RT-PCR primers for *TIMP3, P16* and *MLH1*. TIMP3-F: 5'-ACGCTGGTCTACACCATCAAGC-3' and TIMP3-R: 5'-CCGAAATTGGAGAGCATGTCG-3'; P16-F: 5'-GTCCCCTTGCCTGGAAAGATAC-3' and P16-R: 5'-GGTCTG TGATTACAAACCCCTTCTG-3'; MLH1-F: 5'-TTCTCAGGTTATCGGAGCCAGCAC-3' and MLH1-R: 5'-CTTCGTCCCAATTCACCTCAGTGG-3'.

COBRA primers for *TIMP3.* TIMP3-BS-F: 5'-TTTGTTTTTTTAGTTTTTGTTTTTT-3' and TIMP3-BS-R: 5'-AATC CCCCAAACTCCAACTAC-3'.

DNA methyltransferase (CpG methyltransferase) assay. DNA methylase activity was assayed by measuring the incorporation of $^{3}H_{1}$-methyl group from Ado-Met into DNA using DE-81 ion exchange filter binding assay (13) with some modifications. Human recombinant DNMT1 (New England Biolabs; 100 ng~10 units), recombinant mouse Dnmt3a/ Dnmt3b (500 ng) was incubated with 500 ng of poly(dI-dC) or hemimethylated DNA duplex and 75 or 150 nM (0.275μCi or 0.55μCi) of [methyl-^{3}H]-S-adenosylmethionine (Ado-Met) as described (24) in a total volume of 50 μl at 37^{0}C for 1hr. or M. Sss I (New England Biolabs, 2 units) was assayed in the supplier's buffer. SGI-1027 or decitabine was added at indicated concentrations. Each reaction was performed in duplicate and included controls with no inhibitor or no DNA. The reaction was stopped by soaking reaction mixture onto a Whatman DE-81 ion exchange filter disc, washed (five times, 10 min each, with 0.5M Na-phosphate buffer; pH 7.0) dried and counted in a scintillation counter (24). The background radioactivity (no DNA control)

was subtracted from the values obtained with reaction mixtures containing DNA and the radioactivity obtained in the reaction without any inhibitor was considered as 100% activity. IC_{50} was determined by interpolation from the plot of percent activity versus inhibitor concentration.

To determine the nature of inhibition of DNMTase activity by SGI-1027, DNMT1 enzyme activity was measured in presence of a fixed concentration of inhibitor (0, 2.5, 5, and 10μM) while one of the two (Ado-Met or DNA) was varied in a particular reaction mixture. At a fixed concentration of DNA (500 ng) varying concentrations of Ado-Met used were from 25-500 nM, respectively. Similarly, final DNA concentrations were varied from (25-500ng) at 75 nM Ado-Met.

Genome wide DNA methylation analysis. Stock solutions of 5mdC, 2dC, 5,6-dihydro-5-azacytidine, and 2dG were prepared by dissolving the accurately weighed drug in 10 mL of methanol and methanol containing 0.2% NH_4OH, respectively, to a final concentration of 1 mg/mL and stored in a glass vial at -80 ^oC. Working solutions were freshly prepared daily by diluting the stock solution with methanol. Volumes of 2dC, 5mdC and 2dG working solution were added into working solution of 5,6-dihydro-5-azacytidine to prepare calibration standards at the following concentrations: 0, 2, 5, 10, 20, 50, 100, 200, and 500, 1000 ng/mL in hydrolytic solution. The enzymatic digestion solutions of the genomic DNA extracted from the RKO control and drug-treated cells were reconstituted in 200 μL water at 4^oC and analyzed immediately by LC-MS. The calibration curve is shown in Figure S1. The ratio of methyl cytosine to cytosine content was determined from the calibration curve.

Figure Legends

Fig.S1. SGI-1027 treatment did not demonstrate genome wide DNA hypomethylation. Global DNA methylation calibration curves using (left) 5mdC, (middle) 2dC and (right) 2dG, (2-1000 ng/mL) spiked into a 1000 ng/mL solution of 5,6-dihydro-5-azacytidine in hydrolysis buffer (0.1 mM ammonium bicarbonate, 0.01 mM ammonium carbonate, 1 U AP and Phosphodiesterase). [Molar ratio of 5mdC/(2dC+5mdC) %] of control and SGI-1027 treated cells were 6.01 and 5.91, respectively.

Fig. S2. SGI-1027 induces depletion of DNMT1 in a wide variety of human cancer cell lines. Cells were treated with decitabine at 5μM and varying concentrations of SGI-1027 for 24h. Control cells were treated with the vehicle (DMSO) alone. Whole cell lysates (250 μg of protein) were subjected to immunoblot analysis with antibodies specific for DNMT1, DMT3A, DNMT3B and GAPDH. A, HepG2 B, HeLa C, LNCAP D, MCF-7