condensin components NCAPG or NCAPH2 and their overlap in MCF7 treated or not with E2. Overlap probability in E2-treated cells according to hypergeometric test is p=6.27e-06 for NCAPG and 2.24e-05 for NCAPH2. (*D*) Box plots comparing the mean of normalized long-range interaction frequencies within 6 estrogen responsive gene loci in control (siNT) and SET-depleted (siSET) and 2 estrogen unresponsive genes in MCF7 cells analyzed using different window sizes. A paired two sample Wilcoxon test was used for analysis. * $p<0.05$; ** $p<0.01$.

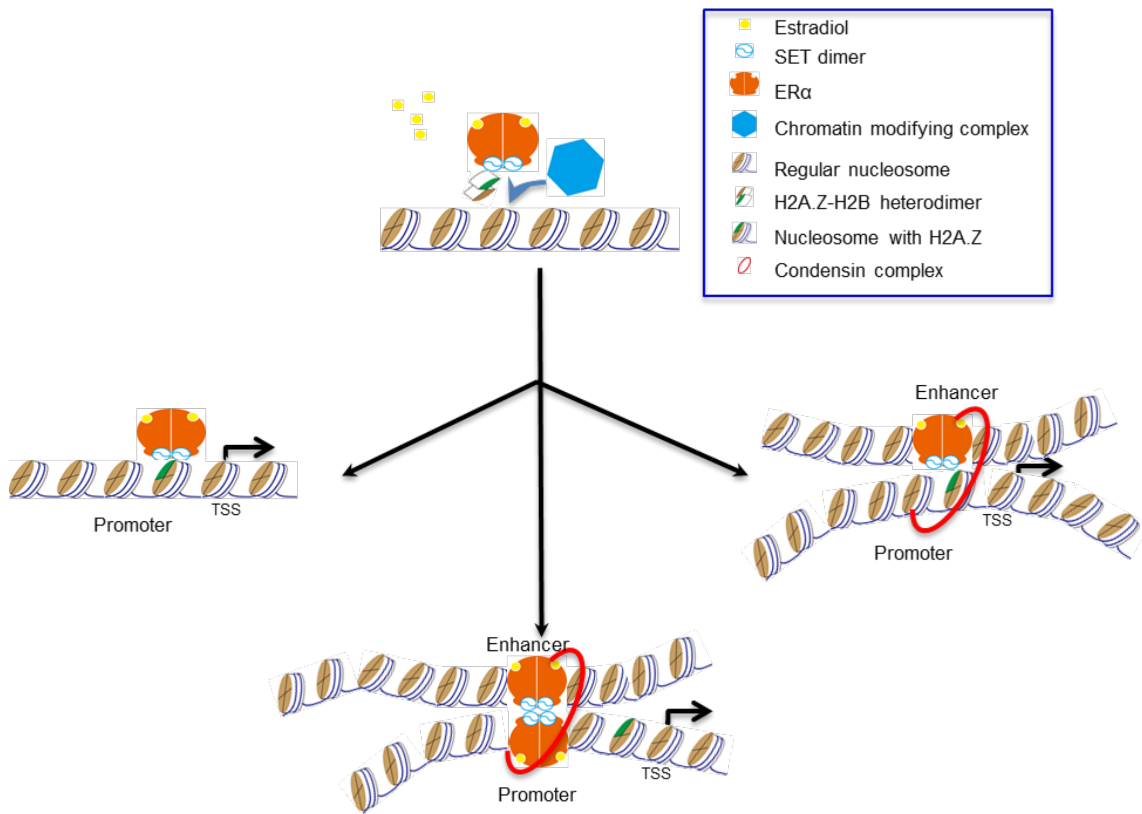

Fig. S8. Working model for the role of SET in estrogen-induced transcriptional activation. After adding estrogen, the histone chaperone SET is recruited to ERα-bound enhancers and promotes transcription of estrogen-responsive genes. SET promotes loading of ERα and histone modifiers, and facilitates the incorporation of the histone variant H2A.Z into nucleosomes in the presence of chromatin remodeling complexes at the promoters; it enhances the formation of enhancer-promoter looping mediated by SET-ERα, SET-H2A.Z and/or ERα-ERα interactions, resulting in enhancer and promoter activation.

Table S1. Position and coverage of RNA baits used in the Capture-C assay

Target ID	Gene Regions	Locus Range	Coverage	High Coverage (≥90%)	Low Coverage (≤90%)
Chr2: 11620000-11686000	*GREB1*	66Kb	86.47596%	0	1
Chr6: 39184000-39255000	*KCNK5*	47Kb	68.09642%	0	1
Chr7: 5540000-5565000	*ACTB*	25Kb	60.913563%	0	1
Chr8: 67588000-67640000	*SGK3*	52Kb	68.53906%	0	1
Chr11: 1770000-1810000	*CTSD*	40Kb	76.290596%	0	1
Chr14: 20910000-20935000	*APEX1*	25Kb	87.572495%	0	1
Chr21: 38578000-38638000	*DSCR9*	60Kb	86.30689%	0	1
Chr21: 43792000-43810000	*TFF1*	18Kb	92.433754%	1	0

Table S2. Primers used in this study

Primer	Sequence	Reference
Primers used in ChIP assay		
TFF1enh ChIP FP TFF1enh ChIP RP	AGGGGATGTGTGTGAGAAGG GCTTCGAGACAGTGGGAGTC	(3)
MYCenh ChIP FP MYCenh ChIP RP	ACTCTGCACTGCCAGACAAA TGGAAACCACATTTTGGTCA	
FOXC1enh ChIP FP FOXC1enh ChIP RP	CTGAGGAACACAAGACTAGCC ACTGGACTCATTTTGGGACATC	(3)
HBBcod ChIP FP HBBcod ChIP rp	AGGACAGGTACGGCTGTCATC TTTATGCCCAGCCCTGGCTC	
GREB1 Enhancer FP GREB1 Enhancer RP	TCTGTGGAGTGCCTGAAGTG GCCAATGCTTTGCCATTATT	(4)
TFF1 ERE2 FP TFF1 ERE2 RP	CCTGTGGTTGCAGATCTTGTT CACACATCCCCTCACTCACTT	(4)
TFF1 ERE1 FP TFF1 ERE1 RP	TGACCATGTCTAGGAAACACCTT CTCTGTTTGCTTAAAGAGCGTTAG	(4)
TFF1 Coding Region FP TFF1 Coding Region RP	TGCCAGCTGTGGGGAGCTGAATAACTT CAGTTCGTTCTGTACACCGAGGCCACT	
TFF1 TSS ChIP FP TFF1 TSS ChIP RP	GCTACATGGAAGGATTTGCTG CATGGGAAAGAGGGACTTTCT	(4)
ACTBTSS ChIP FP ACTBTSS ChIP RP	CCTCGATGCTGACCCTCATCC GACACTGCCCCATTCAATGTCTC	
Primers used in qRT-PCR		
GREB1 TR FP GREB1 TR RP	GTGGTAGCCGAGTGGACAAT AAACCCGTCTGTGGTACAGC	
PGR TR FP PGR TR RP	CTTAATCAACTAGGCGAGAG AAGCTCATCCAAGAATACTG	
MYC TR FP MYC TR RP	TCCGTCCTCGGATTCTCTGCTCT GCCTCCAGCAGAAGGTGATCCA	
ACTB RT FP ACTB RT RP	GGACT TCGAGCAAGAGATGG AGC ACTGTGTTGGCGTACAG	
SET TR FP SET TR RP	CTGTCCCTGCTTTCTGGTATAA TGCATTAGGGTGCTTCTCTG	
ER TR FP ER TR RP	CCCACTCAACAGCGTGTCTC CGTCGATTATCTGAATTTGGCCT	
CTSD TR FP CTSD TR RP	GCGAGTACATGATCCCCTGT CTCTGGGGACAGCTTGTAGC	
H2AZ TR FP H2AZ TR RP	CCTTTTCTCTGCCTTGCTTG CGGTGAGGTACTCCAGGATG	
TFF1 TR FP TFF1 TR RP	CCCTCCCAGTGTGCAAATAAG GAACGGTGTCGTCGAAACAG	
H2AX RT FP H2AX RT RP	GGCCTCCAGTTCCCAGTG TCAGCGGTGAGGTACTCCAG	
RPLP0 TR FP RPLP0 TR RP	TGGCAGCATCTACAACCCTGAA ACACTGGCAACATTGCGGACA	
GAPDH TR FP GAPDH TR RP	CTGGGCTACACTGAGCACC AAGTGGTCGTTGAGGGCAATG	
TFF1e FP TFF1e RP	GTTTGTGACCCAGGCATCTT CAGGGTCCTGTCATTGTGTG	(3)
FOXC1e FP FOXC1e RP	CATGAAAGGTGAAGCGGAAATAC TGAAGGAGCAGGTGAAACG	(3)
CA12e FP CA12e RP	ATTCTCACTGCATACCTGACAC AGCTGTTATTCCCCACTCAAC	(3)
PGRe FP PGRe RP	ACGACTCAGTCTCAGTTTTAGC GTATACAGGCCCAGAGTCAC	(3)
Primers for gene expression and mutation		
SET MT1 FP SET MT1 RP	CAGTACTACTTGGAAGATATTGACGAAGAA GGG GTCAATATCTTCCAAGTAGTACTGTAATGGGTT	
SET MT2 FP SET MT2 RP	TGAAGAGGAGAGGATGAAGGAGAAGATGAC CCTTCATCCTCTCTTCATCATCATCATCAT	

SWR1 CD FP SWR1CD Flag RP	GCAGGATCCATGACTTACG AGGAGTCAGA AAAG GCAGTCGACTTATCActtgtcatcgtcatccttgtagtcgatgtcatgatctttataatcaccgtcatggtctttgtagtcCCATTCATCACTAGTTCCTTCC	
Primers for cloning probe for mononucleosome assembly		
Probe FP Probe RP	Bio-GCTCGTCCCACCCGCAGGGCCCTG GCCACCCGTCAGGTAGCCCG	
sgRNA sequences for CRISPRi		
CRiα1 FP CRiα1 RP CRiα2 FP CRiα2 RP CRiβ1 FP CRiβ1 RP CRiβ2 FP CRiβ2 RP	CACCGGGGGGTTTGGGAACGAACTG AAACCAGTTCGTTCCCAAACCCCCC CACCGAAGAGGATAAGTTCTCAGTA AAACTACTGAGAACTTATCCTCTTC CACCGGGATCGCCGAGCGCGAGTGA AAACTCACTCGCGCTCGGCGATCCC CACCGTGGATCGCCGAGCGCGAGTG AAACCACTCGCGCTCGGCGATCCAC	

Table S3. The performance of the ChIP-seq and CUT&Tag analyses

Sample	Source	Total reads	Reads after filtering	% reads after filtering	Peaks
MCF7-Input.fastq	This paper	49,040,937	39,461,126	80.46%	NA
MCF7-IgG.fastq	This paper	44,705,544	34,596,385	77.38%	139
MCF7-SET-EtOH.fastq	This paper	35,139,346	25,646,208	72.98%	537
MCF7-SET-E2.fastq	This paper	34,429,920	25638476	74.46%	842
MCF7-ERa-siControl-EtOH.fastq	This paper	48,334,400	39,522,445	81.76%	16,523
MCF7-ERa-siControl-E2.fastq	This paper	38,840,816	33,324,260	85.79%	15,436
MCF7-ERa-siSET-EtOH.fastq	This paper	42,058,934	35,935,709	85.33%	6,245
MCF7-ERa-siSET-E2.fastq	This paper	39,295,722	33,621,396	85.55%	10,271
T47D-ERα EtOH.fastq	This paper	59,662,350	47,275,438	79.24%	6,249
T47D-ERα E2.fastq	This paper	52,609,358	40,334,426	76.67%	7,961
T47D-SET EtOH.fastq	This paper	66,673,778	62,474,612	93.7%	31,267
T47D-SET E2.fastq	This paper	53,928,914	44,353,184	82.24%	29,631
MCF7-Input-Kraus.fastq	GSE59530	102,796,826	81,857,972	79.63%	NA
MCF7-ERa-EtOH-Kraus.fastq	GSE59530	52,607,215	38,792,911	73.74%	902
MCF7-ERa-E2-Kraus.fastq	GSE59530	50,310,685	36520309	72.58%	26,183
MCF7-H3K27ac-EtOH-Gevry.fastq	GSE57436	14,707,638	9855985	67.01%	76,868
MCF7-H3K27ac-E2-Gevry.fastq	GSE57436	18,164,081	14133803	77.81%	78,097
MCF7-Input-Ma.fastq	GSE62229	70,774,650	57,248,157	80.89%	NA
MCF7-p300-EtOH-Ma.fastq	GSE62229	27,912,313	9,603,746	34.41%	1,314
MCF7-p300-E2-Ma.fastq	GSE62229	50,284,761	32,647,764	64.93%	4,617
MCF7-NCAPG-EtOH-Ma.fastq	GSE62229	21,933,397	13,494,024	61.52%	236
MCF7-NCAPG-E2-Ma.fastq	GSE62229	25,188,613	16,495,144	65.49%	5,963
MCF7-NCAPH2-EtOH-Ma.fastq	GSE62229	34,849,326	28,413,608	81.53%	898
MCF7-NCAPH2-E2-Ma.fastq	GSE62229	42,924,210	34,818,481	81.12%	6,125

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