ONLINE METHODS

Cell culture, protein stability, GST pull-down, immunoprecipitation and immunocytochemistry. Cell lines (HeLa, HEK293, NIH3T3, Jurkat and COS-7) were from ATCC. Cells were synchronized in G1/S with a 2-mM thymidine block followed by 0.4 mM mimosine. For DNMT1 protein stability studies, Jurkat cells were treated with 20 μg ml^{-1} cycloheximide (Sigma), lysed and assayed by western blotting using anti-DNMT1, anti–DNMT1-Lys142me1 and anti–DNMT1-pSer143 (New England Biolabs).

Immunoprecipitations of nuclear extract were performed as described[28] with 5 μg of anti-DNMT1 (Imgenex) or anti–phospho-AKT1 (Cell Signaling Technologies), or 5 μl of antiserum raised against DNMT1K142me1 peptide (New England Biolabs), and with 5 μg of purified anti-GFP (Roche) or rabbit purified IgG (Cell Signaling Technologies) as a negative control. Nuclear extracts were done as described[32].

GST pull-downs were performed as described[33]. Recombinant purified DNMT1 (ref. 34) and AKT1 were obtained from New England Biolabs and Millipore, respectively.

For immunofluorescence studies, COS-7, NIH3T3 and HeLa cells were cultured on coverslips and transfected with a mixture of DsRed-DNMT1 plasmid and Transpass D2 reagent (New England Biolabs) at a ratio of 1:8 μg/μl for 24 h. The cells were synchronized in G1/S, fixed and visualized using a Zeiss confocal microscope LSM510, ×63 objective, at 568 nm for DsRed-DNMT1 and 460 nm for nuclei (Hoechst 33342). Endogenous AKT1, DNMT1 and PCNA were detected using their respective primary and AlexaFluor 488–conjugated secondary antibodies.

Methyltransferase and kinase assays. Methyltransferase assays were carried out at 37 °C for 10 min in duplicate, with a total volume of 25 μl of reaction mix[15]. Peptides representing unmodified DNMT1 (DNMT1K142; sequence, RTPRRSKSDGEA) and phospho-DNMT1 (DNMT1pS143; sequence, RTPRRSKpSDGEA) were dissolved in water for assay. Kinase assays were performed using recombinant AKT1 (Millipore) with 1 μg of DNMT1K142me1, DNMT1K142(S143A) or wild-type DNMT1K142 peptide. For baculovirus-purified DNMT1, 1 μg of enzyme was first dephosphorylated using PP1 (New England Biolabs). After 1 h at 30 °C, PP1 activity was blocked with protein phosphatase inhibitor-2 (New England Biolabs) and calyculin A (Cell Signaling Technology). We used 40 ng or 100 ng of DNMT1 in the AKT1 assay for 20 min at 37 °C. DNMT1 phosphorylation was monitored with anti–DNMT1-pSer143.

Proteasome and AKT1 inhibition, ubiquitin detection, western blotting, siRNA knockdown and quantitative PCR analysis. HeLa cells were treated with the proteasome inhibitor MG132 (Calbiochem) and/or the AKT1 inhibitor LY294002 (Cell Signaling Technology) (20 μM) for up to 24 h. Cells were lysed with RIPA buffer containing 100 μM *N*-ethylmaleimide (Sigma), 1 mM Na_3VO_4 (New England Biolabs), 50 mM NaF (New England Biolabs), 2.5 mM β-glycerophosphate (Sigma) and 100 nM calyculin A (Cell Signaling Technology) and assayed by western blotting.

For ubiquitin detection, COS-7 cells were transiently transfected with GFP-SET7, HA-ubiquitin and DsRed-DNMT1FL or DsRed-DNMT1(S143A) plasmids, and treated with 10 μM MG132 for 24 h. Nuclear extracts were made in presence of 100 μM *N*-ethylmaleimide[32]. After DNMT1 immunoprecipitation, ubiquitin was revealed by an antibody to the HA tag (Cell Signaling Technology).

Western blots were performed as described[28]. Antibodies and antisera against DNMT1, DNMT1-pSer143 and DNMT1-Lys142me1 were made in-house. Anti–phospho-AKT1, anti-AKT1, anti–cyclin A and anti-HA were from Cell Signaling Technology; monoclonal antibodies to actin and GFP were from Sigma and Roche, respectively. Polyclonal antibody to DsRed was from Biovision. Densitometric analysis was done with ImageJ (NIH; http://rsbweb.nih.gov/ij/).

For *AKT1* gene knockdown, HeLa cells were transfected using Transpass HeLa reagent (New England Biolabs) with 20 nM of AKT siRNA mix (New England Biolabs). For the control, siRNA mix against Litmus 28i was used (New England Biolabs). Total RNA was purified by RNeasy kit (Qiagen). The ProtoScript First Strand cDNA Synthesis kit (New England Biolabs) was used to produce cDNA. RNA was quantified by real-time PCR, using a MyiQ Cycler (Bio-Rad) and iQ SYBR Green Supermix (Bio-Rad), with the following primers: for DNMT1, 5′-GGCTGAGATGAGGCAAAAAG-3′ (forward) and 5′-ACCAACTCGGTACAGGATGC-3′ (reverse); for AKT1 isoforms, 5′-TGTGTCAGCCCTGGACTACC-3′ (forward) and 5′-TGAGCAGCCCTGAAAGCAAG-3′ (reverse). RNA levels were normalized to human ALDOA RNA expression, which was measured using the primers 5′-CGGGAAGAAGGAGAACCTG-3′ (forward) and 5′-GACCGCTCGGAGTGTACTTT-3′ (reverse). The percentage of mRNA remaining was compared with that of cells treated with the siRNA mix against Litmus 28i as a negative control.

Transient transfection, constructs and genomic DNA isolation. COS-7 cells were transfected for 24 h with a mixture of (i) plasmids expressing GFP or GFP-SET7, (ii) plasmids expressing DsRed-DNMT1FL or DsRed-DNMT1(S143A), and (iii) Transpass D2 transfection reagent (New England Biolabs) at a ratio of 1 μg plasmids/8 μl transfection reagent.

GFP-SET7 and DsRed-DNMT1FL constructs have been described[15]. HA-AKT1 cDNA was cloned into pCDNA3.1 vector (Invitrogen). GST-AKT1 domains and GFP-AKT1 were cloned into pGEX5.1 and pEGFP-C2, respectively. DsRed-DNMT1(S143A), DsRed-DNMT1(S143D) and HA-AKT1(T308D S473D), GFP-AKT1(K179M) point mutants were created using the Phusion Site-Directed Mutagenesis kit (New England Biolabs).

HeLa genomic DNA was isolated using the DNeasy Blood and Tissue kit (Qiagen). We digested 2 μg of DNA overnight using micrococcal nuclease (New England Biolabs), Antarctic phosphatase (New England Biolabs) and venom phosphodiesterase (Sigma), and then analyzed it by HPLC[35].

Crystallography. The expression and purification of human SET7 have been described[36]. The protein, concentrated to ~15 mg ml^{-1} (in 20 mM Tris pH 7.5, 5% (v/v) glycerol, 0.1% (v/v) β-mercaptoethanol, 100 mM NaCl), was incubated with a 10-residue peptide derived from DNMT1 (residues 137–146, NH_2-TPRRS**K**SDGE-COOH (Lys142 shown in bold)) and methyl donor AdoMet on ice for 30 min at a molar ratio of 1:3:3 (protein:peptide:AdoMet). Crystals were obtained in 40% (w/v) PEG 3350 and 100 mM Tris pH 7.5, cryoprotected with mother liquor supplemented with 20% (v/v) ethylene glycol and flash-frozen in liquid nitrogen. X-ray diffraction data was collected at the SER-CAT 22-BM beamline at the Advanced Photon Source. Diffraction data were integrated and scaled with HKL2000 (ref. 37). The protein coordinates from the SET7–ER complex (PDB 3CBM)[36] were used to search for the molecular-replacement solution with AmoRe[38]. Manual model building was carried out in O[39] and subsequent refinement with CNS[40].

Expression and purification of DNMT1 N-terminal fragment The N-terminal 350 residues of DNMT1 were cloned into the pGEX-4T1 vector (Amersham Biosciences). The K142R mutant construct was generated by standard PCR-based strategies. Resulting GST-tagged fusion protein constructs were expressed in *Escherichia coli* BL21 (DE3)-Gold cells (Stratagene) with RIL-Codon plus plasmid. The GST fusion tag was removed on the column by digestion with thrombin[41], and eluted protein was loaded onto a HiTrap SP column (GE Healthcare). Bound protein was eluted using a linear gradient of NaCl (50 mM to 1 M) and further purified on a Superdex 200 (16/60) gel-filtration column (GE Healthcare).

32. Andrews, N.C. & Faller, D.V. A rapid micropreparation technique for extraction of DNA-binding proteins from limiting numbers of mammalian cells. *Nucleic Acids Res.* **19**, 2499 (1991).
33. Kim, G.D., Ni, J.W., Stark, N., Roberts, R.J. & Pradhan, S. Co-operation and communication between the human maintenance and de novo DNA (cytosine-5) methyltransferases. *EMBO J.* **21**, 4183–4195 (2002).
34. Pradhan, S., Bacolla, A., Wells, R.D. & Roberts, R.J. Recombinant human DNA (cytosine-5) methyltransferase. I. Expression, purification, and comparison of de novo and maintenance methylation. *J. Biol. Chem.* **274**, 33002–33010 (1999).
35. Ehrlich, M. DNA hypomethylation, cancer, the immunodeficiency, centromeric region instability, facial anomalies syndrome and chromosomal rearrangements. *J. Nutr.* **132**, 2424–2429 (2002).
36. Subramanian, K. *et al.* Regulation of estrogen receptor alpha by the SET7 lysine methyltransferase. *Mol. Cell* **30**, 336–347 (2008).
37. Otwinowski, Z., Borek, D., Majewski, W. & Minor, W. Multiparametric scaling of diffraction intensities. *Acta Crystallogr. A* **59**, 228–234 (2003).
38. Navaza, J. Implementation of molecular replacement in AMoRe. *Acta Crystallogr. D* **57**, 1367–1372 (2001).
39. Jones, T.A., Zou, J.Y., Cowan, S.W. & Kjeldgaard, M. Improved methods for building protein models in electron density maps and the location of errors in these models. *Acta Crystallogr. A* **47**, 110–119 (1991).
40. Brünger, A.T. *et al.* Crystallography & NMR system: a new software suite for macromolecular structure determination. *Acta Crystallogr. D* **54**, 905–921 (1998).
41. Birktoft, J.J. & Breddam, K. Glutamyl endopeptidases. *Methods Enzymol.* **244**, 114–126 (1994).