Supporting Information

Estève et al. 10.1073/pnas.0810362106

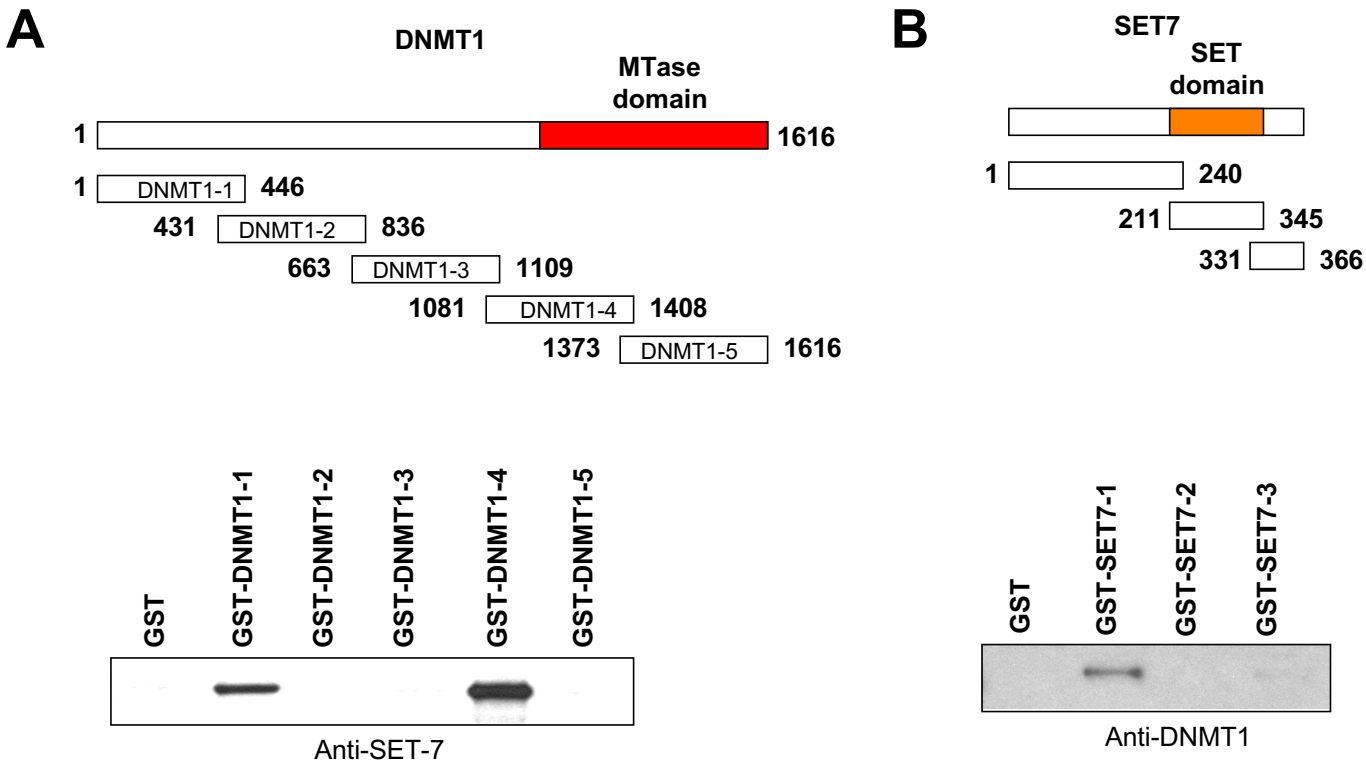

Fig. S1. Direct interaction between SET7 and DNMT1. (*A*) Interacting domain mapping between DNMT1 and SET7. (*Upper*) GST fusion fragments are schematically below the full-length enzyme with amino acids numbers. (*Lower*) Western blot analysis of the interacting fragments of DNMT1 and SET7 using anti-SET7 antibody. (*B*) Interacting domain mapping between SET7 and DNMT1. Experiments similar to *A* were performed by using GST fusions of SET7 with full-length DNMT1. Western blot analysis was performed with anti-DNMT1.

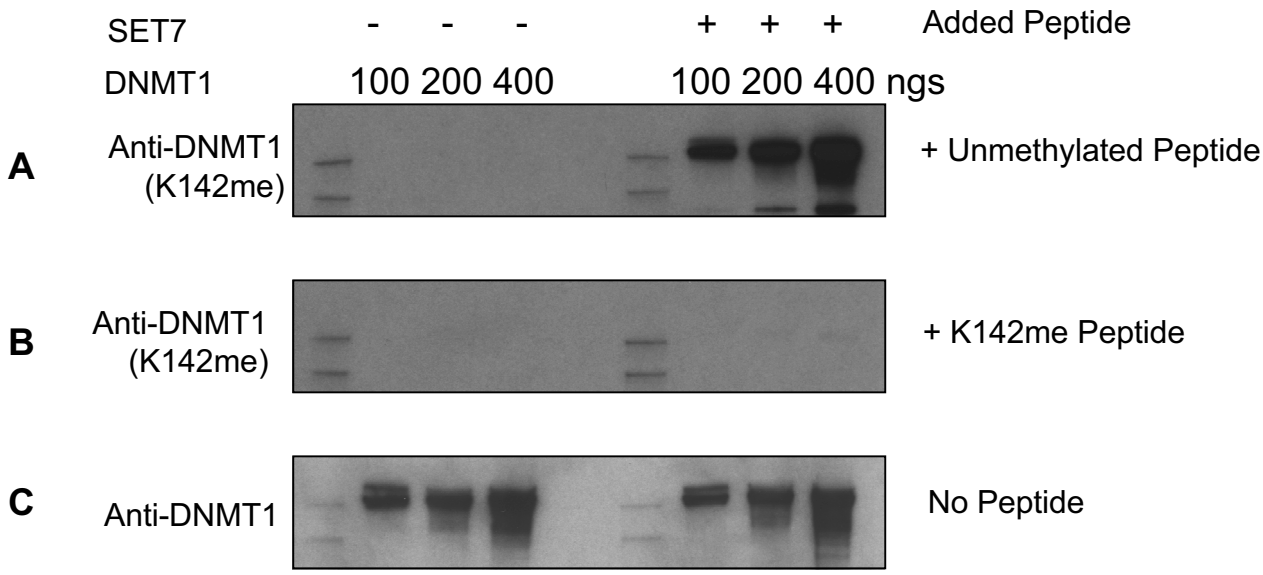

Fig. S2. Specificity of DNMT1 (K142me) antibody. (*A* and *B*) Recombinant DNMT1 from baculovirus expression system was mock methylated (−) or methylated (+) by SET7 and analyzed by Western blot. The Western blots were probed with anti-DNMT1 (K142me) antibody in the presence of the competitor unmethylated (*A*) or K142me peptide (*B*). Note in the absence of K142me peptide, but in the presence of identical unmethylated peptide the antibody recognized methylated DNMT1. Competition with methylated peptide abolished antibody binding in *B*. (*C*) Control Western blot with anti-DNMT1 reveals equal loading of both unmethylated and methylated DNMT1.

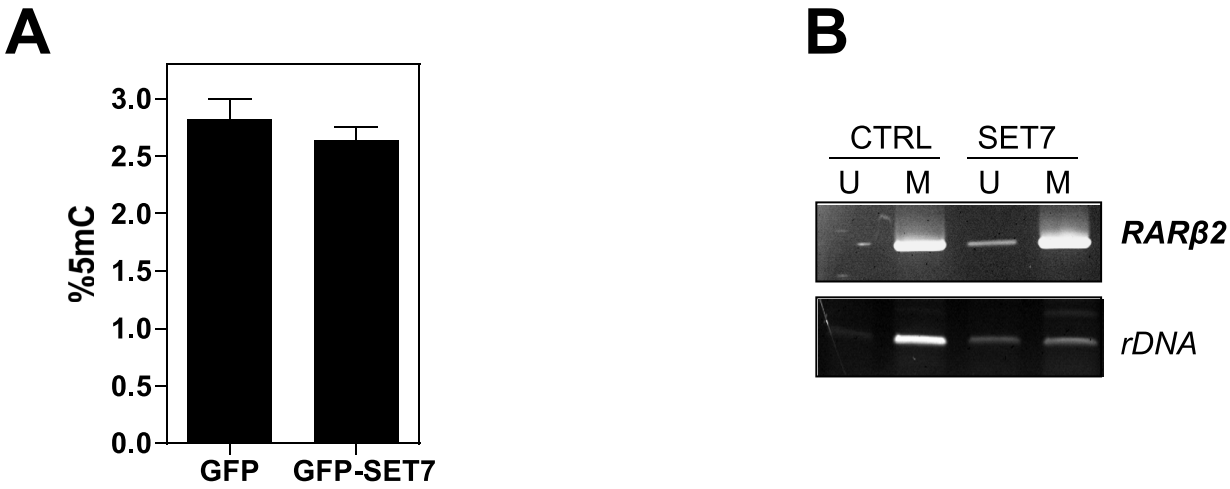

Fig. S3. Decrease DNA methylation by SET7 overexpression. (*A*) HPLC analysis of 5-methyl cytosine in the genomic DNA of HeLa cells transfected with GFP control or GFP-SET7 expression plasmids. The genomic DNA was isolated after 3-successive transfection (98 hrs) and analyzed by HPLC (1). The quantitative data was measured from 3 independent measurements. The content of % 5mC for control GFP and GFP-SET7 overexpressed cells were 2.81 $\pm$ 0.36 (*P* value: 0.0006; $\alpha = 0.05$) and 2.63 $\pm$ 0.26 (*P* value 0.0002; $\alpha = 0.05$) respectively. (*B*) Methyl-sensitive PCR assay for demethylation in RAR2 and rDNA loci in SET7-overexpressing cells. Primers specific for unmethylated (U) and methylated (M) are indicated on top.

1. Ehrlich M (2002) DNA hypomethylation, cancer, the immunodeficiency, centromeric region instability, facial anomalies syndrome and chromosomal rearrangements. *J nutrition*, 132(Suppl 8):2424S–2429S.

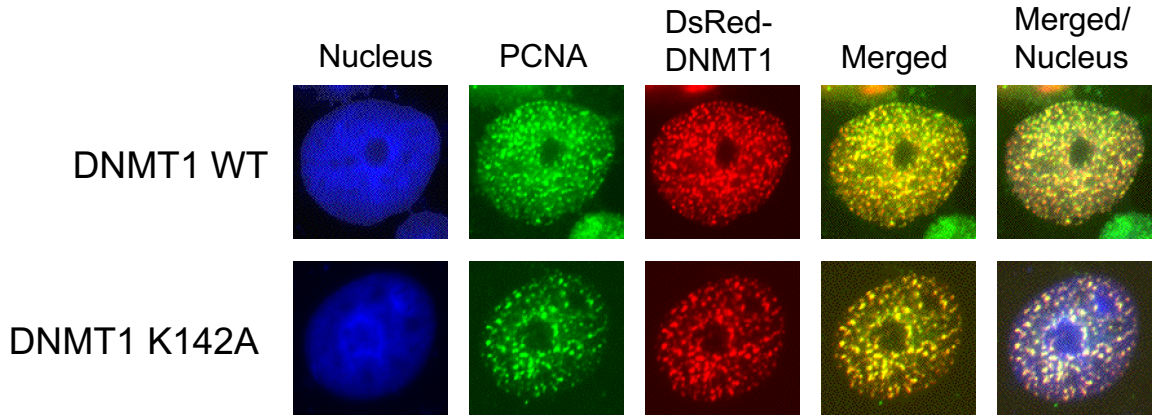

Fig. S4. PCNA loading of DNMT1. Both WT DNMT1 (red) and K142A mutant DNMT1 (red) associate with DNMT1 loading factor PCNA (green) during S phase. Nuclear staining was performed with Hoechst stain.

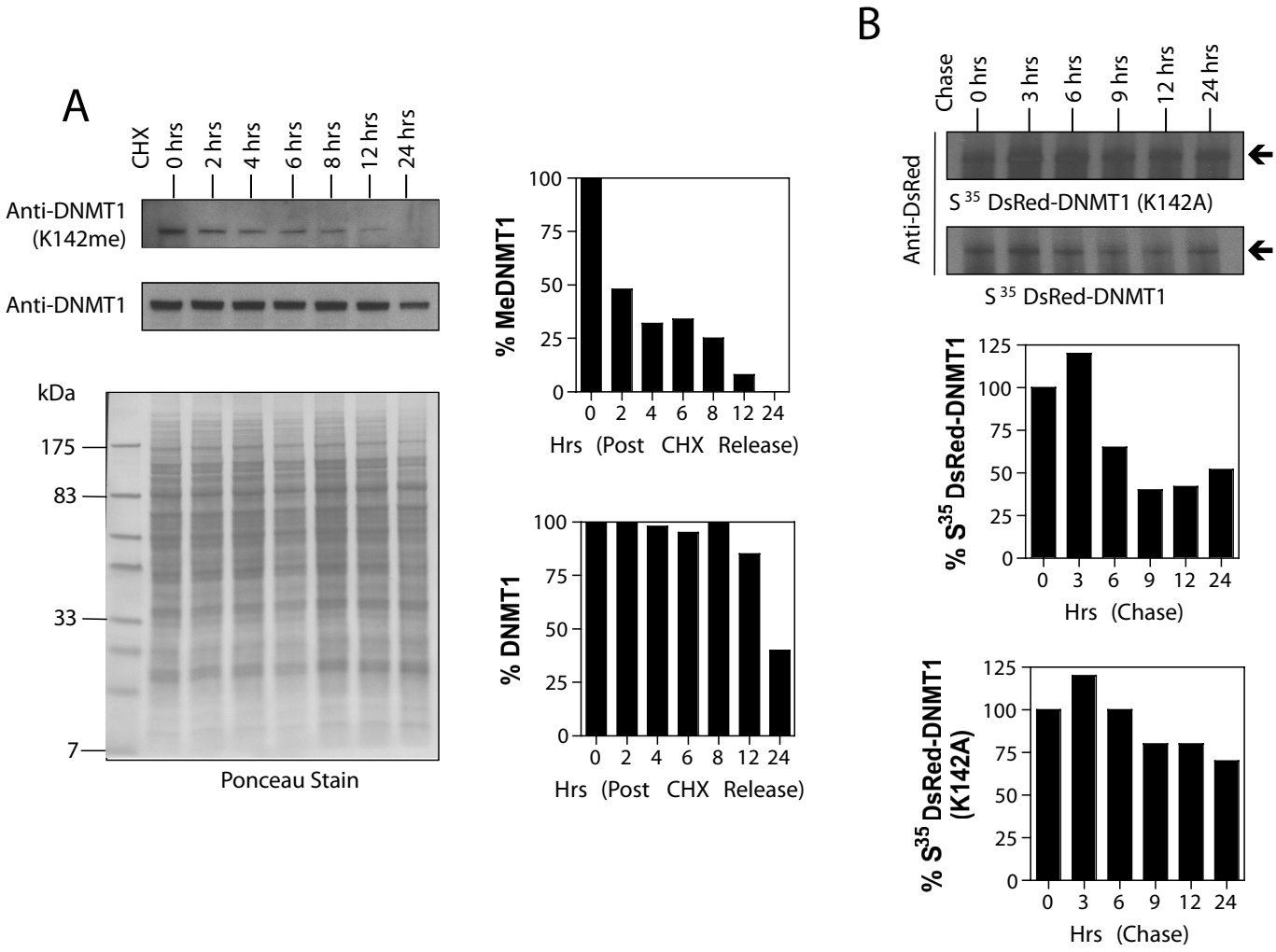

Fig. S5. Half-life studies of DNMT1. (*A*) Half-life study of endogenous methylated and unmethylated DNMT1 in Jurkat cells. CHX (cylohexamide) was used for protein synthesis monitoring. The hrs depict the cell sample withdrawal after specific time of CHX treatment. Antibodies are indicated at the left, and the ponceau-stained gel before Western blot analysis is shown for loading control. At the right side densitometric quantitation of meDNMT1 and DNMT1 is shown. (*B*) Pulse–chase with radioactive S35 Met after the COS-7 cells were transfected with DsRed-DNMT1 or DsRed-DNMT1 (K142A). The overexpressed proteins were immunoprecipitated with anti-DsRed antibody. Fluorography is shown at the top, and quantitative bar corresponding to the Western blots is at the bottom.