Supporting Information

Jin et al. 10.1073/pnas.1404303111

SI Methods

Cell Culture and Transfection. Prostate cancer LNCaP and CWR22Rv1 cells were obtained from American Type Culture Collection and cultured in RPMI-1640 containing 10% (vol/vol) FBS, and 293T cells were cultured in DMEM high glucose containing 10% (vol/vol) FBS. siRNA and plasmid transfections in 293T cells or siRNA transfection in LNCaP cells were carried out using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. The KDM4C construct was previous described (1). *KDM4C* siRNA (sc-92765) was from Santa Cruz Biotechnology and negative control siRNA (1027281) was from Qiagen.

Antibodies. Specific antibodies were purchased from the following commercial sources: anti-AR (N-20), anti-AR (441), and anti-jumonji domain-containing histone demethylase JMJD2C (S-15) were from Santa Cruz Biotechnology; anti–γ-Tubulin (T5326) and Monoclonal ANTI-FLAG M2 antibody (F1804) from Sigma-Aldrich Prestige Antibodies; anti-H3K9me2 (39375) from Active Motif; other histone mark antibody from Abcam; and anti-JMJD2C (A300-885A) from Bethyl Laboratories.

Cell Lysis and Immunoblotting. Cells were washed twice with cold PBS and lysed in lysis buffer (50 mM Tris·HCl, pH 7.4, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100). For histone mark Western blot, samples were briefly sonicated, followed by boiling in SDS sample buffer (Invitrogen), and separated by 4–12% (vol/vol) Bis-Tris NuPAGE gel (Invitrogen). Then proteins were transferred to nitrocellulose membrane (Bio-Rad) and Western blotting was performed following standard protocols.

RNA Purification and RT-PCR. Total RNA was isolated from cells using RNeasy Plus Mini Kit (Qiagen) following the manufacturer's protocol. First-strand cDNA synthesis from total RNA was carried out using iScript cDNA Synthesis Kit (Bio-Rad). Resulting cDNA was then analyzed by quantitative real-time PCR (qPCR) using the SsoAdvanced SYBR Green Supermix (Bio-Rad) on Stratagene Mx3000 or the CFX Connect Real-Time PCR Detection System. For expression level detection, GAPDH or Actin was used as the endogenous control. Primers were specific for the genes tested and their sequences are listed in Table S1.

Drug Screen and Induced Gene Translocation Assay. LNCaP cells were cultured in hormone-deficient media for 3 d before treatment with a vehicle or compounds (10-μM final concentration) for 2 h, followed with combined treatment of dihydrotestosterone (DHT) (100 nM) and irradiation (IR) (50 Gy) for 24 h. For certain experiments, LNCaP cells were transfected with validated siRNAs or expression vectors before DHT and irradiation treatment. The gene fusion events of TMPRSS2:ERG and TMPRSS2:ETV1 were examined by RT-qPCR using SYBR Green Master mix on an Mx3000 Real Time PCR system (Stratagene). Relative quantities of TMPRSS2:ERG and TMPRSS2:ETV1 fusion transcript were normalized to Actin and to the expression level of TMPRSS2. The relative amount of each fusion transcript was then calibrated to DHT+IR-treated control samples as appropriate. Samples without detectable fusion transcript after 40 cycles of amplification were indicated by 0 (2).

Biotinylated SD70 ChIP-Sequencing. Synthesized biotinylated SD70 (Biotin-SD70) was used to treat LNCaP cells (10-μM final concentration) for 2 h, followed by DHT (100 nM) for 1 h. The ChIP-sequencing (ChIP-seq) experiment was performed as previously described (3). Briefly, ~10^7 treated cells were cross-linked with 1% formaldehyde at room temperature for 15 min. After sonication, the soluble chromatin was incubated with 50 μL Streptavidin Dynabeads (Life Technologies) for 2 h. Complexes were washed and DNA extracted and purified by QIAquick Spin columns (Qiagen). Other ChIP assays and ChIP-seq were performed as described (4). Each reaction of KDM4C, androgen receptor (AR), or H3K9me2 ChIP-seq was performed by using 3 μg anti-JMJD2C (A300-885A; Bethyl Laborataries), 3 μg AR (N-20) antibody, or 10 μL H3K9me2 (39375; Active Motif) antibody, respectively. Anti-KDM4C (S-15) was used for ChIP-qPCR to confirm the ChIP-seq result. For ChIP-seq, the extracted DNA was ligated to specific adaptors followed by HT-sequencing (HT-seq) on an Illumina HiSEq 2000 system according to the manufacturer's instructions. The sequencing reads were aligned to the hg18 (www.1000genomes.org/category/assembly) assembly by using Bowtie2 (http://bowtie-bio.sourceforge.net/bowtie2/index.shtml). The HT-seq data were visualized by preparing custom tracks for the University of California Santa Cruz genome browser by using Hypergeometric Optimization of Motif Enrichment (HOMER). The total number of mappable reads was normalized to 10 million for each sample. The ChIP-seq peaks were identified by using HOMER with the default parameter settings. For SD70 ChIP-seq, the peaks were filtered by the peak score: Only the peaks with a peak score >10 were kept for downstream analysis. The cobound peaks were defined by a 100-bp overlap window. The read density (RD) profiles were computed by counting the average within a 6-kb window by using HOMER, and were displayed in Microsoft Office Excel. The heatmap matrices were computed using HOMER and displayed in Multiple Experiment Viewer.

The statistical significance of the difference between ChIP-seq RD profiles was assessed by Kolmogorov–Smirnov test in R [ks.test()] and by *t* test [t.test()].

Global Run-On Followed by HT-Seq. LNCaP cells (10^7) were treated with synthesized SD70 or vehicle DMSO for 2 h followed by DHT (100 nM) for 1 h. The Global Run-on followed by HT-seq (GRO-seq) experiment was performed as described (5). The image analysis and base calling were performed by using Illumina's standard computational analysis pipeline. The sequencing reads were aligned to the hg18 assembly using Bowtie2. To estimate gene expression, the sequencing reads were counted on the first 60 kb of the gene bodies by using HOMER. A RD threshold (i.e., the normalized GRO-seq read counts per kilobase) was used to exclude genes with low counts. A fold change (FC) higher than 1.5 was used to call the differentially regulated genes. Multiple gene isoforms were considered, as provided by HOMER genome annotation files (http://homer.salk.edu). The Gene Ontology (GO) analyses were carried by using HOMER as well.

RNA-Seq. *KDM4C* or negative control siRNA was transfected into LNCaP cells once every other day in stripping media, 100 nM DHT, or vehicle 0.1% EtOH was added to the cell culture for 6 h after 4-d knockdown of *KDM4C*. Total RNA was extracted by RNeasy Plus Mini Kit (Qiagen) following the manufacturer's protocol. Residual DNA was removed by DNase I on column digestion (Qiagen). Knockdown efficiency was detected by qPCR. The ribosome RNA was removed by a Ribo-Zero Magnetic Kit (epicentre) and then subjected to library construction by ScriptSeq v2 RNA-Seq Library Preparation Kit (epicentre), followed by deep sequencing. The RNA-seq reads were aligned to the human genome hg18 using Bowtie2 with ultrasensitive parameters. The RNA-seq reads were counted over gene exons and normalized to 10 million. EdgeR (www.bioconductor.org/packages/release/bioc/vignettes/edgeR/inst/doc/edgeRUsersGuide.pdf) was used for statistical analyses of siCTL and si*KDM4C* samples, and an FC of 1.5 was used to call the differentially expressed genes. For RNA- and GRO-seq computational analyses, multiple gene isoforms were included, as provided by HOMER genome annotation files (http://homer.salk.edu).

In Vitro Demethylation Assay. Briefly, 100 ng recombinant KDM4C (1-460aa, ab167940; Abcam) was incubated with 2 μg histone from calf thymus (Sigma) at 37 °C for 3 h in histone demethylation buffer: 20 mM Tris·HCl (pH 7.4), 150 mM NaCl, 50 μM $Fe(SO4)_2$, 1 mM a-ketoglutarate, and 2 mM ascorbate. Substrate methylation levels were analyzed by immunoblotting with specific antibodies. The membrane was subjected Pouceau S staining to quantify the loading amount. For nondemethylation control, SDS loading buffer (Invitrogen) was added directly to stop the reaction at time 0. Quantitive demethylase assay was performed with an EpiSeeker KDM4/JMJD2 Activity Quantification Assay Kit (Fluorometric) (ab113462; Abcam) following manufacturers' instruction. The 530 nm excitation/590 nm emission was read by Infinite M1000 PRO (TECAN).

DNA-Dependent DNA Polymerase or RNA Polymerase Activity Assay. In vitro DNA-dependent DNA polymerase or RNA polymerase activity assay was performed as described (6) with some modifications. For DNA polymerase activity, qPCR with a gradient concentration of SD70 was performed on ∼200-bp ActB transcript using LNCaP cDNA as template. qPCR was done by VeriQuest Fast Sybr qPCR Master Mix (Affymetrix). For RNA polymerase activity assay, 25 ng PCR product of a 548-bp lncRNA *PRNCR1* fragment with 5′ additional T7 polymerase binding sequence was used as the template for in vitro transcription (Promega). Titrated SD70 at the indicated concentration was mixed with the reaction and the RNA product was denatured and followed by electrophoresis after reacting at 37 °C for 2 h.

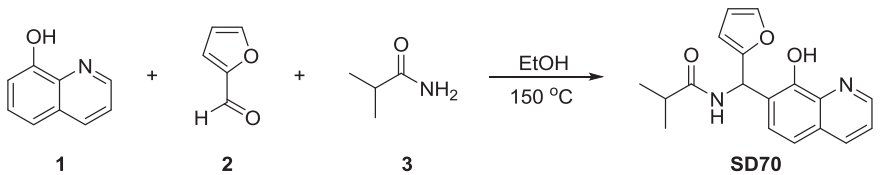
Topoisomerase Assay. One unit of topoisomerase I (M0301S; New England Biolabs) or topoisomerase II (TH2000-1; TopoGEN) was incubated with 200 ng Supercoiled pHOT-1 plasmid (TopoGEN) at 37 °C for 1 h according to manufacturers' instructions. The reaction was terminated by adding GelPilot Loading Dye (Qiagen). The reaction products were analyzed by ethidium bromide-free electrophoresis on 1% agarose gel, then stained by ethidium bromide (1 μg/mL), and photographed using a short-wavelength UV lamp (Syngene Imaging System; Imgen Technologies).

Cell Proliferation Assay. The cell proliferation assay was performed using CellTiter 96 AQueous One Solution Cell Proliferation Assay (MTS; Promega). Briefly, cells were distributed in a 96-well plate with six replicates for each sample. After removing media and having been rinsed once by PBS, cells were supplied with 100 μL PBS mixed with 4 μL MTS reagent, followed by a 1-h incubation at 37 °C in a 5% CO_2 incubator. After incubation, 490-nm absorbance on each well was measured by a light absorbance reader.

Xenografts and Animals. Animal studies were conducted in accordance with the protocols approved by the Institutional Animal Care and Use Committee (IACUC) of the University of California, Davis. Three million of CWR22Rv1 cells were suspended with matrigel in the ratio of 2:1 for s.c. injection into male athymic nude mice. Tumor progression was monitored by caliper measurement twice a week and the tumor volume was calculated according to the equation, $v = \text{length} \times \text{width}^2 \times 1/2$. When the tumor size reached between 150 and 200 mm^3, animals were randomly distributed to groups receiving 10 mg/kg of SD70 via i.p. injection or vehicle once a day with continuous tumor monitoring until the tumor burden exceeded the limit indicated by the IACUC humane endpoints (less than 20 mm in one dimension) for 3–4 wk. For SD70 drug preparation, SD70 powder was first dissolved in DMSO at 50 mg/mL, then diluted into 75% PEG300:25% D5W to arrive at 2.5 mg/mL; 100 μL per animal was used for around 25 g of body weight.

General Information for Characterization of Compounds. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Varain Inova 400 spectrometer at 400 MHz. The chemical shifts are given in parts per million (ppm) on the delta (δ) scale. The solvent peak was used as a reference value (for ^{1}H NMR: $CDCl_3$ = 7.26 ppm). The following abbreviations were used to designate multiplicities: s, singlet; d, doublet; t, triplet; dd, doublet of doublets; m, multiplet. Coupling constant (J) were expressed in Hz.

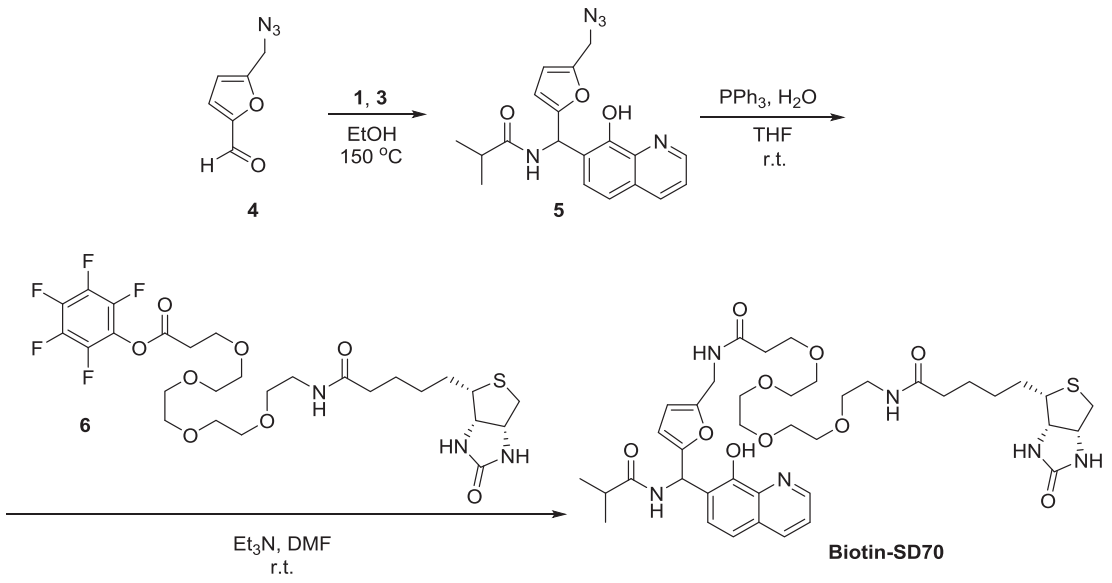
Synthesis of SD70.



8-Hydroxyquinoline (**1**, 580 mg, 4.0 mmol), furfural (**2**, 768 mg, 8.0 mmol), and isobutyramide (**3**, 523 mg, 6.0 mmol) were mixed in ethanol (2 mL) in a glass pressure tube. The mixtures were heated at 150 °C for 3 h, cooled to room temperature, and concentrated

under reduced pressure. The residue was chromatographed on silica gel using gradient elution [hexane/ethyl acetate, first 6/1 (vol/vol) then 1/1 (vol/vol)]. The eluted fractions containing the desired product were combined and concentrated to dryness under reduced pressure. **SD70** was thus obtained as a tan solid (420 mg, 34% yield). The tan solid thus obtained can be further purified into an off-white solid by recrystallization in a 1:1 mixture of hexane:ethyl acetate. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.78 (d, J = 3.2 Hz, 1H), 8.15 (d, J = 8.3 Hz, 1H), 7.48 (d, J = 8.5 Hz, 1H), 7.45 (dd, J = 8.3, 4.3 Hz, 1H), 7.35 (d, J = 8.3 Hz, 1H), 7.09 (d, J = 8.5 Hz, 1H), 6.60 (d, J = 8.7 Hz, 1H), 6.29 (m, 1H), 6.12 (m, 1H), 2.47 (m, 1H), 1.22 (d, J = 6.9 Hz, 3H), and 1.18 (d, J = 6.9 Hz, 3H).

Synthesis of Biotin-SD70.



5-(Azidomethyl)furfural (**4**, 450 mg, 3.0 mmol) was mixed with 8-hydroxyquinoline (**1**, 580 mg, 4.0 mmol) and isobutyramide (**3**, 523 mg, 6.0 mmol) in ethanol (1 mL) in a glass pressure tube. The mixtures were heated at 150 °C for 3 h, cooled to room temperature, and concentrated under reduced pressure. The residue was chromatographed on silica gel using gradient elution [hexane/ethyl acetate, first 6/1 (vol/vol) then 1/1 (vol/vol)]. The eluted fractions containing the desired product were combined and concentrated to dryness under reduced pressure. Compound **5** was thus obtained as a tan solid (120 mg, 33% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.78 (dd, J = 4.2, 1.5 Hz, 1H), 8.15 (dd, J = 8.3, 1.4 Hz, 1H), 7.48 (d, J = 8.6 Hz, 1H), 7.44 (m, 1H), 7.34 (dd, J = 8.5, 4.1 Hz, 1H), 7.08 (d, J = 8.5 Hz, 1H), 6.58 (d, J = 8.0 Hz, 1H), 6.24 (d, J = 3.1 Hz, 1H), 6.09 (d, J = 2.4 Hz, 1H), 4.21 (d, J = 3.4 Hz, 2H), 2.50 (m, 1H), 1.22 (d, J = 6.9 Hz, 3H), 1.18 (d, J = 6.9 Hz, 3H).

Triphenylphosphine (150 mg, 0.57 mmol) and water (0.1 mL) were added to *N*-((5-(azidomethyl)furan-2-yl)(8-hydroxyquinolin-7-yl) methyl)isobutyramide (**5**, 100 mg, 0.27 mmol) dissolved in THF (3 mL). The resulting solution was allowed to stand at room temperature for 8 h and then concentrated under reduced pressure. The residue was dissolved in anhydrous dimethylformamide (2.0 mL), to which was added perfluorophenyl 17-oxo-21-((3a*S*,4*S*,6a*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)-4,7,10,13-tetraoxa-16-azahenicosanoate (**6**, 200 mg, 0.30 mmol) and triethylamine (50 mg, 0.50 mmol). The resulting solution was concentrated under reduced pressure. The residue was chromatographed on silica gel using an eluent composed of dichloromethane/methanol [20/1 (vol/vol)]. The eluted fractions containing the desired product were combined and concentrated to dryness under reduced pressure. **Biotin-SD70** was thus obtained as a heavy oil (15 mg, 7% yield over two steps). ^{1}H NMR (400 MHz, CD_3OD): δ 8.83 (s, 1H), 8.43 (d, J = 7.9 Hz, 1H), 7.56 (s, 1H), 7.26 (d, J = 8.1 Hz, 1H), 7.11 (s, 1H), 7.04 (d, J = 8.6 Hz, 1H), 6.84 (s, 1H), 6.79 (s, 1H), 6.23 (d, J = 3.1 Hz, 1H), 6.12 (d, J = 3.1 Hz, 1H), 4.47 (m, 1H), 4.29 (m, 1H), 3.69 (t, J = 6.2 Hz, 2H), 3.59–3.53 (m, 12H), 3.50 (t, J = 5.5 Hz, 3H), 3.34 (t, J = 5.5 Hz, 3H), 3.16 (m, 1H), 2.90 (dd, J = 12.8, 5.0 Hz, 1H), 2.69 (d, J = 12.6 Hz, 1H), 2.53 (m, 1H), 2.44 (t, J = 6.1 Hz, 2H), 2.19 (t, J = 7.4 Hz, 2H), 1.70–1.30 (m, 6H), 1.14 (d, J = 6.9 Hz, 3H), and 1.08 (d, J = 6.9 Hz, 3H).

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2. Tomlins SA, et al. (2007) Distinct classes of chromosomal rearrangements create oncogenic ETS gene fusions in prostate cancer. *Nature* 448(7153):595–599.
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5. Li W, et al. (2013) Functional roles of enhancer RNAs for oestrogen-dependent transcriptional activation. *Nature* 498(7455):516–520.
6. Momparler RL, Karon M, Siegel SE, Avila F (1976) Effect of adriamycin on DNA, RNA, and protein synthesis in cell-free systems and intact cells. *Cancer Res* 36(8):2891–2895.

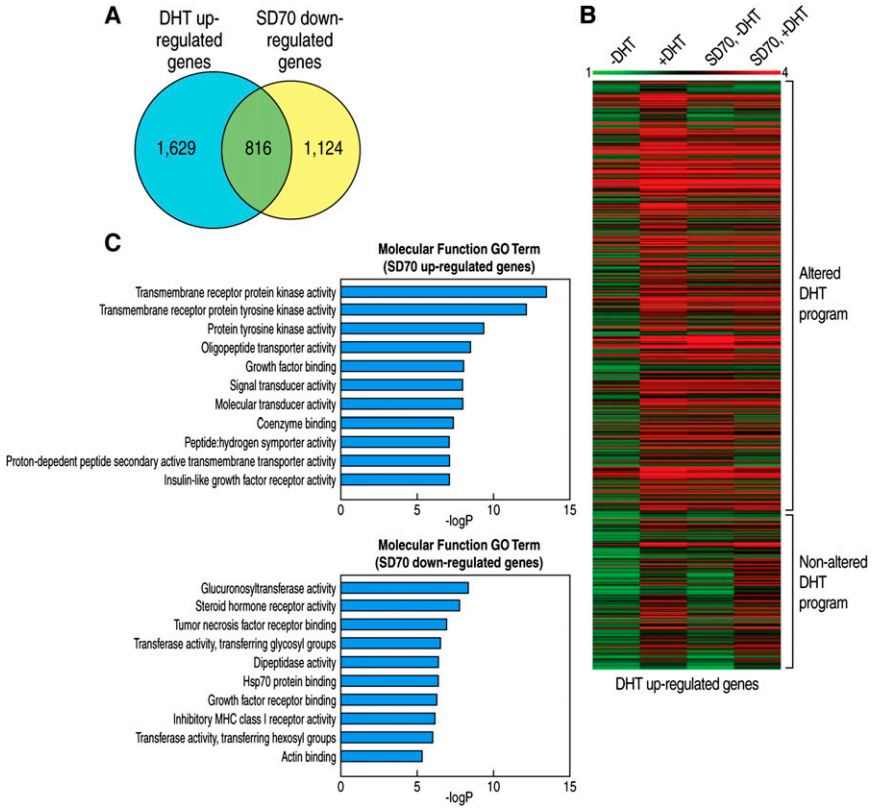

Fig. S1. GRO-seq analysis reveals the altered AR program by SD70. (*A*) Schematic diagram of SD70-suppressed genes overlapping with DHT-induced genes. (*B*) Hierarchical clustering of 2,445 DHT up-regulated genes in four GRO-seq samples. (*C*) Top GO-term of SD70 up- or down-regulated genes in the DHT-treated condition.

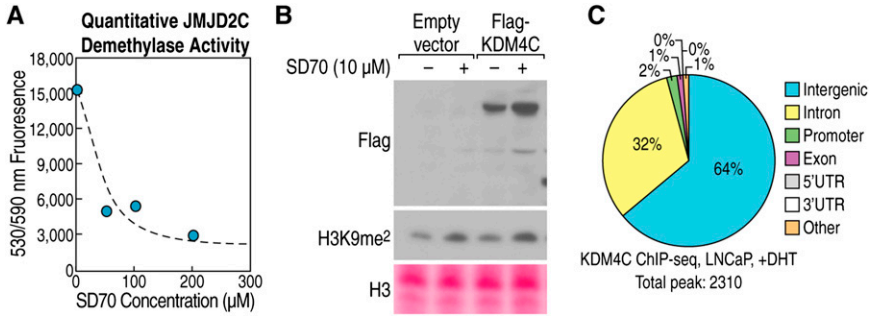

Fig. S2. SD70 is KDM4C (JMJD2C) enzymatic inhibitor. (*A*) Quantitative in vitro demethylase assay of different SD70 concentrations (0, 50, 100, and 200 μM) and the different 200-ng KDM4C (1–460 aa) 530-nm/590-nm fluorescence intensity is an indicator of demethylase activity (using EpiSeeker KDM4/JMJD2 Activity Quantification Assay Kit, ab113462). (*B*) Western blot in 293T cells to detect the whole-cell H3K9me2 level changed by SD70 in control or the KDM4C overexpression condition. (*C*) The KDM4C peaks distribution revealed by ChIP-seq.

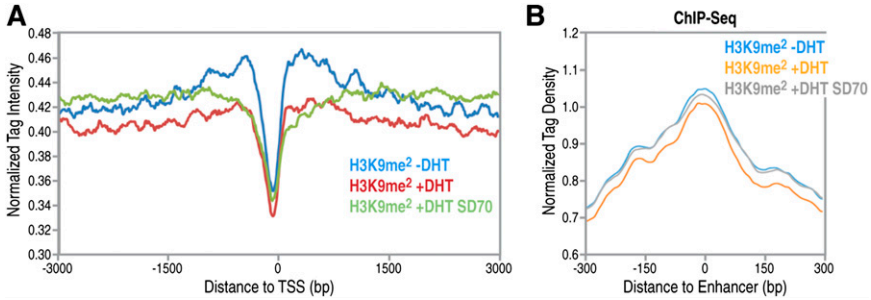

Fig. S3. SD70 rescued the DHT-induced H3K9me2 lost in the gene transcriptional regulatory region. H3K9me2 ChIP-seq tag density profile of *A*, the transcriptional start site ±3-kb region by bin size of 10 nt; and *B* showing the enhancer region with or without indicated SD70 (10 μM, 24 h) or DHT (100 nM, 1 h) treatment in LNCaP cells.

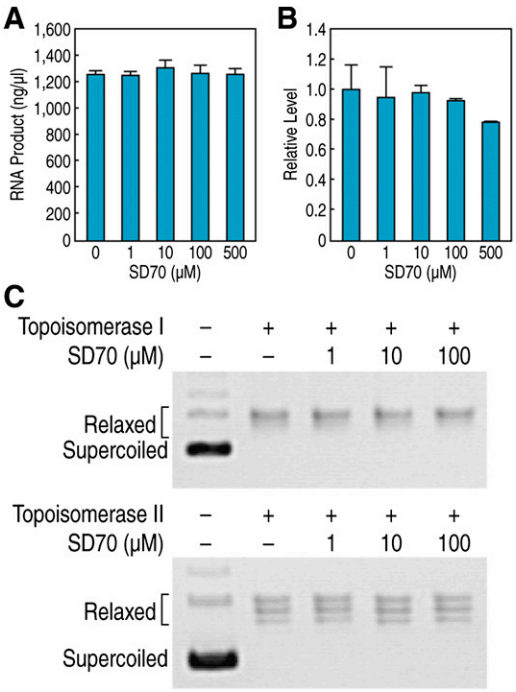

Fig. S4. SD70 exerts no major effects on DNA-dependent DNA/RNA polymerase or topoisomerase activity. (*A*) Measurement of the SD70 effect on RNA synthesis. In vitro transcription assay for equal amounts of template was incubated at the indicated concentrations of SD70, and the amount of final RNA product was analyzed by concentration measurements. (*B*) qPCR for the ActB gene fragment at the indicated concentration of SD70, as an indicator of DNA polymerase activity. All reactions were loaded with the same amount of cDNA template sample, and the results were normalized by untreated sample. Error bars represent SD of three repeats. (*C*) In vitro plasmid conformation capture assay for the detection of topoisomerase I (*Upper*) or topoisomerase II (*Lower*) activity with supercoiled pHOT-1 plasmid at the indicated concentrations of SD70.

Other Supporting Information Files

Table S1 (XLSX)