Supporting Information

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SI Methods

DNA Synthesis and Purification. Oligonucleotides were either synthesized on an Applied Biosystems 3400 DNA synthesizer or purchased from IDT. The terminal C6 alkyne moiety that was incorporated into the 5′ end of one of the strands was purchased from Glen Research. Complementary unmodified strands were purchased from IDT. DNA was deprotected and cleaved from solid support with ammonium hydroxide (60 °C for 12 h). Following a preliminary round of HPLC, oligonucleotides were treated with 80% acetic acid in water for 20 min. Each oligonucleotide was purified by HPLC using a gradient of acetonitrile and 50 mM ammonium acetate. Following purification, oligonucleotides were desalted by ethanol precipitation and quantified by UV-visible spectrophotometry based on their extinction coefficients at 260 nm (IDT Oligo Analyzer). Oligonucleotide masses were verified by matrix-assisted laser desorption mass spectrometry. DNA duplexes were formed by thermally annealing equimolar amounts of single-stranded oligonucleotides in deoxygenated phosphate buffer (5 mM phosphate, 50 mM NaCl, pH 7.0) at 90 °C for 5 min followed by slowly cooling to 25 °C.

The following sequences were prepared:

Alkyne: 5′-C_2-$(CH_2)_6$-GA CTG AGT ACT GCG CGC ACT GAT AGC-3′

Complement: 5′-GCT ATC AGT GCG CGC AGT ACT CAG TC-3′

Methylated complement: 5′-GCT ATC AGT GCG C^mGC AGT ACT CAG TC-3′

The *BssHII* restriction site is underlined.

Western Blot Analysis of Lysate for DNMT1. DNMT1 expression was confirmed by Western blot. Samples were mixed with Laemmli reagent and betamercaptoethanol and probe sonicated for 10 s, followed by heat inactivation at 90 °C for 5 min. Samples were loaded onto 4–12% polyacrylamide gels in Mops buffer and run at 175 mV for 1.5 h. Gels were subsequently transferred to membranes with a dry transfer procedure for 1.5 h. Membranes were then blocked with 5% (vol/vol) milk in Tris buffered saline with Tween 20 (TBST) at room temperature for 1 h, followed by overnight incubation with a 1° antibody in milk and 3% BSA (wt/vol) [1:2,000 for DNMT1 (New England Biolabs) and 1:1,000 for Lamin A (Santa Cruz Biotechnology)] for either DNMT1 or Lamin A. The membranes were then rinsed with TBST buffer. Membranes were incubated with goat anti-rabbit 2° antibody (Abcam Incorporated) [1:7,500 in 5% (vol/vol) milk in TBST] for 1 h and then rinsed with TBST, followed by scanning on an Odyssey infrared gel scanner. Resulting Western blots are shown in Fig. S3.

^{3}H-SAM Methyltransferase Activity Assay. Methyltransferase activity was additionally tested using the conventional method of a ^{3}H-SAM incorporation activity assay. The activity assay followed a published protocol (1, 2). Briefly, 20-μL total reaction volumes were used for the ^{3}H-SAM activity assay. DNA (20 μM), identical to that used as a substrate for the electrochemical assay including the hexynyl terminus, was used. Then, 0.5 μCi ^{3}H-SAM was added, and the reactions were run in DNMT1 activity buffer. BSA (100 μg/mL) was included for the purified DNMT1 reaction, which was used as a positive standard, along with a negative standard that contained no protein. For the lysate samples, ∼2 μL of lysate was included in the reaction mixture, bringing the total protein content for the reaction mixture to 3,500 μg/mL. Reactions were incubated at 37 °C for 2 h, followed by stopping with 30 μL of 10% trichloroacetic acid in water. The resulting solutions were spotted onto DE81 filter paper (Whatman) and air-dried for 15 min. Filter papers were then individually soaked in 10 mL of 50 mM Na_2HPO_4 for 15 min and rinsed with both 50 mM Na_2HPO_4 and 95% ethanol. Filter papers were then heated to 37 °C to dry for 15 min before liquid scintillation counting. The DNMT1 assay, measured using radioactivity, is shown in Fig. S4.

1. Pradhan S, Bacolla A, Wells RD, Roberts RJ (1999) Recombinant human DNA (cytosine-5) methyltransferase. I. Expression, purification, and comparison of *de novo* and maintenance methylation. *J Biol Chem* 274(46):33002–33010.

2. Jurkowska RZ, Ceccaldi A, Zhang Y, Arimondo PB, Jeltsch A (2011) DNA methyltransferase assays. *Methods Mol Biol* 791:157–177.

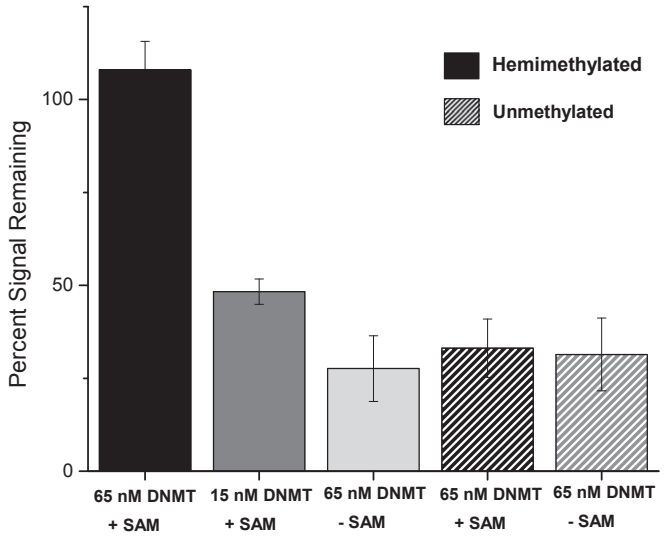

Fig. S1. Substrate specificity and detection limits for purified DNMT1. The data are reported as the percent signal remaining by comparing the signal after protease treatment and after restriction enzyme treatment. DNMT1 (65 nM) on hemimethylated DNA with 160 μM SAM afforded full protection of the DNA on the surface (black), whereas electrodes treated with 15 nM DNMT1 on hemimethylated DNA with 160 μM SAM maintained 48 ± 3% signal (dark gray). DNMT1 (65 nM) on hemimethylated DNA without SAM (light gray), on unmethylated substrate with 160 μM SAM (black hashed), or on unmethylated substrate without 160 μM SAM (gray hashed) showed no signal protection. The constant potential amperometry was performed for 90 s with an applied potential of 320 mV to the patterning/detection electrode array and −400 mV to the substrate electrode array relative to a AgCl/Ag reference electrode with a platinum auxiliary electrode. All scans were performed in Tris buffer (10 mM Tris, 100 mM KCl, 2.5 mM $MgCl_2$, 1 mM $CaCl_2$, pH 7.6) with 4 μM MB+ and 300 μM potassium ferricyanide. Error bars represent the SD over three measurements for three experiments.

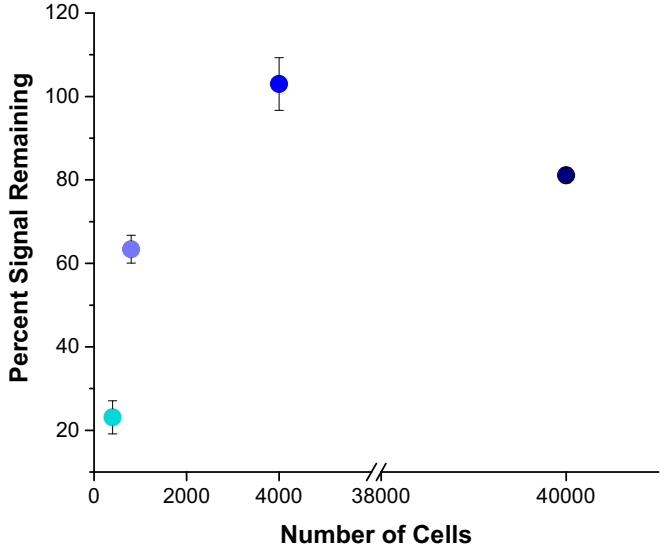

Fig. S2. Signal protection from differing amounts of lysate. All electrodes on this array were modified with hemimethylated DNA, and values are normalized to 100% protection of the DNMT1-treated electrodes. All samples include 160 μM SAM. The darkest blue is lysate from ~40,000 cells. The subsequent points are from 4,000, 800, and 400 cells. Full protection is afforded from ~4,000 cells. The constant potential amperometry was performed for 90 s with an applied potential of 320 mV to the patterning/detection electrode array and −400 mV to the substrate electrode array relative to a AgCl/Ag reference electrode with a platinum auxiliary electrode. All scans were performed in Tris buffer (10 mM Tris, 100 mM KCl, 2.5 mM $MgCl_2$, 1 mM $CaCl_2$, pH 7.6) with 4 μM MB+ and 300 μM potassium ferricyanide. Error bars represent the SE for three electrodes over three measurements.

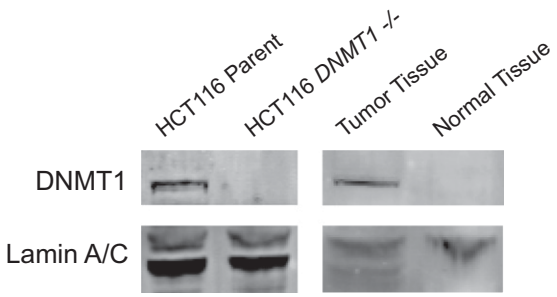

Fig. S3. Western blot for DNMT1. Shown are gel lanes for HCT116 parent cell lysate, the HCT116 *DNMT1*$^{-/-}$ lysate, the tumor tissue lysate, and the adjacent normal tissue lysate. Loading control Lamin A is also shown for all samples. DNMT1 is only detectable from the HCT116 parent lysate and the tumor tissue lysate; no DNMT1 is observable in the HCT116 *DNMT1*$^{-/-}$ lysate or the normal tissue lysate.

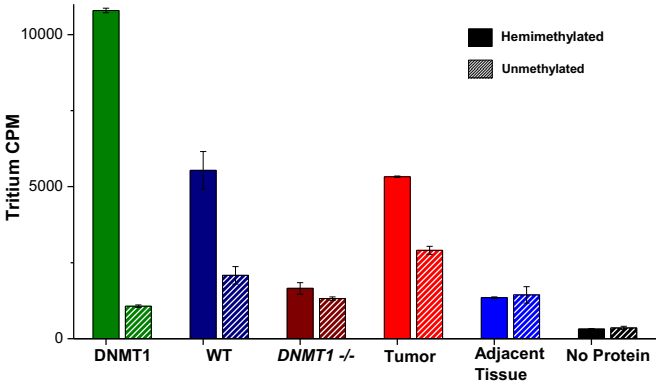

Fig. S4. ^{3}H-SAM DNMT1 activity assay. The results of a ^{3}H-SAM DNMT1 activity assay are shown, with the cpm per sample given. In all cases, the solid bar is data from hemimethylated substrate, and the hashed bar is data from unmethylated substrate. In green is 65 nM DNMT1, in dark blue is parent HCT116 lysate, and in dark red is DNMT1$^{-/-}$ lysate. In red is the tumor lysate, and in blue is lysate from adjacent healthy tissue. In black is the negative control that contains no protein. Error bars represent triplicate scintillation measurements for two replicates of each experimental condition.