Supplementary Information

Enhanced anticancer effects of a methylation inhibitor by inhibiting a novel DNMT1 target, CEP 131, in cervical cancer

Dong Hyun Kim1,5,#, Hye-Min Kim1,#, Pham Thi Thu Huong1,8,#, Ho-Jin Han1,5, Joonsung Hwang1, Hyunjoo Cha-Molstad1, Kyung Ho Lee1, In-Ja Ryoo1, Kyoon Eon Kim2, Yang Hoon Huh3, Jong Seog Ahn1,5,* Yong Tae Kwon4,* Nak-Kyun Soung1,5,* and Bo Yeon Kim1,5,*

1Anticancer Agent Research Center, Korea Research Institute of Bioscience and Biotechnology (KIRIBB), Ochang, Cheongju, 28116, Korea.
2Department of Biochemistry, College of Natural Science, ChungNam National University, Daejeon, 34134, Korea.
3Center for Electron Microscopy Research, Korea Basic Science Institute, Ochang, Cheongju, 28119, Korea.
4Protein Metabolism Medical Research Center, Department of Biomedical Sciences, College of Medicine, Seoul National University, Seoul, 03080, Korea.
5Department of Biomolecular Science, University of Science and Technology, Daejeon, 34113, Korea.

#The first three authors contributed equally.
*Correspondence: Jong Seog Ahn (jsahn@kribb.re.kr; Tel.: +82-43-240-6160; Fax: +82-240-6169), Yong Tae Kwon (yok5@snu.ac.kr; Tel.: +82-2-740-8547; Fax: +82-2-3673-2167), Nak-Kyun Soung (soungnak@kribb.re.kr; Tel.: +82-43-240-6165; Fax: +82-43-240-6259), Bo Yeon Kim (bykim@kribb.re.kr; Tel.: +82-43-240-6100; Fax: +82-43-240-6259).
Materials and Methods

Plasmid Construction and siRNA

LZRS-DNMT1 was a generous gift from Paul Khavari (Addgene plasmid #24952; Cambridge, MA). Using EcoR I restriction sites, DNMT1 was cloned into the pHRI’-CMV SV40 puromycin vector. Additionally, DNMT1 was cloned into the pUC19 vector (NEB, Ipswich, MA) for mutagenesis. The C1242S DNMT1 enzymatic mutant was generated using PCR-based mutagenesis and the primer set DNMT1 C1242S forward (5′-GCTGTGGCGGGCCGCCCAGGAGGCAATTCAGCGGCA-3’) and DNMT1 C1242S reverse (5′-CATGCGGTAAGCCTGCTGGGGCGGCCCAGCACAGC-3’); the mutation site is indicated in bold. The PCR product (230 bp insert) and pUC19 DNMT1 wild-type plasmid were digested with SacII prior to ligation and cloning. The cloning results were verified by sequencing (Cosmogenetech, Seoul, Rep. of Korea). The DNMT1 C1242S mutant was cloned into the pHRI’-CMV SV40 puromycin vector. To generate a lentiviral construct expressing HA-tagged Cep131, Cep131 cDNA was purchased from Open Biosystems (Huntsville, AL, USA), followed by amplification of the Cep131 gene using the following primers: forward (5′-CCCAGATATCATGAAAGCCACCCAGCCGC-3’) and reverse (5′-CCCAGCGCGCTCAGGTGACCTTTGATGCTGGCGG-3’). The PCR product and pCI Neo HA vector were digested with EcoRV, followed by ligation and cloning. The results of cloning were verified by sequencing and confirmed that nucleotide 1828 was changed to T, destroying the BspI enzyme site without altering the amino acid sequence. The pCI-Neo HA Cep131 plasmid was digested with AscI (end-filled)-SalI and then ligated with a BamHI (end-filled)-SalI treated pHRI’-CMV SV40 puromycin vector.

To generate lentivirus-based shRNAs, the following oligonucleotides targeting the indicated gene sequences (targets indicated in bold) were used: i) nucleotides 5114-5162 of DNMT1 (NM_001130823): forward 5′-CCGGGTATGAGTGAAAATTAAGAGCTAGCTCTTAATTCCACTCATACTTTTTG-3’ and reverse 5′-AATTCAAAAAATGATGAGTGAAAATTAAGAGCTAGCTCTTAATTCCACTCATACATTTTTG-3’, ii) nucleotides 2005-2023 of human Cep131 (NM_014984): forward 5′-CCGGCAGCAGTAGTGAGATTAGCTAGCTAACTCCAGCTGTG CTGTTTTT G-3’ and reverse 5′-
ii) nucleotides 124-142 of EGFP (of CVU55763); forward 5′-CCGGGCAAGCTGACCTGAAAGTTGCTAGCAACTTCAGGCCAGCTTGCTTTTGC-3′ and reverse 5′-AATTCAAAAAAGCAAGCTGACCTGAAAGTTGCTAGCAACTTCAGGCCAGCTTG-3′. PRC products were digested with AgeI and EcoRI and cloned into the pLKO.1-puro vector (Addgene, Cambridge MA., USA). All clones were verified by sequencing.

To generate pGL3-CEP131 promoter reporter plasmids of varying lengths (-3,000 bp, -1,935 bp, -893 bp, and delta -893 bp), the Bac clone (CH17-202A3) containing the CEP131 promoter region was purchased from BACPAC RESOURCES (Oakland, CA). The -3,000 bp promoter plasmid was generated using the following primers: forward (5′-TTTgcggccgcCTAgtcgacGTCCTTAGCAGCTTCCTCC-3′) and reverse (5′-CCCAAGCTTCCTGGCGCCCCGCCAC-3′) constructs with smaller promoter regions were generated using the -3,000bp vector as a template and the following primer: forward (5′-CCCTCAGCTTCTAGTAGGCCGATGTGGTG-3′) (-1,935bp), forward 5′-GGGCTCGAGATCCGGGAGAATGTTC-3′ (-895bp), reverse (same as listed above for the -3000 bp construct). As a negative control, a -895 bp deletion construct was generated using the same forward primer as for the -3,000 bp construct and the reverse primer (5′-CTGGGGATTCCGCCGG-3′). Forward and reverse primers contain XhoI and HindIII restriction enzyme sites, respectively. All constructs were verified by sequencing. All primers were purchased from Cosmogentech Inc. (Seoul, Rep. of Korea) and their sequences are listed in Supplementary Table 2.

**Quantification by Real-Time PCR and Reverse Transcription PCR**

Real-time PCR analysis was carried out using the Platinum SYBR Green qPCR SuperMix UDG kit (Invitrogen, Carlsbad, CA) on a CFX96 Real Time System (Bio-Rad, Hercules, CA). The mix included: 10 µl platinum mix, 0.8 µl primer (10 µM), 0.6 µl Rox reference dye, 3.6 µl H2O, and 5 µl template. Reactions were carried out as follows: samples were incubated at 50 °C (2 minutes) and 95 °C (10 minutes), followed by 40 cycles of 95 °C (10 seconds) and 60 °C (1 minute). The dissociation curves were measured for each sample. The relative level of the DNMT1 or Cep131 sequence against the reference sequence (GAPDH) was measured using the ΔΔCt method with calculated real efficiencies. The sequences of primers
used were as follows: DNMT1 forward (5′-CCGAGTGCAGTGCTGCAGTGTCAGT-3′) and reverse (5′-GCTCCTGTGTTAGCAGTGCAGTGG-3′); Cep131 forward (5′-GTTGCAGTCCTCTGTGAAAC-3′) and reverse (5′-GCTCCTGTGTTAGCAGTGCAGTGG-3′); GAPDH forward (5′-TAGACGGGAAGCTCAGCTGCAG-3′) and reverse (5′-AGGTCCACCACCGTTGGCT-3′). All primers were purchased from Cosmogentech Inc. (Seoul, Rep. of Korea) and their sequences are listed in Supplementary Table 2.

**Chromatin Immunoprecipitation Assay (ChIP)**

ChIP was performed with an anti-DNMT1 antibody (Imgenex, San Diego CA) on formaldehyde cross-linked HeLa CCL2 cells (4 × 10^8 cells). At 12 hours after re-plating, the cells were fixed in 1% formaldehyde for 10 minutes at room temperature; 0.25 mM glycine was added for blocking. The cells were washed in PBS, resuspended in Buffer A (50 mM Tris-Cl pH 8.0, 140 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.1% sodium deoxycholate, 1 mM PMSF), sonicated (Branson sonifier 250 set at 25% output; 30 seconds on, 1 minute off for 5 cycles), and centrifuged for 20 minutes at 13,000 rpm at 4 ºC to remove solid materials. The DNA content of the pooled samples was measured at A_260, and the samples were stored at -80 ºC. These fractions represent the cross-linked whole-cell extracts ready for immunoprecipitation. Lysates were precleared in Buffer A with Protein G, salmon sperm DNA (sonicated to an average size of 2-5 kb, 10 µg/ml), and BSA (500 ng/ml). After preclearing, aliquots containing equal amounts of DNA were used for immunoprecipitation with the indicated antibodies. Genomic DNA was used to normalize the PCR. Lysates were diluted with 9 volumes of dilution buffer (50 mM Tris pH 8.0, 5 mM EDTA, 200 mM NaCl, 0.5% NP40). After immunoprecipitation overnight, antibody-bound complexes were isolated by addition of protein G for 30 minutes at 4 ºC. Precipitates were washed twice with 1 ml of washing buffer (WB; 20 mM pH 8.0, 2 mM EDTA, 500 mM NaCl, 0.1% SDS, 1% NP40) for 20 minutes at 4 ºC, twice with 1 ml buffer NiCl for 20 minutes at 4 ºC, and twice with 1 ml TE buffer for 20 minutes at 4 ºC. Extraction of antigen/antibody complexes was performed with 200 µl of elution buffer (1× TE containing 2% SDS). Complexes were then eluted from the beads followed by incubation at 65 ºC for 5 hours to reverse cross linking. DNA was purified using a Qiaquick PCR purification kit and eluted with 50 µl of TE. For detection of immunoprecipitated p16^{INK4A}, GAPDH, and Cep131 promoter region, 2 µl of eluted DNA was used. As a positive control, the human p16^{INK4A} promoter region primers were used: forward (5′-TTGAAGCTCGTTGCTTTGGAT-3′) and reverse (5′-
ATCGAAATCACCTGTACGACT-3'); expected size of PCR product is 401 bp. As a negative control, the GAPDH promoter region primer set was used: forward (5'-ATGGTTGCCACTGGGGATCT-3'), and reverse (5'-TGCCAAAGCCTAGGGGAAGA-3'); expected size of the PCR product is 176 bp (ActiveMotif, Carlsbad, CA). For the human CEP131 promoter region, the following primers were used Cep131 F (5'-CTATATGACCCGCCCCTT-3') and Cep131 R (5'-GTCCTTCGGGAAGTTCAAGC-3'); expected size of the PCR product is 103 bp. Immunoprecipitations were performed in duplicate. All primer sequences are summarized in Supplementary Table 2.

**Bisulfite Sequencing**

Two micrograms of genomic DNA from shGL2 or shDNMT1 HeLa cells were bisulfate modified according to the manufacturer’s protocol (Zymo Research, Irvine, CA) in 30 µl volumes. Two microliters of converted DNA were used for bisulfite sequencing. The primers were designed using the MethPrimer program and are summarized in Supplementary Table 2. Final PCR products were eluted from 2% agarose gels and cloned into the pGEM T easy vector system (Promega, Madison, WI). At least 20 colonies from each plate were confirmed by sequencing analysis. The data were analyzed using the BiQ Analyzer program (1, 2).

**Cell culture, Transfection, and Lentivirus Generation and Infection**

Cell lines were cultured as recommended by the American Type Culture Collection (Manassas, VA). Transfections were carried out using Lipofectamine 2000 (Invitrogen) for plasmids and Oligofectamine (Invitrogen) for siRNA. To produce the shRNA lentiviruses, pHR’-CMV delta R 8.2 delta vpr and pHR’-CMV-VSV-G (protein G of vesicular stomatitis virus) were cotransfected into 293T cells containing pLKO.1 puro-shGL2, shDNMT1, and shCep131.

To generate lentiviruses expressing wild type DNMT1, DNMT1 C1242S mutant, or HA-tagged Cep131, 293T cells were cotransfected with pHR’-CMV delta R 8.2 delta, pHR’-CMV-VSV-G, and the respective pHR’-CMV-SV40 puromycin-based constructs described
above. To select a lentivirus-integrated population, HeLa CCL2 or NIH3T3 cells were infected with the indicated viruses and treated with 2 µg/ml puromycin for 2-3 days.

The lentivirus expressing shLuciferase (GL2) has been previously described (3). All other target sequences for lentivirus-based shRNA and synthetic siRNA (Luciferase, DNMT1, DNMT3A, and DNMT3B) are summarized in Supplementary Table 3 (4-6). Synthetic siRNAs were purchased from Bioneer Inc. (Daejeon, Rep. of Korea).

**Luciferase Assay**

293T cells and HeLa CCL2 cells overexpressing wild type DNMT1, C1242S mutant, or empty vector were transfected with 5 µg of the pGL3 Cep131 promoter reporter plasmid. At 24-48 hours after transfection, cells were lysed in 100 µl of Reporter Lysis Buffer (Promega) for 10-20 minutes. Samples were frozen at −80 °C for at least 1 hour. Luciferase assays were performed using the Luciferase Assay System (Promega). Luciferase activity was normalized for protein concentration (Bradford method).

**Immunoblotting**

Protein samples were separated by SDS-PAGE, transferred to a PVDF membrane and detected by immunoblotting with the indicated antibodies using the Enhanced Chemiluminescence (ECL) detection system (Pierce, Rockford, IL). All antibodies used in this study are listed in Supplementary Table 4.

**Immunofluorescence Microscopy**

Cells were grown on Poly-L-lysine (Sigma)-coated glass coverslips, fixed with 4% paraformaldehyde (EMS) for 10 minutes, and washed four times with PBS. The coverslips were then incubated for 2 hours in PBS plus 5% bovine serum albumin containing mouse anti-DNMT1 antibody (Imgenex INC), rabbit or mouse anti–γ-tubulin antibody (Sigma), rabbit anti-Cep131 antibody (Bethyl lab. Montgomery, TX), mouse anti-Centrin2 antibody (Millipore), rabbit anti-HA antibody (Santa Cruz Biotechnology), or rabbit anti-ninein antibody (Supplementary Table 4). After two washes with PBS for 5 minutes, the coverslips were further incubated for 1 hour with the appropriate secondary antibody. All secondary antibodies [Alexa Fluor 488 (green)- and Texas red (red)-conjugated antibodies] were
purchased from Invitrogen. To stain chromosomes, cells were treated with PBS containing 0.1 µg/ml of 2,5'-Bi-1H-benzimidazole, 2'-(4-ethoxyphenyl)-5-(4-methyl-1-piperazinyl) (Heochst33342; Invitrogen). To visualize primary cilia, hTERT-RPE cells cultured under serum starvation conditions for 2 days were stained with a mouse anti-acetylated tubulin antibody (Sigma) and a rabbit anti-Cep131 antibody (Bethyl Lab). Confocal fluorescent images were collected with a Zeiss LSM 710 confocal microscope or with a Zeiss Axiovert 100M microscope.

Cell growth and Xenograft assays
Indicated cells were cultured for indicated time. After 1 hour of Cell Counting Kit – 8 (CCK-8) treatment (10 µL), plates were analyzed at 450 nm (650 nm reference) using a 96-well plate recorder (Molecular Devices, CA, USA), or cell counter for direct counting (Thermo Fisher Scientific, MA, USA). Also, indicated cells were collected by centrifugation, suspended in 0.2 ml (3.0 X 10^6 cells) of PBS and injected into 4-week-old male nude mice subcutaneously. Tumor tissues were measured every week until their size became 2.0 cm^3.

Statistical analysis
Values are presented as mean ± standard deviation (SD) of three or more experiments. Data were analyzed using Student's t-test or one-way analysis of variance (ANOVA) for comparisons between two mean values. A value of \( P < 0.05 \) was considered significant.
Supplementary Table 1. The list of centrosome related genes were analyzed in this study, the most of genes are listed form Jakobsen L. et al. (7)

For quantification analysis, housekeeping genes (R1-R5) were used as references.

Genomic DNA Contamination was confirmed by GDC primer set (company supplied).

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<td>NM_025180.3, NM_001042400.1, NM_001042384.1, NM_001042383.1</td>
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<td>94</td>
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<td>TUBGCP6</td>
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<td>R1</td>
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<td>NM_001101.3</td>
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<td>R2</td>
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<td>NM_004048</td>
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<tr>
<td>R3</td>
<td>GAPDH</td>
<td>NM_002046</td>
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<td>R4</td>
<td>HMBS</td>
<td>NM_000190</td>
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<tr>
<td>R5</td>
<td>HPRT1</td>
<td>NM_000194</td>
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GDC: Genomic DNA check
<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer sequence</th>
<th>Product size</th>
<th>Purposes</th>
<th>Reference</th>
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<tbody>
<tr>
<td>DNMT1 C1242S</td>
<td>F1: 5'-ACAGTGTTTCACAGAGGACTG-3' R2: 5'-GTCATCCACCACCACTGCT-3'</td>
<td>425bp</td>
<td>RT</td>
<td>Osong Kwon, et al., 2010</td>
</tr>
<tr>
<td></td>
<td>GCTGTGCCGCGGCGGAGGGCCGAGGACCGGCTTACAAGGCGATG-3'</td>
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<td>qRT</td>
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<tr>
<td></td>
<td>CATGCCGCTAGACCGCTTGCGGCGGCCCCGCGCCAC-3'</td>
<td></td>
<td>ChIP</td>
<td>This Study</td>
</tr>
<tr>
<td>Cep131 Promoter (-3000bp)</td>
<td>F: 5'-CCCGTGACGTCCCTTTAGGAGCTTCTCTCC-3' R: 5'-CCCAAGCTCTCGTGCGGCCCCGAC-3'</td>
<td>3084bp</td>
<td>cloning</td>
<td>This Study</td>
</tr>
<tr>
<td>Cep131 Promoter (-1935bp)</td>
<td>F: 5'-CCCCCTCGAGCTTTAGGCGGATGTTGAG-3' R: the same with -3000bp construct</td>
<td>2019bp</td>
<td>cloning</td>
<td>This Study</td>
</tr>
<tr>
<td>Cep131 Promoter (-893bp)</td>
<td>F: 5'-GGGCTCGAGATCCGGGAGAATGTTC-3' R: the same with -3000bp construct</td>
<td>977bp</td>
<td>cloning</td>
<td>This Study</td>
</tr>
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<td>Cep131 Promoter (-600bp)</td>
<td>F: 5'-CCCTCGAGAAGGAGGTTAGGATGCT-3' R: the same with -3000bp construct</td>
<td>684bp</td>
<td>cloning</td>
<td>This Study</td>
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<td>Cep131 Promoter (-400bp)</td>
<td>F: 5'-CCCTCGAGCTTGGGCCGCCCCGAC-3' R: the same with -3000bp construct</td>
<td>484bp</td>
<td>cloning</td>
<td>This Study</td>
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<tr>
<td>Cep131 Promoter (-300bp)</td>
<td>F: the same with -3000bp construct R: 5'-CTGGGGATTTCCGCCAGG-3'</td>
<td>2119bp</td>
<td>cloning</td>
<td>This Study</td>
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<tr>
<td>Cep131 Promoter (Δ-893bp)</td>
<td>F: 5'-CAATGCCGCGGAGTTCCAGT-3' R: 5'-GCTCTCTTTGTAGTTACGGAAGT-3'</td>
<td>100bp</td>
<td>RT, qRT</td>
<td>This Study</td>
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<tr>
<td>DNMT1</td>
<td>F: 5'-CCGAGTGTCGCTCCAGTTAGT-3' R: 5'-GTTGGCTCTCTTGAAGA-3'</td>
<td>371bp</td>
<td>ChIP</td>
<td>Witcher et al., 2009</td>
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<tr>
<td>DNMT3A</td>
<td>F: 5'-TATTTGAGACGCACACAGAGAGAC-3' R: 5'-GGTTGCTCCAGGAATACATTGAG-3'</td>
<td>111bp</td>
<td>RT</td>
<td>Girault et al., 2003</td>
</tr>
<tr>
<td>DNMT3B</td>
<td>F: 5'-GGCAAGTTTCAAGGCTCTCG-3' R: 5'-TTGGTACATGGCTTGGATAAGA-3'</td>
<td>113bp</td>
<td>RT</td>
<td>Girault et al., 2003</td>
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<tr>
<td>GAPDH</td>
<td>F: 5'-TAGACCGGGAGCTCATCGGCC-3' R: 5'-AGGTCCACACCCGTGGTCTG-3'</td>
<td>226bp</td>
<td>RT, qRT</td>
<td>Chuang et al., 2000</td>
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<td>p16INKA</td>
<td>F: 5'-TGAAGCTGTCTTGTGGAT-3' R: 5'-ATCGAAATCAGCTGTAGAAGT-3'</td>
<td>401bp</td>
<td>ChIP</td>
<td>Wither et al., 2009</td>
</tr>
<tr>
<td>Cep131</td>
<td>F: 5'-CTATATGACCCCGCCCTTCTC-3' R: 5'-GTCCTCTGGGAGAATCGAC-3'</td>
<td>103bp</td>
<td>ChIP</td>
<td>This Study</td>
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<tr>
<td>GAPDH</td>
<td>F: 5'-ATGTTGGCCACTGGGGATCT-3' R: 5'-TGCCAAGCCTAGGGGAAGA-3'</td>
<td>176bp</td>
<td>ChIP</td>
<td>Active Motif kit</td>
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<tr>
<td>Bisulfite converted Cep131 promoter (From -541 to -391nt)</td>
<td>F1; 5' - GTTTTCTAGTGTATTGAGTAACG-3'</td>
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<tr>
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<td>F2; 5' - GTTTTTGTATTGGTAGTTGAATAG-3'</td>
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<td>F3; 5' - TTTTCTAGTGTATTGAGTAACGA-3'</td>
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<td>F4; 5' - TTTTTGTATTGTAATGTTGAATAG-3'</td>
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<td>R1; 5' - ACAAAAAAATATAAACGAAACACG-3'</td>
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<tr>
<td></td>
<td>R2; 5' - AAAACAAATAAACACAAAACACACCA-3'</td>
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<tr>
<td></td>
<td>R3; 5' - AAAAAAAACATATAAACGAAACACG-3'</td>
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<td>Bisulfite converted Cep131 promoter (From -137 to +11nt)</td>
<td>198bp~201 bp Bisulfite sequence This Study</td>
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<td>F1; 5' - GTTTGAATTTTTCGAAGGATAGTC-3'</td>
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<td>F2; 5' - GTTTGAATTGGTAGTTGAATAGTTC-3'</td>
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<td>F3; 5' - ATTTTTCAAGGATAGTTCGAG-3'</td>
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<td>F4; 5' - AATTTTTTAGTAGTTGATGG-3'</td>
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<td>F5; 5' - TTGAAATTTCGAAGGATAGTC-3'</td>
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<td></td>
<td>F6; 5' - GAATTGTTGAAGGATAGTGAGTC-3'</td>
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<tr>
<td></td>
<td>R1; 5' - GATAAACTAAAACCGAGGACCGAAG-3'</td>
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<td>R2; 5' - AATAAACACACACACACACACACACAC-3'</td>
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<td>R3; 5' - GAAAACCGATAAACAAACACACACACACAC-3'</td>
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<td>R4; 5' - CAAAACCAATCAATATACAAACACACACACACAC-3'</td>
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<td>R5; 5' - CAATAAACACACACACACACACACACACACACACACACAC-3'</td>
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<td>R6; 5' - CCAATAAACACACACACACACACACACACACACACACACAC-3'</td>
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<td>193bp~198 bp Bisulfite sequence This Study</td>
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## Supplementary Table 3. shRNA or siRNA sequences used in this study

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<tr>
<th>Gene</th>
<th>Sequence (nt positions from the start codon)</th>
<th>Reference</th>
<th>Method</th>
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</thead>
<tbody>
<tr>
<td>Luciferase(GL2)</td>
<td>CGTACGGGAATACTTCGA</td>
<td>(Elbashir et al., 2001)</td>
<td>lentivirus,</td>
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<td></td>
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<td>synthetic</td>
</tr>
<tr>
<td>DNMT1</td>
<td>GTATGAGTGGAAATTAAGA (5114-5162; 3’UTR)</td>
<td>(Sen GL et al., 2010)</td>
<td>lentivirus,</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>synthetic</td>
</tr>
<tr>
<td>DNMT3A</td>
<td>ACTGACGTCTCCAACATGA (2623-2641)</td>
<td>This study</td>
<td>synthetic</td>
</tr>
<tr>
<td>DNMT3B</td>
<td>AGATGACGATGCTAGAGTT (3961-3981; 3’UTR)</td>
<td>(Leu YW et al., 2003)</td>
<td>synthetic</td>
</tr>
<tr>
<td>Cep131</td>
<td>CAGCAGCAGCTGGAGATTA (2005-2023)</td>
<td>(Graser S et al., 2007)</td>
<td>lentivirus,</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>synthetic</td>
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Supplementary Table 4. The list of Antibodies used in this study

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<tr>
<th>Antibodies</th>
<th>Species</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-α-tubulin</td>
<td>Mouse</td>
<td>Sigma, St. Louis, MO</td>
</tr>
<tr>
<td>Anti-Cep131</td>
<td>Rabbit</td>
<td>Bethyl Laboratories, Montgomery, TX</td>
</tr>
<tr>
<td>Anti-DNMT1</td>
<td>Mouse</td>
<td>BD bioscience, Franklin Lakes, NJ</td>
</tr>
<tr>
<td>Anti-DNMT1*</td>
<td>Mouse</td>
<td>Imgenex, San Diego, CA</td>
</tr>
<tr>
<td>Anti-γ-tubulin</td>
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<td>Sigma, St. Louis, MO</td>
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<tr>
<td>Anti-SP1</td>
<td>Rabbit</td>
<td>Santa Cruz Biotechnologies, Santa Cruz, CA</td>
</tr>
<tr>
<td>Anti-SP1*</td>
<td>Rabbit</td>
<td>Millipore, St. Louis, MO</td>
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<tr>
<td>Anti-TNFAIP3</td>
<td>Mouse</td>
<td>Imgenex, San Diego, CA</td>
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<tr>
<td>Anti-TUBGCP3</td>
<td>Rabbit</td>
<td>Proteintech Group, Rosemont, IL</td>
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<tr>
<td>Anti-SHKBPI</td>
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<tr>
<td>Anti-MAPRE1</td>
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</tr>
<tr>
<td>Anti-GAPDH</td>
<td>Rabbit</td>
<td>Santa Cruz Biotechnologies, Santa Cruz, CA</td>
</tr>
</tbody>
</table>

*; These antibodies used for ChIP assay
References


