**Supplementary Information**

**Supplementary Materials and Methods, Supplementary Figure Legends and Supplementary Tables**

**Supplementary Materials and Methods**

**Cell lines, cell culture, clinic sample, plasmids, and gene knockdown**

The breast cancer cell lines MCF7 and T47D were maintained in DMEM containing 10% FBS, non-essential amino acids (HyClone), sodium pyruvate (HyClone), and antibiotics (HyClone). Other cell lines were maintained in their respective media according to ATCC protocol. Matched tissue samples were obtained from Abcam (ab43503, ab43504, ab43507 and ab43508). HEK293T cells for virus packaging were co-transfected with packaging plasmid (pCMV-Δ8.91), envelope (pMDG), and hairpin pLKO-RNAi vectors (National RNAi Core Facility, Institute of Molecular Biology / Genomic Research Centre, Academia Sinica, Taiwan, ROC). The specific oligo sequences of shRNA are listed in Table S2. Virus-containing supernatants were collected at 48 hours post-transfection. To perform gene knockdown, the cells were treated with virus plus medium containing polybrene (8 μg/ml) for 16 hours. The transduced cells were selected with puromycin (1 μg/ml).

***In vitro* HMT assay**

GST-tagged human SMYD3 and mutant SMYD3Y239F proteins were purified from *E. coli*,and an *in vitro* HMT assay was performed with 10 μg of calf thymus histones (Sigma), recombinant histones or purified nucleosomes as substrates. The HMT buffer containing 20 mM Tris-HCl, pH8.0, 100 mM NaCl, 2 mM MgCl2, 1 mM CaCl2, 10 mM DTT and protease inhibitor with 0.5μCi SAM, *S*-adenosyl-L-[methyl-3H]-methionine, (PerkinElmer Life Sciences) as the methyl donor and was incubated with enzymes and substrates for 2hours at 30°C. The samples were resolved by SDS-PAGE and stained with Coomassie blue. To determine the methylated site of human H2A.Z.1, recombinant His-tagged H2A.Z.1 and GST-tagged SMYD3 were co-incubated with cold SAM. HPLC-MS/MS was employed to identify the methylated sites of histones. To perform an *in vitro* HMT assay using nucleosomes as substrates, GST-tagged SMYD3 and SMYD3Y239F proteins were co-incubated with SAM, *S*-adenosyl-L-methionine, (A7007, Sigma) and nucleosomes isolated from shSMYD3 MCF7 cells ([1](#_ENREF_1)). Reactions were subjected to Western blot analyses using specific antibodies against SMYD3, H2A.ZK101me2, H2A.Z, H4K5me, H4K20me3, H4, H3K4me3 and H3. Nucleosome contents wereexamined by Coomassie blue staining.

**Constructs for SMYD3, H2A.Z, ANP32E and cyclin A1 expression**

SMYD3WT and SMYD3Y239F cDNAs were inserted into *EcoR*I and *Xho*I sites of the pCDNA3.1/myc-His vector. HEK293T and MCF7 cells were seeded and transfected with control vector and SMYD3 expression plasmids using the Maestrofectin transfection reagent (Maestrogen Inc.). Plasmids for exogenous H2A.Z (H2A.Z.1) expression were a gift from Dr. Gaudreau ([2](#_ENREF_2)). The mutant constructs pWPI-H2A.ZK101Q and pWPI-H2A.ZK101R were generated by mutagenesis PCR. The primers used for mutagenesis are listed in Table S2. HEK293T cells were transfected with GFP-tagged pWPI constructs and the packaging vectors to produce viruses. Virus-infected H2A.ZWT, H2A.ZK101Q and pWPI-H2A.ZK101R MCF7 and T47D cells were subjected to FACS for selecting stable GFP-expressing cells. The ANP32E constructs were generated by inserting ANP32E cDNA from the GST-ANP32E plasmid (a gift from Dr. Ali Hamiche ([3](#_ENREF_3))) into the pCDNA3.1/myc-His vector. For cyclin A1 complementation, cyclin A1 cDNA was inserted into *Eco*RV and *Eco*RI sites of the pLAS3w.Pneo vector (National RNAi Core Facility, Institute of Molecular Biology / Genomic Research Centre, Academia Sinica, Taiwan, ROC). Lentiviruses of control vector RFP and cyclin A1 were produced as described above and infected cells were selected with neomycin (400 μg/ml) for 7 days.

**Generation of antibodies, dot blot analysis, and peptide competition assays**

The peptide DEELDSLIKme2ATIAGGGC was designed for the dimethylation of H2A.Z at residue K101. A cysteine residue was added to the C-terminus to facilitate conjugation with a carrier protein for higher immunogenicity. Pre-immune serums were collected before boost. To generate antibodies, rabbits were boosted with carrier conjugated-peptides once every 20 days and blood samples were collected every 10 days. Blood samples were incubated at 37°C for 30 min. Serum and blood cells were separated by high-speed centrifugation. Clarified serum was incubated at 56°C for 30 min to remove complement.

The specificity of antibodies was verified by means of peptide dot blot analysis and peptide competition assays. Uncropped blots were provided in Fig. S2F. For dot blot analysis, two biotin-labeled H2A.Z peptides DEELDSLIKATIAGGGVggk-biotin (with ggk as a linker, labeled as K101) and DEELDSLI*K*me2ATIAGGGVggk-biotin (with ggk as a linker, labeled as K101me2) were serially diluted and spotted onto a nitrocellulose membrane (162-0112, Bio-Rad), and the specificity of antibodies was verified. ExtrAvidin-Peroxidase (E2886, Sigma) was used in the detection as a loading control. For peptide competition assays, MCF7 cell lysates were subjected to Western blot analyses using H2A.ZK101Ame2 antibody in the presence or absence of 1 μg/ml H2A.ZK101me2 peptides.

**Western blot analyses and antibodies used**

Cells were collected, washed twice with phosphate-buffered saline (PBS) buffer, and then dissolved in sample buffer. After centrifugation, the supernatantswereseparated by SDS*-*PAGE*,* transferred to polyvinylidene diﬂuoride (PVDF) membrane with a 0.22-μm pore size (Immobilon PSQ, Millipore), and blocked overnight with 5% milk in TBS-T (Tris-buffered saline with 0.1% Tween-20) at 4°C. The primary antibodies usedwere anti-histone H2A.ZK101me2 (this study), histone H2A.Z (ab4174, Abcam), histone H2A (ab18255, Abcam), histone H2B (07-371, Millipore; ab1790, Abcam), histone H3 (ab18521, Abcam), histone H4 (ab10158, Abcam), histone H4K5me (PTM-696, PTM Biolabs), H4K20me3 (ab177190, Abcam), H3K4me3 (ab8580, Abcam) , cyclin A1 (ab53699, Abcam), nuclear matrix protein p84 (NB100-174, Novus), c-Myc (11667203001, Roche), His (27-4710-01, GE Healthcare), Flag (F3165, Sigma), ANP32E (SAB2100124-50UG, Sigma), YL1 (GTX102152, GeneTex), SRCAP (sc-133312, Santa Cruz), GST (sc-138, Santa Cruz), RUVBL1 (GTX111294, GeneTex), Tip60 (10827-1-AP, Proteintech), p400 (ab70301, Abcam), SMYD3 (GTX121945, GeneTex) and GAPDH (GTX100118, GeneTex). The quantification of protein expression was performed using ImageJ software (Image Processing and Analysis in Java). All protein expression levels were normalized against the corresponding control protein levels as indicated. Images were representatives of n ≥ 3 for each experiment.

**Histone stability and salt extraction assay**

MCF7 cells expressing H2A.ZWT, H2A.ZK101Q and H2A.ZK101R were treated with 10 and 20 μM MG132 for 24 hours and harvested for mRNA and protein expression level measurements. MCF7 cells with shLuc or shSMYD3 knockdown were treated with 100 μg/ml of cycloheximide (CHX) for the indicated time periods and H2A.Z protein expression was analyzed. The histone extraction and stability assay were performed as previously described ([4](#_ENREF_4)). Briefly, histone extracts were prepared and then washed several times in cold-filtered sterilized buffer A (0.25 M sucrose, 3 mM CaCl2, 1 mM Tris-HCl, pH 8.0, 0.1% Triton X-100). The salt stability assays were modified as previously described ([5](#_ENREF_5)). Briefly, the nuclei were isolated by centrifugation at 3900 rpm for 5 min at 4°C. The supernatant was removed leaving the pellet of nuclei. After washing with buffer A, chromatin was incubated with buffer B (10 mM Tris-HCl, pH 7.5, 1 mM DTT, 1 mM EDTA, 1x protease inhibitors (Roche Applied Science), 0.1% Triton X-100) containing different salt concentrations ranging from 100 mM to 800 mM NaCl for 30 min at room temperature. The chromatin was pelleted, solubilized in the same manner as in the fractionation experiments and analyzed by immunoblotting.

**MNasesensitivity assay**

The MNase sensitivity assay was performed as previously described ([6](#_ENREF_6)) with modifications. In brief, nuclei were isolated using a hypotonic buffer (Tris-Cl, pH 7.5, 10 mM NaCl, 1 mM CaCl2, 3 mM MgCl2, 0.5% Nonidet P-40) and treated with increasing dosages of MNase (M0247S, NEB) for 15 min at 37°C. Reaction was terminated by addition of 5 mM of EDTA. DNA was then purified by ethanol in the presence of 0.3 M sodium acetate (pH 5.2) and dissolved in Tris-EDTA buffer. The purified DNA was then separated on a 2% Tris-acetate EDTA agarose gel post-stained with EtBr. Quantification analysis was done by ImagJ software as previously described ([7](#_ENREF_7)).

**Immunoprecipitation assay**

MCF7 cells stably expressing Flag-H2A.ZWT, Flag-H2A.ZK101Q and Flag-H2A.ZK101R were lysed in salt lysis buffer (50 mM Tris-HCl, pH 7.9, 10 mM EDTA, 1% SDS, 1 mM DTT, 1 mM EDTA, 0.5 M NaCl and a complete protease inhibitor (Roche Applied Science)) with sonication, and then diluted 10 times with dilution buffer (16.7 mM Tris-HCl, pH 7.9, 1.2 mM EDTA, 0.01% SDS, 1.1% Triton X-100 and 125 mM NaCl). In each immunoprecipitation reaction, 2 mg of whole-cell extracts from these cells were mixed with 1 μg anti-Flag or anti-c-Myc antibody for 1 hour at 4°C, and the antibody/protein complexes were co-incubated with 50 μl of protein G-coupled sepharose beads (GE Healthcare) overnight. After extensive washing with dilution buffer for three times, the bound proteins were eluted with 40 μl of SDS sample buffer and used for a Western blot analysis. For the ANP32E co- immunoprecipitation assays, MCF7 cells with 2 days SMYD3 knockdown were transfected with the pCDNA3.1/Myc-His ANP32E plasmid for 24 hrs. Cells were harvested and immunoprecipitation assays were performed with anti-IgG or anti-Flag antibodies as described above.

**Pull-down assay**

H2A.Z peptides containing amino acids 93-109re generated according to the following sequences: DEELDSLIKATIAGGGVggk-biotin (with ggk as a linker, labeled as K101) and DEELDSLI*K*me2ATIAGGGVggk-biotin (with ggk as a linker, labeled as K101me2). For the GST pull-down assay, GST and GST-ANP32E (a gift from Dr. Ali Hamiche ([3](#_ENREF_3))), proteins were immobilized onto 50 µl of Glutathione Sepharose™ 4B beads (GE Healthcare), incubated with 40 µg of biotinylated peptides for 16 hours at 4°C with rotation in binding buffer (50 mM Tris-HCl, pH 7.9, 150 mM NaCl, 0.1% NP-40), and then washed 3 times in the same buffer. The bound proteins and peptides were eluted with 20 μl of SDS sample buffer, and 2 μl of the samples were used for dot blotting. Anti-GST (sc-138, Santa Cruz) antibody and ExtrAvidin-Peroxidase (E2886, Sigma) were used for detection.

For His tagged histone H3 interaction, histone H3 was cloned into pET28a (Novagen) and expressed in the *E. coli* BL21-CodonPlus strain (Agilent Technologies). Recombinant His6-tagged proteins were purified with 50 μl of Ni-NTA-agarose beads (Qiagen) according to the manufacturer’s instructions. MCF7 cells stably expressing Flag-H2A.ZWT and Flag-H2A.ZK101Q were extracted as above, and 2 mg of protein lysates were co-incubated with His6-tagged H3 overnight at 4°C with rotation in binding buffer (50 mM NaH2PO4, pH 7.9, 250 mM NaCl, 20 mM imidazole, 0.5% Triton X-100), and then washed three times in the same buffer. Specific antibodies against His (27-4710-01, GE Healthcare) and Flag were used for a Western blot analysis to detect pulled down H3 and co-pulled down H2A.ZWT or H2A.ZK101Q.

For the peptide pull-down assay, 10 µg of biotinylated peptides were immobilized onto 50 µl of avidin beads (Thermo Scientific), and incubated with T47D cell lysates. Peptide-protein complexes were processed as described above. Specific antibodies were employed to detect individual protein signals.

**ITC experiments**

Calorimetric titrations of GST-ANP32E with H2AZK101 and H2A.ZK101me2 peptides were performed on an iTC200 microcalorimeter (GE) at 20 and 30°C for determination of binding enthalpy and affinity. Protein samples were extensively dialysed against the ITC buffer containing 25 mM KPi (pH 8.0), 250 mM KCl and 1 mM Tris(2-carboxyethyl)phosphine (TCEP). All solutions were filtered using membrane filters (pore size 0.45 µm and thoroughly degassed for 20 min by gentle stirring under vacuum. The 200-µl sample cell was filled with a 100 µM solution of protein and the 40-µl injection syringe, with 1.6 mM of the titrating peptides. Each titration involved a preliminary 0.2-µl injection followed by 18 subsequent 2-µl injections. Binding isotherms were generated by plotting heats of reaction normalized by the moles of injectant versus the ratio of total injectant to total protein per injection. The titration data were analyzed using the program Origin 7.0 and non-constrained fitting was performed for the interaction of GST-ANP32E with peptides, in which the directly measured heat changes upon addition of small volumes of each ligand permit extraction of the enthalpy (Δ*H*), the binding affinity (*K*a), and the stoichiometry (*N*) of the interaction. The remaining thermodynamic parameters, including the binding free energy (Δ*G*) and the entropy (Δ*S*) of the interaction were calculated using the relationship

*ΔG = ΔH – TΔS = -RT* ln *Ka*

where T is the absolute temperature and R is the gas constant.

**Histone eviction assay on bead-bound nucleosomes**

Nuclei were purified as previously described and treated with MNase (0.2 Units for 30 min at 37oC) to harvest mononucleosomes. Eviction assay was performed using immunoprecipitated Flag-H2A.Z containing nucleosomes which were incubated with increasing amount of recombinant GST-ANP32E (1 and 2 μg) in 30 μl eviction reaction buffer (20 mM Tris-HCl pH 7.65, 50 mM NaCl, 3 mM MgCl2, 10% glycerol, complete protease inhibitor) for 30 min at 37°C. The unbound material was collected and dissolved in SDS sample buffer. The non-evicted nucleosomes and H2A.Z-Flag remaining bound on the beads were directly eluted in SDS sample buffer. Bound and unbound materials were separated in a 14% SDS-PAGE and then transferred onto the PVDF membrane and blotted either with anti-H3 or anti-Flag antibodies.

**Cell proliferation, colony formation and soft agar assays**

At the start of the experiment, 1 × 104 cells were seeded in a 12-well dish. At the indicated time point, the number of cells was counted. For the colony formation assay, 1 × 103 cells were seeded in a 10-cm dish. After 2 weeks, the plates were washed with PBS, fixed with 4% paraformaldehyde for 10 min, stained with 0.1% crystal violet for 10 min at room temperature, and washed twice with PBS followed by rinsing thoroughly in distilled water. The plates were dried at room temperature, and the total number of colonies was counted. For the soft agar assay, cells (1 × 104) were mixed with 0.4% top agar and seeded into 6-well plates with 0.6% base agar (214010, BD Bioscience). These cells were then cultured in an incubator at 37°C for 30 days in 5% CO2 at 95% humidity. After 30 days, visualized colonies in the soft agar were captured and counted using a bioluminescence imaging system (UVP BioSpectrum AC Imagine System, UVP). Each data point presented is the average value of triplicate samples.

**Cell synchronization and cell cycle analysis**

To synchronize cells at the G1 phase, cells were serum-starved for 18 hours, released in medium with 10% FBS for 10 hours and serum starved again for 18 hours. To measure the progression of the cell cycle into the S phase, cells were released in medium with 10% FBS combined with 0.5 μg/μl nocodazole. Cells were labeled with 10 μM bromodeoxyuridine (BrdU) for 1 hour at 37°C before collection. Then, the cells were fixed and stained with anti-BrdU-APC (eBioscience) and propidium iodide (PI). BrdU incorporation was compared with the amount of PI stained using flow cytometry (BD Biosciences).

**Mouse xenograft model**

For each condition, 1 × 106 MCF7 cells were harvested in 100 μl PBS and injected subcutaneously on both sides of the back in 4-week-old female nude mice (n = 6). Tumor size was measured weekly and calculated with the formula (length × width2 /2). Tumor incidence was defined when the tumor mass reached 100 mm3, and growth was monitored until it reached 500 mm3. After 10 weeks, the mice were sacrificed and the xenografts were excised and weighed. Error bars indicate SD.

**Gene expression microarray**

Two microarray analysis experiments were performed in parallel, including H2A.ZWT vs. H2A.ZK101Q in endogenous H2A.Z knockdown cells and shLuc vs. shSMYD3 in MCF7 cells. Total RNA was extracted as described above, and a RNA expression array was performed with an Affymetrix GeneChip Human Genome U133 Plus 2.0 Array. The results were analyzed using MetaCore 5.0 software (GeneGO pathways analysis; http://www.genedo.com) and selected using a cut-off ratio of absolute normalized fold change > 2 or < 0.5 (log2-normalized ratios > 1 or < -1). Details about the experiments, raw data, and several tables of results from this study that are not included in this paper were submitted to the NCBI Gene Expression Omnibus (GEO) public repository, which is available online at www.ncbi.nlm.nih.gov/geo with the GEO accession numbers GSE58047 for the H2A.ZWT vs. H2A.ZK101Q group and GSE58048 for the shLuc vs. shSMYD3 group. The cross-referenced comparison of the two datasets was sorted by 19 genes in a common set. Both up- and down-regulated gene expressions were confirmed using real-time TaqMan (PCR) analyses. Optimal oligonucleotide primers and TaqMan probes from the universal probe library (UPL) database were designed with ProbeFinder software (Roche Applied Science) and were listed in Table S2. TaqMan PCR was performed in 20 μl final volume with TaqMan Master mix, 200 nM forward and reverse primers, 100 nM Universal ProbeLibrary probe, and cDNA template. The cycling conditions for real-time TaqMan PCR were 95°C for 10 s, 60°C for 30 s, and 72°C for 1 s for 40 cycles, and the reaction was performed using an ABI 7500 Fast Real Time PCR system (Applied Biosystems). All PCR reactions were performed in duplicate, and gene expression signals were normalized to the GAPDH signal in the same reaction.

**RNA analysis and qRT-PCR**

Total RNA was isolated using the RNeasy kit (Qiagen). RNA was reverse-transcribed into first-strand cDNA using AMV reverse transcriptase (Promega). cDNA was amplified with KAPA SYBR fast PCR mix (Kapa Biosystems, Woburn, MA) and subjected to analysis using a CFX ConnectReal-Time System thermal cycler (Bio-Rad). qRT-PCR for *RPL30* mRNA (which encodes the ribosomal protein L30) was used as an internal control. The relative abundance ofmRNA was calculated after normalization with *RPL30* mRNA using the CT equation. To verify target genes of microarray data, primers were designed using the Roche webpage and the Universal Probe Library following the manufacturer’s recommendation. The primers used are listed in Table S2.

**ChIP and re-ChIP**

ChIP assays were performed according to the manufacturer’s protocol (Upstate Biotechnology). Briefly, approximately 2 × 106 cells were fixed by adding formaldehyde at a final concentration of 1% and incubated for exactly 10 min. The cells were washed twice with ice-cold PBS containing protease inhibitors and harvested on ice. The lysates were sonicated to shear the DNA to fragment lengths of 200 to 500 basepairs. Complexes were immunoprecipitated overnight with 2 μg of antibodies specific for SMYD3 (GTX121945, Genetex), rabbit IgG (GTX35035, Genetex), H2A.Z (ab4174, Abcam), H2A.ZK101me2, Flag (F3165, Sigma), H2A (ab18255, abcam), H2B (ab1790, abcam), H3 (ab1791, abcam), and H4 (ab10158, abcam). Input samples were processed in parallel. Antibody/protein complexes were collected on 50 μl of protein G-coupled sepharose beads (GE Healthcare) and washed as follows: once with Tris/EDTA-150 mM NaCl, twice with Tris/EDTA-500 mM NaCl and once with PBS. Immune complexes were eluted with 1% SDS and TE buffer. After decrosslinking, DNA was purified using a PCR cleanup kit (Qiagen) and analyzed by qRT-PCR. For re-ChIP experiments, after primary immunoprecipitation, the crosslinked complexes were eluted by incubation at 37°C with 10 mM DTT for 30 min. The supernatant was removed and diluted 20 times using ChIP dilution buffer and re-immunoprecipitated with the second antibody as described above. The results were expressed as the percentage of the initial inputs. The primer sets used for the ChIP assay are listed in Table S2.

**Supplementary References**

1. Schnitzler GR. Isolation of histones and nucleosome cores from mammalian cells. Current protocols in molecular biology / edited by Frederick M Ausubel [*et al*] 2001;Chapter 21:Unit 21 5.

2. Svotelis A, Gevry N, Grondin G, Gaudreau L. H2A.Z overexpression promotes cellular proliferation of breast cancer cells. Cell cycle 2010;9:364-70.

3. Obri A, Ouararhni K, Papin C, Diebold ML, Padmanabhan K, Marek M, *et al*. ANP32E is a histone chaperone that removes H2A.Z from chromatin. Nature 2014;505:648-53.

4. Bonisch C, Schneider K, Punzeler S, Wiedemann SM, Bielmeier C, Bocola M, *et al*. H2A.Z.2.2 is an alternatively spliced histone H2A.Z variant that causes severe nucleosome destabilization. Nucleic acids research 2012;40:5951-64.

5. Subramanian V, Mazumder A, Surface LE, Butty VL, Fields PA, Alwan A, *et al*. H2A.Z acidic patch couples chromatin dynamics to regulation of gene expression programs during ESC differentiation. PLoS genetics 2013;9:e1003725.

6. Hashimoto H, Takami Y, Sonoda E, Iwasaki T, Iwano H, Tachibana M, *et al*. Histone H1 null vertebrate cells exhibit altered nucleosome architecture. Nucleic acids research 2010;38:3533-45.

7. Pai CC, Deegan RS, Subramanian L, Gal C, Sarkar S, Blaikley EJ, *et al*. A histone H3K36 chromatin switch coordinates DNA double-strand break repair pathway choice. Nature communications 2014;5:4091.

**Supplementary Figure Legends**

**Fig. S1. Representative mass spectrometry data of methylated H2A.Z.** GST-tagged human SMYD3 protein was purified from *E. coli* and *in vitro* HMTassay was performed with recombinant His-tagged human H2A.Z.1 as substrates. The LTQ Orbitrap mass analyzer was employed to identify methylated H2A.Z following in-gel digestion with trypsin. Annotated collision-induced dissociation spectrums of the A, unmethylated, B, monomethylated (me) and C, dimethylated (me2) state of the tryptic peptide encompassing Gly93-Lys101 in H2A.Z were presented singly and doubly charged N-terminal *b* ions and C-terminal *y* ions are indicated in blue and red, respectively.

**Fig. S2. Methylation activity of SMYD3 on histones in the context of nucleosome and evaluation of the H2A.ZK101me2 antibody.**

A, an *in vitro* HMT assay of GST-tagged human SMYD3WT and SMYD3Y239F. Nucleosomes isolated from MCF7 cells were used as substrates. CB staining was shown at the left and autoradiography was shown at the right. B, rabbit polyclonal antibodies specifically against H2A.ZK101me2 were generated using the indicated peptide. A cystine at the C-terminus was designed for its conjugation to the carrier protein. A dot blot spotting analysis was performed using two biotin-labeled H2A.Z peptides with serial dilution and avidin-HRP was used as a loading control. C, peptide competition assay was performed by Western blot analyses using MCF7 cell lysates and hybridized with H2A.ZK101Ame2 antibody. Left panel, without peptides. Right panel, with 1 μg/ml H2A.ZK101me2 peptides. D, MCF7 cells expressing H2A.ZWT or mutant H2A.Zproteins were hybridized with anti-H2A.ZK101me2 or anti-H2A.Z antibodies. GAPDH was used as a loading control. E, an *in vitro* HMT assay of GST-tagged human SMYD3WT and SMYD3Y239F. Nucleosomes isolated from 3-day SMYD3 knockdown MCF7 cells were used as substrates. Western blot analyses were conducted using indicated antibodies. CB staining was shown at the bottom to indicate equal loading of GST-tagged human SMYD3WT, SMYD3Y239F and isolated nucleosomes. F, full-sized gel images of figure 1E. All data were representative of n ≥ 3 for each experiment.

**Fig. S3. Dimethylation of H2A.Z is critical for its protein interaction.**

A, co-immunoprecipitation from endogenous H2A.Z-knocked down H2A.ZWT-, H2A.ZK101Q- and H2A.ZK101R- expressing MCF7 cells with an anti-Flag antibody. The histograms showed the ratio of p400, TIP60, YL1 and Flag-H2A.Z protein signal to their input signal in Western blots. B, pulled-down recombinant His-tagged H3 was incubated with H2A.ZWT- or H2A.ZK101Q-expressing cell lysates and co-pulled down proteins were subjected to a Western blot analysis to detect co-purified H2A.Zproteins. C, biotinylated H2A.Z peptides were co-incubated with T47D cell lysates to pull down endogenous ANP32E and H3. Pulled-down proteins were subjected to a Western blot analysis. A portion of the reaction was employed to the avidin-HRP dot blot assay to demonstrate the equivalent amounts of input peptides. D, immunoprecipitated H2A.Z proteins from SMYD3 knockdown MCF7 cells with overexpression of ANP32E-Myc proteins were subjected to a Western blot analysis to detect co-immunoprecipitated Myc tagged ANP32E and H3proteins. All values in the histogramswere means ± SD of triplicatesand data were representative of n ≥ 3 for each experiment. \**P* < 0.05.

**Fig. S4. Dimethylation of H2A.Z is critical for promoting cell growth.**

A, expression levels of SMYD3, H2A.ZK101me2 and H2A.Z protein were elevated in human cancer cell lines compared to a lung fibroblast cell line, IMR-90. Western blot analyses were performed using indicated antibodies. GAPDH was used as a loading control. B, Western blot analyses of SMYD3, H2A.ZK101me2 and H2A.Z protein in two sets of breast tissues. Matched tissue samples were obtained from Abcam (1: ab43503, 2: ab43504, 3: ab43507, 4: ab43508). N: normal tissues; T: tumor tissues. CB staining was shown at the bottom to indicate equal loading of samples. C, a Western blot analysis confirmed H2A.Z knockdown efficiency on day 12 in H2A.ZWT, H2A.ZK101Q- and H2A.ZK101R-expressing MCF7 cells. D, H2A.ZWT-promoted MCF7 cell tumorigenicity was abolished in SMYD3 knockdown cell of colony formation on soft agars. E, MCF7 cells overexpressing empty vector, SMYD3WT or SMYD3Y239F were subjected to cell proliferation assay. Data were collected on day 12. Specific antibodies against indicated proteins were used. \**P* < 0.05 vs. SMYD3WT value. F, SMYD3-promoted MCF7 cell proliferation was not enhanced in H2A.ZWT-expressing cells. Cell proliferation was measured on day 12 and Western blot analyses showed the overexpression of SMYD3 and H2A.Z. \**P* < 0.05; \*\**P* < 0.01. n.s.: not significant. All values in the histograms were means ± SD of triplicates and data were representative of n ≥ 3 for each experiment.

**Fig. S5. Genes affected by SMYD3 knockdown and H2A.ZK101Q expression.**

A-C, whole-genome microarray analysis of RNA isolated from two parallel pairs of experiments B, shLuc vs. shSMYD3 and C, H2A.ZWT vs. H2A.ZK101Q expression in endogenous H2A.Z knockdown MCF7 cells were conducted. Cross-referenced shSMYD3/shLuc and H2A.ZK101Q/H2A.ZWT datasets showed that 35 transcripts were co-regulated. These 35 transcripts are expressed from 19 genes. With a cut-off of absolute normalized fold change > 2 or < 0.5 (log2 normalized ratios > 1 or < -1), the results were analyzed using GeneGO MetaCore software to reveal their gene ontology process. D and E, the confirmation of the microarray analyses for the expression of genes, including 8 down-regulated genes, D, and 11 up-regulated genes, E, in both shSMYD3/shLuc and H2A.ZK101Q/H2A.ZWT datasets using qRT-PCR. \**P* < 0.05, \*\**P* < 0.01, \*\*\**P* < 0.001 vs. shLuc or H2A.ZWT control, respectively. All values in the histograms were means ± SD of triplicates and data were representative of n ≥ 3 for each experiment.

**Fig. S6. Cyclin A1 is one of the downstream targets co-regulated by SMYD3 and H2A.ZK101me2.**

A, mRNA expression of cyclin A1 and cyclin A2 in H2A.Z knockdown cells. mRNA expressions were measured using qRT-PCR. B, a re-ChIP assay demonstrated that dimethylated H2A.Z was recruited to the cyclin A1 promoter, indicating that anti-dimethylated antibodies were indeed bound to dimethylated K101 of H2A.Z. MCF7 cells were harvested and the chromatin complexes were first precipitated with IgG or anti-H2A.Z antibodies and then subjected to re-ChIP assay with anti-H2A.ZK101me2 or IgG antibodies. Immunoprecipitated chromatin was quantified at indicated regions by qRT-PCR. C and D, ChIP assays were performed with SMYD3-repressed MCF7 cells using specific antibodies against H2A.Z and H2A (C), or H2B, H3 and H4 (D). E and F, MCF7 cells expressing H2A.ZWT, H2A.ZK101Q or H2A.ZK101R were subjected to ChIP assay using specific antibodies against H2A.Z and H2A (E), or H2B, H3 and H4 (F). In C-F, immunoprecipitated chromatin was quantified by qRT-PCR. \**P* < 0.05. Primer sequences are listed in Table S2. G, MCF7 cells expressing empty vector, H2A.ZWT and H2A.ZK101Q were infected with lentiviruses carrying shLuc and shCCNA1 plasmids. Cell proliferation assay was performed. A Western blot analysis indicated knockdown efficiency of cyclin A1. \**P* < 0.05, \*\**P* < 0.01 vs. shLuc control value. H, MCF7 cells were infected with lentiviruses carrying shLuc control vector or shSMYD5. A Western blot analysis was conducted using indicated antibodies. All values in the histograms were means ± SD of triplicatesand data were representative of n ≥ 3 for each experiment. I, alignment of H2A.Z, H2A and other H2A variants. The red color indicates the position of K101.

**Supplementary Table S1. List of methylated histone proteins identified from in vitro HMT assay on calf thymus extracts followed by LC-MS/MS analysis**

**Supplementary Table S2. Candidate genes regulated by both SMYD3 and H2A.Z, shRNA oligo sequences, and primer sequences.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Down-regulated genes in both shSMYD3/shLuc and H2A.ZK101Q/H2A.ZWT array data (genes with < 0.5 fold differences). | | | | |
| ID | | shSMYD3/  shLuc | H2A.ZK101Q/  H2A.ZWT | Gene Title/Gene Symbol | Location of predicted SMYD3 binding sites (5’-CCCTCC-3’) | |
| 205899\_at | | 0.46 | 0.38 | Cyclin A1/*CCNA1* | -547~-542, -404~-399, +445~+460 | |
| 231766\_s\_at | | 0.33 | 0.27 | Collagen, type XII, alpha 1/*COL12A1* | +428~+433, +446~+451 | |
| 224646\_x\_at | | 0.50 | 0.33 | H19, imprinted maternally expressed transcript (non-protein coding)  /*H19* | -975~-970, -739~-734, -709~-704,  -658~-653, 549~-544, -276~--271, +71~+76, +207~+212 | |
| 1557961\_s\_at | | 0.29 | 0.38 | Hypothetical protein LOC100127983 */LOC100127983* | No | |
| 207414\_s\_at | | 0.50 | 0.47 | Proprotein convertase subtilisin/kexin type 6/*PCSK6* | -340~-335, -279~-274, +129~+134 | |
| 219494\_at | | 0.50 | 0.33 | *RAD54* homolog B (*S. cerevisiae*)/*RAD54B* | No | |
| 220177\_s\_at | | 0.47 | 0.50 | Transmembrane protease, serine 3/*TMPRSS3* | -988~-983, -968~-963, -948~-943, -908~-903, -868~-863, -848~-843,  -828~-823, -808~-803, -771~-766, -631~-626, -611~-606, -438~-433,  -418~-413, -358~-353, -338~-333, -298~-293, -258~-253, -238~-233,  -218~-213 | |
| 216228\_s\_at | | 0.50 | 0.31 | WD repeat and HMG-box DNA binding protein 1/*WDHD1* | No | |
| 202664\_at | | 0.44 | 0.44 | WAS/WASL interacting protein family, member 1/*WIPF1* | +174~+179 | |

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| Up-regulated genes in both shSMYD3/shLuc and H2A.ZK101Q/H2A.ZWT array data (genes with > 2 fold differences). | | | | |
| ID | shSMYD3/  shLuc | H2A.ZK101Q/  H2A.ZWT | Gene Title/Gene Symbol | Location of predicted SMYD3 binding sites (5’-CCCTCC-3’) |
| 209160\_at | 3.73 | 4.29 | Aldo-keto reductase family 1, member C3  (3-alpha hydroxysteroid dehydrogenase, type II)/*AKR1C3* | No |
| 37547\_at | 2.63 | 3.03 | Bardet-Biedl syndrome 9/*BBS9* | No |
| 18285\_s\_at | 2.14 | 2.14 | 3-hydroxybutyrate dehydrogenase, type 2/*BDH2* | No |
| 238625\_at | 2.46 | 2.64 | Chromosome 1 open reading frame 168/*C1orf168* | No |
| 228915\_at | 2.46 | 2.30 | Dachshund homolog 1 (Drosophila)/*DACH1* | No |
| 20625\_s\_at | 2.64 | 2.14 | E74-like factor 5 (Ets domain transcription factor)/*ELF5* | -971~-966, -820~-815,  -309~-304 |
| 227778\_at | 2.14 | 4.93 | Maestro heat like repeat family member 1/*MROH1* | -984~-979, -845~-840, -751~-746, -460~-455, +213~+218, +411~+416, +445~450 |
| 205303\_at | 2.00 | 2.29 | Potassium inwardly-rectifying channel, subfamily J, member 8/*KCNJ8* | -779~-774, +352~+357 |
| 206241\_at | 2.00 | 2.14 | Karyopherin alpha 5 (importin alpha 6)/*KPNA5* | No |
| 238067\_at | 7.46 | 2.14 | TBC1 domain family, member 8B (with GRAM domain)/*TBC1D8B* | -17~-12, +24~+29, +131~+136, +289~+294, +415~+420 |
| 209676\_at | 3.25 | 2.64 | Tissue factor pathway inhibitor (lipoprotein-associated coagulation inhibitor)/*TFPI* | No |
| 202688\_at | 2.64 | 3.73 | Tumor necrosis factor (ligand) superfamily, member 10/*TNFSF10* | -839~-834 |

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| Oligo sequences for shRNA-mediated gene knockdown. | | | |
| Clone ID | Gene Symbol | Target Sequence | Region |
| TRCN0000123290 | *SMYD3* | GCTTCCCGATATCAACATCTA | CDS |
| TRCN0000123291 | *SMYD3* | CAACTCTTTCACCATCTGTAA | CDS |
| TRCN0000155095 | *SMYD5* | GCTATGGGAATTACAACCCAT | 3’UTR |
| TRCN0000154724 | *SMYD5* | GCCAATGAAGAGGAGGAAATT | CDS |
| TRCN0000291923 | *H2AFZ* | GCTTCAAAGAAGCTATTGATT | 3’UTR |
| TRCN0000291924 | *H2AFZ* | CGTGGAGATGAAGAATTGGAT | CDS |
| TRCN0000435992 | *CCNA1* | GTGATAAATGTGACTGAATAT | CDS |
| TRCN0000421104 | *CCNA1* | TTGCCTGAGTGAGCTTCATAA | CDS |

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| Primers used in this study. | | | |
| Genes | Forward sequence | Reverse sequence | Assay |
| *AKR1C3* | CATTGGGGTGTCAAACTTCA | CCGGTTGAAATACGGATGAC | TaqMan qPCR (27a) |
| *BBS9* | CCGTCTCTGCCTAAGTACCG | TTCTAGGTCTGACTCTGGGATTG | TaqMan qPCR (66) |
| *BDH2* | CGTGATTGGCCTCACAAAA | TTGTAGAGATGGCGTATCAACTG | TaqMan qPCR (42) |
| *CCNA1* | TCAGTACCTTAGGGAAGCTGAAA | CCAGTCCACCAGAATCGTG | TaqMan qPCR (71) |
| *COL12A1* | TGGCACAAGACCCTCAGAAT | AATCAGCCGCTCCAGATG | TaqMan qPCR (72) |
| *DACH1* | GCGTGAACAAGCAGAACAGA | ATCTCTGGGGTCAGAGAGTCAT | TaqMan qPCR (27) |
| *ELF5* | TCTGCGGCGAGTACCTGT | CTTGCTTTCTTCAGCGTCATT | TaqMan qPCR (88) |
| *H19* | GCAAGAAGCGGGTCTGTTT | TTGAGCTGGGTAGCACCATT | TaqMan qPCR (69) |
| *MROH1* | TGCATTCCTTGTCTCAGAGC | CCCAGTGTGAACATGGACAA | TaqMan qPCR (88) |
| *KCNJ8* | GGAGTCCACTGTGTGTGTGAC | AAAACCGTGATGGCCAAA | TaqMan qPCR (88) |
| *KPNA5* | CTGCATACCAAGGTAGTGATTGA | ACCAAGTGCCCAAACAGC | TaqMan qPCR (45) |
| *PCSK6* | TTACTTCAACGACCCCATTTG | CCTGTGTAGCCCCTCTTCC | TaqMan qPCR (27) |
| *RAD54B* | GCCTTTTGTTCAACTCTATAAAGGA | TTCAGTAAACAGGAGAGGGTTGT | TaqMan qPCR (18) |
| *TBC1D8B* | CCAACGACTACTTCGTGCTG | TGAATCAAGAGTCCCAACCA | TaqMan qPCR (69) |
| *TFP1* | AAGAGCGGAAATGTCAAACG | GGCACACCATCTTCCAGTTT | TaqMan qPCR (71) |
| *TMPRSS3* | TGGGACTCGGGAATTATGAG | CCCCCATGGTGACTATTTCA | TaqMan qPCR (39) |
| *TNFSF10* | CCTCAGAGAGTAGCAGCTCACA | CAGAGCCTTTTCATTCTTGGA | TaqMan qPCR (5) |
| *WDHD1* | TTGATATTTCAATGCTAAAAACTGGT | TTGTGAATGCTGCCTTCTTG | TaqMan qPCR (69) |
| *WIPF1* | TTACCTTCGCCAGGACGTT | GCCGTTTCTGCTTACTGGAG | TaqMan qPCR (69) |
| *CCNA1* | AATGGGCAGTACAGGAGGAC | CCACAGTCAGGGAGTGCTTT | SYBR green qPCR |
| *CCNA2* | CTCTACACAGTCACGGGACAAAG | CTGTGGTGCTTTGAGGTAGGTC | SYBR green qPCR |
| *H2AFZ* | TGCAACTTGCTATTCGTGGA | TGGATGTGTGGAATGACACC | SYBR green qPCR |
| *SMYD3* | TTACTGCGAGCAGTCCGAGACA | TTGTCCTGGGTTTGGCAACGGA | SYBR green qPCR |
| *CCNA1* | GGAGGTTGGCTTCTTGATGA | GGGTGACAGAGCAAGATTCC | ChIP a: -5145~-4907 bp |
| *CCNA1* | GAGCCAGGGTTCTCAGGA | GAAGGGACTGTTTCCGTGAC | ChIP b: -623~-532 bp |
| *CCNA1* | TTGGTGCTCTCCCTCCTAGA | TGTAGCCAGCACAACTCCA | ChIP c: +445~+605 bp |
| *CCNA1* | GTTGGGATTACAGGCGTCAG | TGCTCTACCTGGAGGAACC | ChIP d: +1312~+1552 bp |
| *CCNA1* | TGGATCAGAAAATGCCTTCC | CTTGAGTGTGCCGGTGTCTA | ChIP e: +5166~+5352bp |
| H2A.ZK101Q | CAATTGTAGCCTGGATGAGACTATCCAATTCTTCATCTCCACGAATAGC | | Mutagenesis |
| H2A.ZK101R | GATGAAGAATTGGATTCTCTGATCAGGGCTACAATTGCTGGTGGTG | | Mutagenesis |
| SMYD3Y239F | GAGAGGAGCTGACCATCTGCTTCCTGGATATGCTG | | Mutagenesis |
| a: TaqMan probe number in universal probe library (Roche) | | | |