Supplemental Data

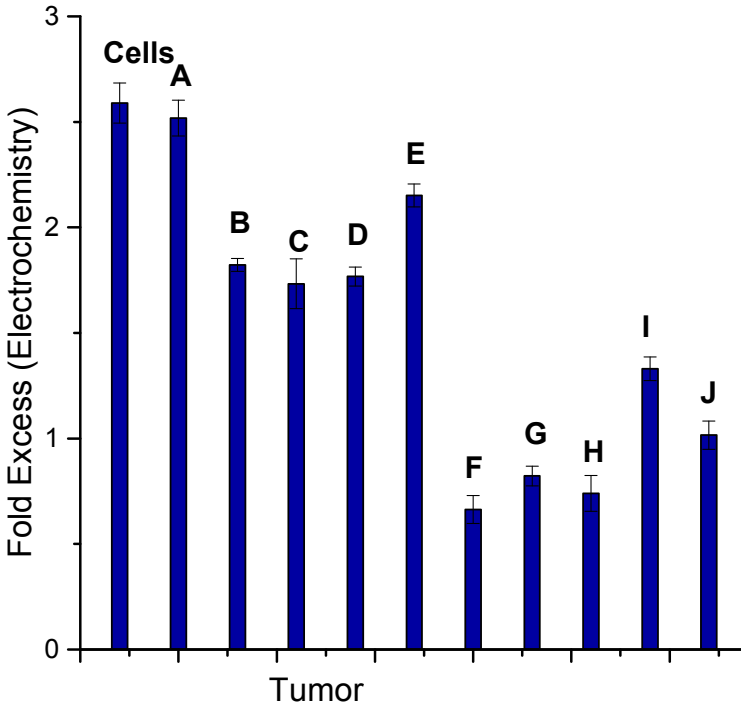

Figure S1. DNMT1 activity measured electrochemically without normalization to the unmethylated substrate, as shown in Figure 4. The fold excess activity measured electrochemically shows hyperactivity in all but two of the tissue samples. Those that do not show hyperactivity have equivalent DNMT1 activity between tumor and normal tissue. The data for activity on the hemimethylated substrate for the tumor tissue are normalized to that of the normal adjacent tissue.

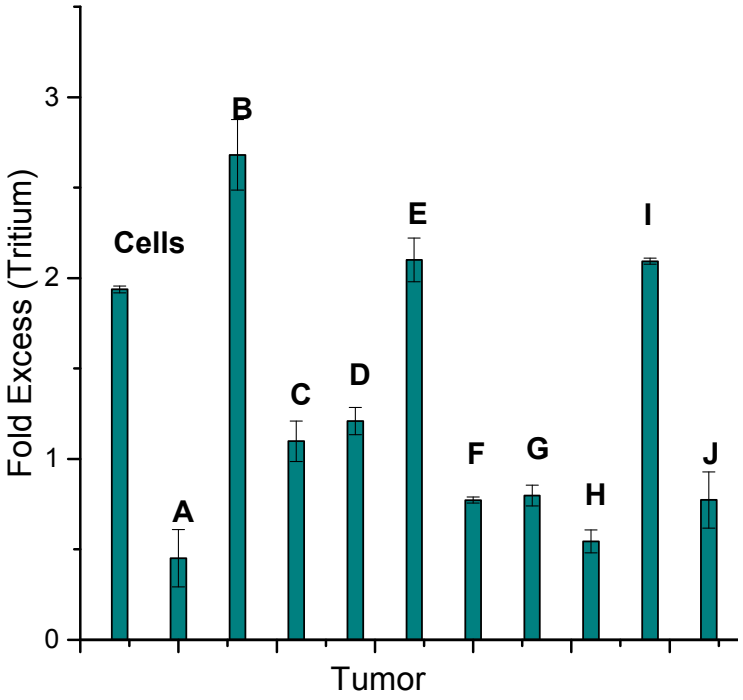

Figure S2. DNMT1 activity measured with radioactivity without normalization to the unmethylated substrate, as shown in Figure 4. The fold excess activity measured with radioactive labeling does not show similar hyperactivity in the tumor sample as when measured electrochemically. The data for activity on the hemimethylated substrate for the tumor tissue are normalized to that of the normal adjacent tissue.

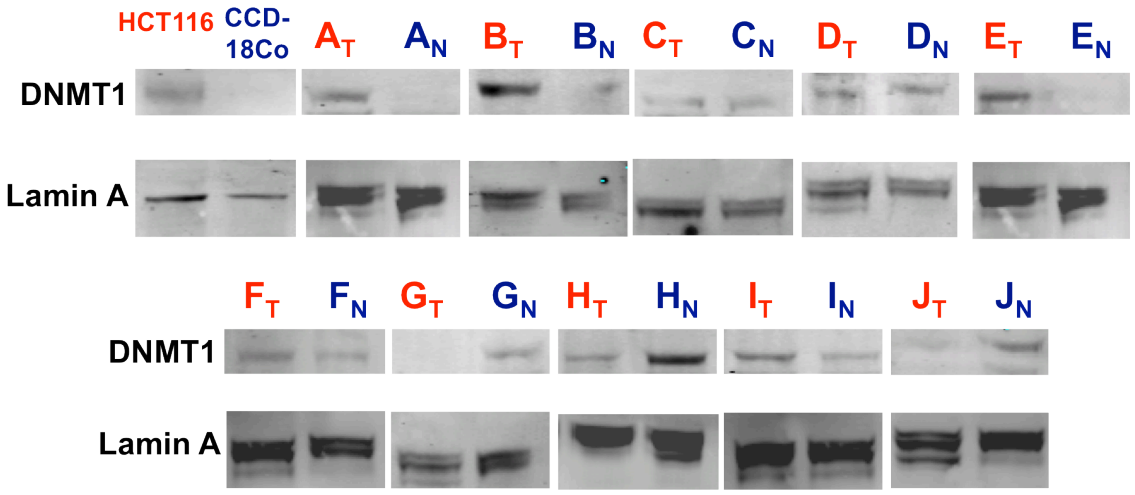

Figure S3. Sample Western blots used for quantification in Figure 5. Shown are the bands used for quantification of differential DNMT1 protein content in the tumor as compared to normal tissue. The top bars show the DNMT1 in each tissue set as well as cell lysate. The bottom bars show the loading control, Lamin A, to which each DNMT1 concentration is normalized. 60 μg of protein per lane were loaded onto the gels. 1^o antibody for DNMT1 (R & D) and Lamin A (Santa Cruz) is incubated overnight. Goat anti-rabbit 2^o antibody (Abcam) or Donkey Anti Sheep for DNMT1 (Santa Cruz) is subsequently incubated, followed by imaging. Quantification is performed with Li-COR Odyssey Image Studio software.

Supplemental Experimental Procedures

Methods and Materials

DNA Synthesis and Purification

Hexynyl-modified oligonucleotides were synthesized on an Applied Biosystems 3400 DNA synthesizer, and unmodified complementary DNA was purchased from IDT. The terminal hexynyl moiety that was incorporated into the 5′ end of one of the strands was purchased from Glen Research. DNA was deprotected and removed from solid support with ammonium hydroxide (60 °C for 16 h). Following a preliminary round of HPLC, oligonucleotides were treated with 80% acetic acid in water for 20 minutes. Each oligonucleotide was purified by high-performance liquid chromatography (HPLC) on a PLRP-S column (Agilent) with a gradient of acetonitrile and 50 mM ammonium acetate. Oligonucleotides were desalted by ethanol precipitation and quantified by ultraviolet-visible spectrophotometry based on their extinction coefficients at 260 nm (IDT Oligo Analyzer). Masses were verified by matrix-assisted laser desorption (MALDI) mass spectrometry. Based on the UV-Vis quantification of the DNA, 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 minutes 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′

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

Methylated Complement: 5′-GCT ATC AGT GCG C^{m}GC AGT ACT CAG TC-3′

The *BssHII* restriction site is shown in red.