Supporting Information

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SI Methods

Cell Culture. Embryonic carcinoma NCCIT cells (American Type Culture Collection) were cultured in RPMI medium 1640 (Life Technologies) supplemented with 10% FBS. Colorectal cancer cell lines HCT116 and DKO1 (a hypomethylated derivative of HCT116, DNMT1$^{\Delta E2-5}$/DNMT3B$^{-/-}$ double knockout) have been previously described (1). Normal human embryonic stem cells (H1), normal human fibroblasts (LD419), and keratinocytes (HaCaT) were cultured under recommended conditions.

Nucleosome Occupancy and Methylome Sequencing (NOMe-seq). Methylase-based single-molecule DNA assay was performed as previously described (2, 3) with minor modifications. After nuclei extraction, GpC methyltransferase (M.CviPI; New England Biolabs) reactions were done in M.CviPI reaction buffer. GpC methyltransferase treatment was followed by DNA extraction, sodium bisulfite conversion, PCR amplification of the regions of interest, cloning, and sequencing of individual clones to reveal the structure of single replicas as functional units. Cells were trypsinized and centrifuged for 3 min at 500 × *g*. Cell pellets were washed in ice-cold PBS, resuspended in 1 mL of ice-cold nuclei buffer [10 mM Tris (pH 7.4), 10 mM NaCl, 3 mM $MgCl_2$, 0.1 mM EDTA, and 0.5% Nonidet P-40, plus protease inhibitors] per 5 × 10^6 cells, and incubated on ice for 5 min. Nuclei were recovered by centrifugation at 900 × *g* for 3 min, washed in nuclei wash buffer [10 mM Tris (pH 7.4), 10 mM NaCl, 3 mM $MgCl_2$, and 0.1 mM EDTA containing protease inhibitors], and resuspended at a concentration of 4.0 × 10^6 cells per mL in 1× M.CviPI reaction buffers. Purified genomic DNA was treated with 200 U of M.CviPI for 15 min at 37 °C. Reactions were stopped by the addition of an equal volume of stop solution [20 nM Tris·HCl (pH 7.9), 600 mM NaCl, 1% SDS, 10 mM EDTA, and 400 μg/mL Proteinase K] and incubated at 55 °C overnight. DNA was purified by phenol/chloroform extraction and ethanol precipitation. Bisulfite conversion was performed with the EpiTect Bisulfite Kit (Qiagen). Molecules were cloned with the TOPO TA Kit (Invitrogen), both according to the manufacturers' instructions. Primers used were as follows. OCT4 distal enhancer: 5′-TGAGAAATATTGGTGTGGAGATTTT-3′, 3′-TTAAAACTTTTCCCCCACTCTTATA-5′; OCT4 proximal promoter: 5′-GTTTGGTTTTTTGAAGGGGAAGTAGGGATT-3′, 3′-CCACACCTCCATATTCTTCAAAAACCCAAA-5′; OCT4 proximal promoter: 5′-TGAAGAATATGGAGGTGTGGGAGTGATTTTAGAT-3′, 3′-TAGCCCATCACCTCCACCACCTAAAAAAA-5′; NANOG proximal promoter (A): 5′-GTTATTTTAAATTAAGAAATTATTTTAATGAGAATTTT-3′, 3′-TCCAACTTTTAAATCAAAAATATAATTCAACAA-5′; and NANOG proximal promoter (B): 5′-TTGAATTATATTTTTGATTTAAAAGTTGG-3′, 3′-TTAAAATCCT AAATCTCTAAATTTATAATA-5′.

Reagents and Antibodies. Commercial primary antibodies used were anti-Oct4, anti-Sox2, anti-Nanog, anti-H3, anti-H3K4me3, and anti-H3K9me3 (Abcam); anti-AcH3 (Millipore Laboratories); and anti-BAF155 and anti-BAF170 (Santa Cruz Biotechnology).

RNA Extraction and RT-PCR. Total RNA was extracted with TRIzol reagent, digested with DNase I, and reverse-transcribed with SuperScript III Reverse Transcriptase (Invitrogen). PCRs were performed with gene-specific primers and probes. Total RNA was extracted with TRIzol reagent, digested with DNase I, and reverse-transcribed with SuperScript III Reverse Transcriptase (Invitrogen). Amplification of cDNA was performed on an Opticon light real-time PCR cycler (Bio-Rad) using KAPA Probe Fast qPCR Mix (Kapa Biosystems) under the following conditions: 95 °C for 3 min, followed by 45 cycles of 95 °C for 3 s, and then 60 °C for 30 s. Analyses were conducted in parallel, using human proliferating cell nuclear antigen and GAPDH mRNA primers for normalization. Total OCT4 RT: 5′-CCCTGGTGCCGTGAAGC-3′, 3′-TTGCTCGAGTTCTTTCTGCAGA-5′, probe—AGCAAAACCCGGAGGAGTCCCAGG; endogenous OCT4 RT: 5′-CCGTCACCACTCTGGGCT-3′, 3′-CCCCCATTCCTAGAAGGGC-5′, probe—TCCCATGCATTCAAACTGAGGTGCC; NANOG RT: 5′-GCAGAAGGCCTCAGCACCTA-3′, 3′-AGTCGGGTTCACCAGGCAT-5′, probe—CTACCCCAGCCTTTACTCTTCCTACCACCA; SOX2 RT: 5′-AGAAGTTTGAGCCCCAGGCT-3′, 3′-CTCTGGCCGATCCTGCC-5′; KLF4 RT: 5′-CCATTACCAAGAGCTCATGCC-3′, 3′-CGATCGTCTTCCCCTCTTTG-5′; C-MYC RT: 5′-ATCCATGAGCAGGACCTGGA-3′, 3′-GATGGCTGTGTCACTTCTGCC-5′; AP RT: 5′-TGGGAGATGGGATGGGTGT-3′, 3′-TTGTGGTGGAGCTGACCCTT-5′; LIN28 RT: 5′-CCGGACCTGGTGGAGTATTCT-3′, 3′-CGCTTCTGCATGCTCTTTCC-5′; REST RT: 5′-GCAACAAAGAAAAGTAGTCGGAGAA-3′, 3′-ATTCGGCCTTCCTCGGTG-5′; CDX2 RT: 5′-TCACTACAGTCGCTACATCACCATC-3′, 3′-ATTTTAACCTGCCTCTCAGAGAGC-5′; PAX6 RT: 5′-CCTATGCCCAGCTTCACCAT-3′, 3′-GGCAGCATGCAGGAGTATGAG-5′; and NEUROG1 RT: 5′-GCAGTGACCTATCCGGCTTC-3′, 3′-GGAGGCTGCCTGTTGGAGT-5′.

Western Blot Analysis. Cell lysates were boiled in Laemmli sample buffer for 3 min, and 30-μg samples of each protein were subjected to SDS/PAGE.

ChIP Assay. ChIP assays were performed according to the Upstate Biotechnology instructions. For each ChIP assay, 100 μg of DNA sheared by a sonicator was precleared with salmon-sperm DNA-saturated protein A Sepharose and then precipitated by H3 antibody and others. After immunoprecipitation, recovered chromatin fragments were subjected real-time PCR. IgG control experiments were performed for all ChIPs and were accounted for in the IP/Input by presenting the results as (IP − IgG)/(Input − IgG). Primers used were as follows. OCT4 distal enhancer: 5′-GAGGATGGCAAGCTGAGAAA-3′, 3′-CTCAATCCCCAGGACAGAAC-5′; OCT4 proximal enhancer: 5′-TCTGTTTCAGCAAAGGTTGGG-3′, 3′-TTGGTCCCTACTTCCCCTTCA-5′; OCT4 proximal promoter: 5′-GCAAACATCCTTCGCCTCAG-3′, 3′-GTGAAATGAGGGCTTGCGAAG-5′; NANOG proximal promoter (A): 5′-ATCCCCCACCCCCCC-3′, 3′-TATCGCGCCACTGTACTCCAG-5′; and NANOG proximal promoter (B): 5′-TTGTTGCTGGGTTTGTCTTCAG-3′, 3′-AAAGTAGCTGCAGAGTAACCCAGACT-5′.

siRNA transfection. Cells were transfected with scrambled or target gene-specific siRNA using Lipofectamine LTX (Invitrogen). siRNAs specifically targeting OCT4 were purchased from Dharmacon.

Introduction of Exogenous OCT4. Cells were transfected with mock or exogenous OCT4 expression vectors using Lipofectamine LTX (Invitrogen). Exogenous OCT4 expression vectors were purchased from Addgene.

1. Egger G, et al. (2006) Identification of DNMT1 (DNA methyltransferase 1) hypomorphs in somatic knockouts suggests an essential role for DNMT1 in cell survival. *Proc Natl Acad Sci USA* 103:14080–14085.
2. Wolff EM, et al. (2010) Hypomethylation of a LINE-1 promoter activates an alternate transcript of the MET oncogene in bladders with cancer. *PLoS Genet* 6:e1000917.
3. Meissner A, et al. (2008) Genome-scale DNA methylation maps of pluripotent and differentiated cells. *Nature* 454:766–770.

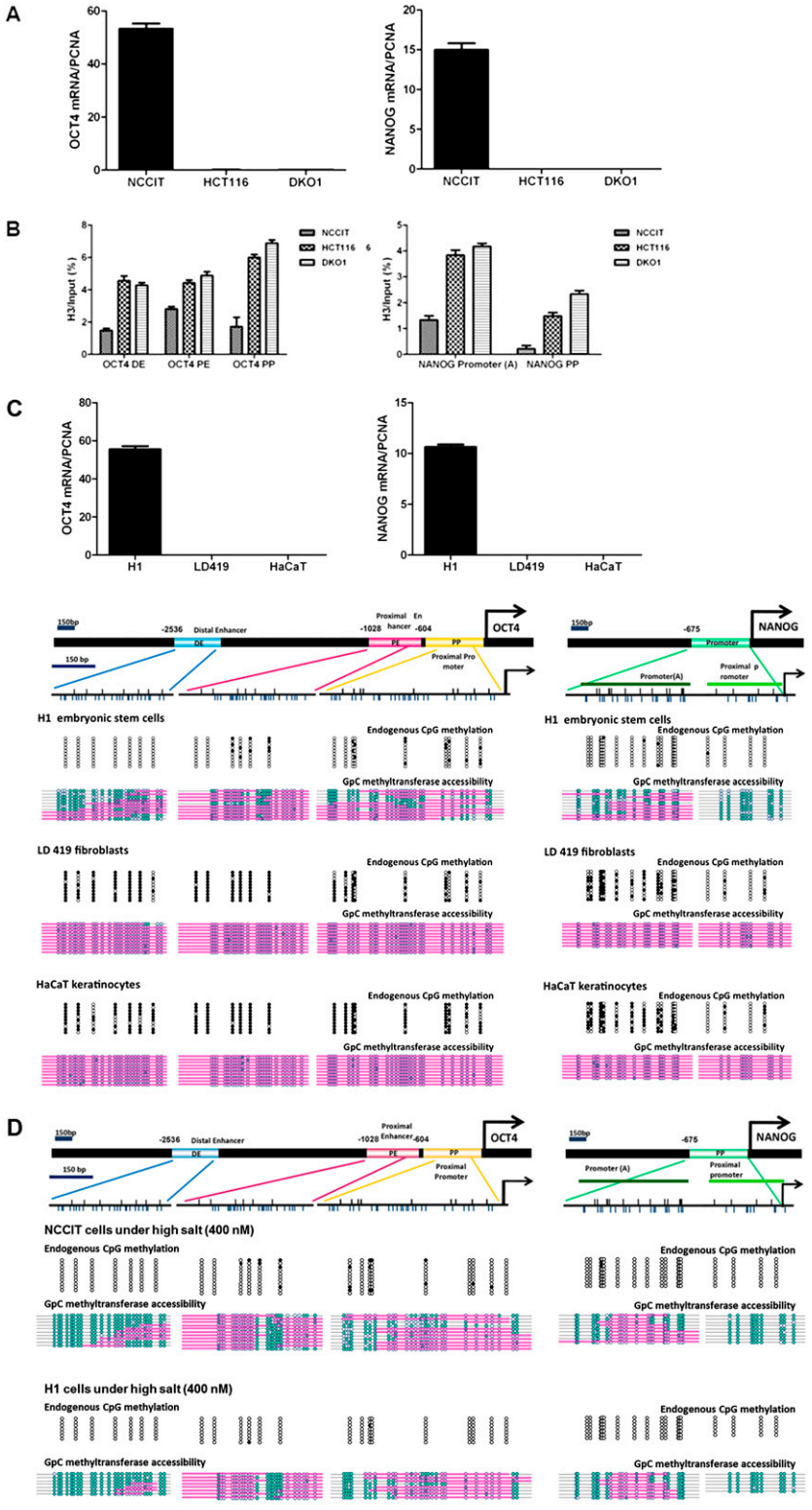

Fig. S1. (*A*) The levels of expression of OCT4 and NANOG in NCCIT, HCT116, and DKO1 cells. OCT4 and NANOG mRNA levels were analyzed. Bars are the means of three biological replicates ± SEM. (*B*) Core histone (H3) ChIP at the OCT4 distal enhancer and NANOG proximal promoter in NCCIT, HCT116, and DKO1 cells. Bars are the means of three biological replicates ± SEM. (*C*) The levels of expression of OCT4 and NANOG (*A*) and endogenous DNA methylation and nucleosome occupancy (*B*) in H1, LD419, and HaCaT cells. (*D*) Endogenous DNA methylation and nucleosome occupancy under high salt concentration in NCCIT and H1 cells.

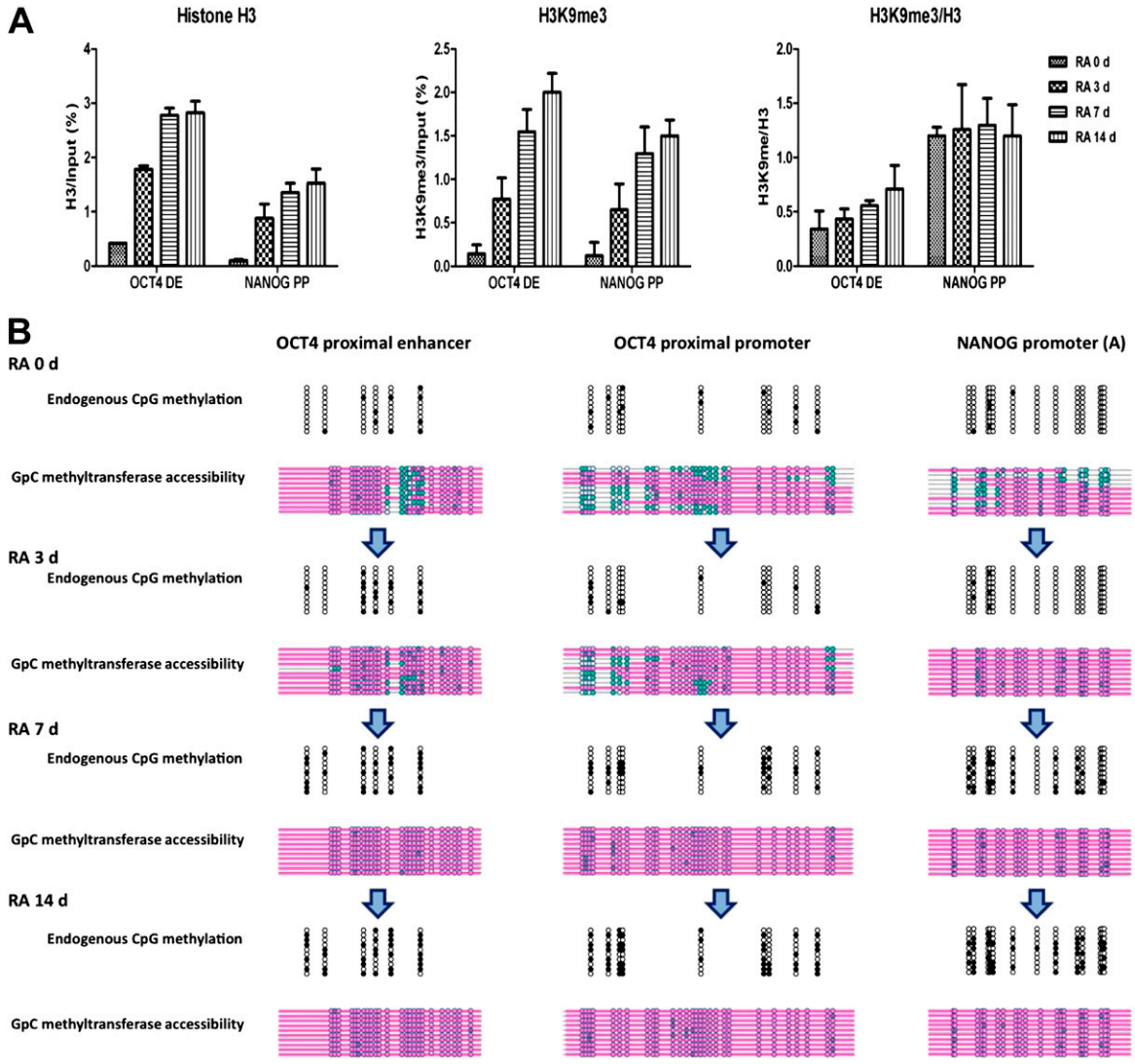

Fig. S2. (*A*) Core histone (H3) and H3K9me3 ChIP at the OCT4 distal enhancer and NANOG proximal promoter during the differentiation. (*B*) Endogenous DNA methylation and nucleosome occupancy changes at the other DNA regulatory regions of OCT4 and NANOG.

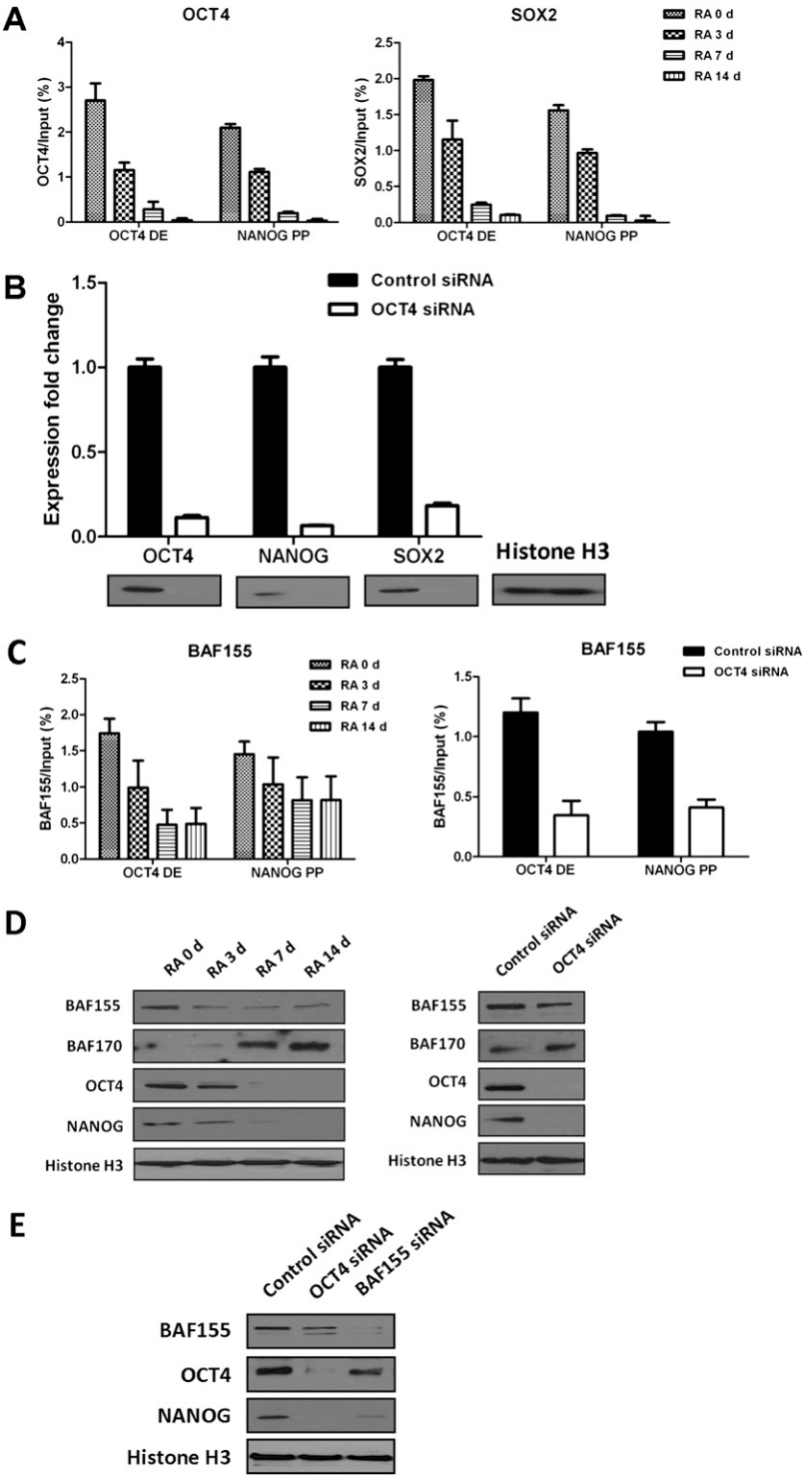

Fig. S3. (*A*) The change of OCT4 and SOX2 binding. ChIP from NCCIT cells was immunoprecipitated with anti-OCT4 and anti-SOX2 antibodies, and their binding to the OCT4 distal enhancer and NANOG proximal promoter was analyzed by quantitative PCR. (*B*) OCT4 knockdown experiments. At 72 h post-transfection with OCT4-targeted and control siRNAs, OCT4, NANOG, and SOX2 mRNA and protein levels were analyzed. Bars are the means of three biological replicates ± SEM. Western blotting data are representative of three biological experiments. (*C* and *D*) The recruitment of the chromatin-remodeling factors. (*C*) The recruitment of the chromatin-remodeling Brg1/Brm-associated factor (BAF) complex was analyzed by ChIP assay using anti-BAF155. Bars are the means of three biological replicates ± SEM. (*D*) The expression of BAF155, BAF170, OCT4, NANOG, and histone H3 were analyzed by Western blotting during retinoic acid (RA)-induced differentiation and OCT4 siRNA knockdown states. The data are representative of three biological experiments. (*E*) At 72 h posttransfection with BAF155 or OCT4 siRNA in NCCIT cells, OCT4, NANOG, and BAF155 were analyzed by Western blot.

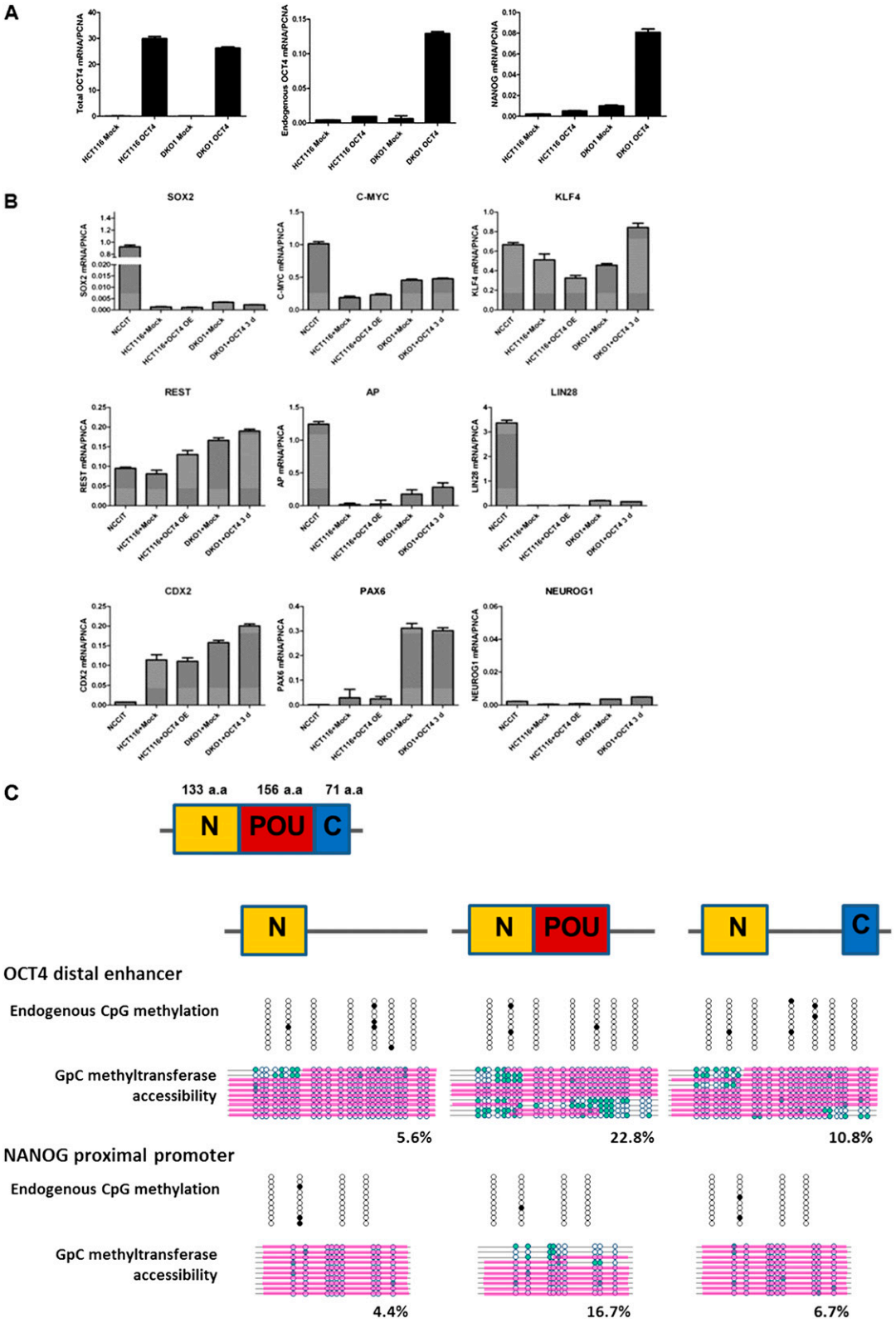

Fig. S4. (*A*) Total and endogenous OCT4 and NANOG expression after exogenous OCT4 induction. For introduction of exogenous OCT4, transfection of HCT116 and DKO1 cells with OCT4 and mock vectors was carried out with Lipofectamine LTX. At 72 h posttransfection, quantitative PCR was performed with specific primers for total and endogenous OCT4 and NANOG expression. Bars are the means of three biological replicates ± SEM. (*B*) The levels of expression of OCT4 downstream target genes after transfection of exogenous OCT4. At 72 h posttransfection of exogenous OCT4, quantitative PCR was performed in HCT116 and DKO1 cells. Bars are the means of three biological replicates ± SEM. (*C*) Endogenous DNA methylation and nucleosome occupancy changes after transfection of deletion mutants of OCT4. At 72 h posttransfection of deletion mutants of OCT4, the NOMe-seq assay was performed in DKO1 cells.

Table S1. OCT4 proximal enhancer putative transcription factors with Genomatix MatInspector (http://www.genomatix.de/online_help/help_matinspector/matinspector_help.html)

Matrix family	Detailed family information	Sequence (5′ → 3′)
SMAD	Vertebrate SMAD family of transcription factors	aGTCTgggc
THAP	THAP domain-containing protein	agtctgGGCAa
LEFF	LEF1/TCF	tgggcaaCAAAgtgaga
SORY	SOx/sRY-sex/testis determining and related HMG box factors	ctgggcaACAAagtgagaccctg
IRFF	IFN regulatory factors	gcaacaaagtGAGAccctgtc
SORY	SOx/sRY-sex/testis determining and related HMG box factors	ggcaacaaagtgagaccCTGTct
ABDB	Abdominal-B type homeodomain transcription factors	ccctgtctTAAAaaata
ABDB	Abdominal-B type homeodomain transcription factors	ttaaaaaaTAAAaataa
MEF2	MEF2, myocyte-specific enhancer binding factor	ttaaaaaaTAAAaataaaaaaag
SALL	Spalt-like transcription factors	aaATAAaaataaa
SALL	Spalt-like transcription factors	aaATAAaaaaagt
HOMF	Homeodomain transcription factors	aaaataaaAAAAgtttctg
MYT1	MYT1 C2HC zinc finger protein	aaaAAGTttctgt
XCPE	Activator-, mediator-, and TBP-dependent core promoter element for RNA polymerase II transcription from TATA-less promoters	gtGGGGgacct

LEF1, lymphoid enhancer-binding factor 1; MYT1, myelin transcription factor 1; SMAD, SMAD family member; TBP, TATA box binding protein; TCF, transcription factor; and THAP, THAP domain containing, apoptosis associated protein.