Supplemental Information

Table 1. Oligonucleotide Sequences and Nomenclature

12bp-hm-lc	GGGCC**X**GCAGGG
	CCCGGGCGTCCC
16bp-hm-lc	GTGGGCC**X**GCAGGGTG
	CACCCGGGCGTCCCAG
20bp-hm-lc	GGATGGGCC**X**GCAGGGTTGG
	CCTACCCGGGCGTCCCAACC
24bp-hm-lc	GGTAATGGGCC**X**GCAGGGTATTGG
	CCATTACCCGGGCGTCCCATAAGG
16bp-hm-la	GTGGGCCCGCAGG**X**TG
	CACCCGGGCGTCCGAG
24bp-nm	AAATTGAGCCCGAGCCTCCCGTTC
	TTTAACTCGGGCTCGGAGGGCAAG
24bp-hm-1	AAATTGAGCC**X**GAGCCTCCCGTTC
	TTTAACTCGGGCTCGGAGGGCAAG
24bp-hm-2	AAATTGAGCC**X**GAGCCTCC**X**GTTC
	TTTAACTCGGGCTCGGAGGGCAAG
24bp'-hm-1	GAGC**X**CGTAAGCCCGTTCAGGTCG
	CTCGGGCATTCGGGCAAGTCCAGC
24bp'-hm-2	GAGC**X**CGTAAGCCCGTT**X**AGGTCG
	CTCGGGCATTCGGGCAAGTCCAGC
24bp-nonCpG	TCCAGGACTTCTCTCAGGTTAACT
	AGGTCCTGAAGAGAGTCCAATTGA

X, 5-methylcytosine

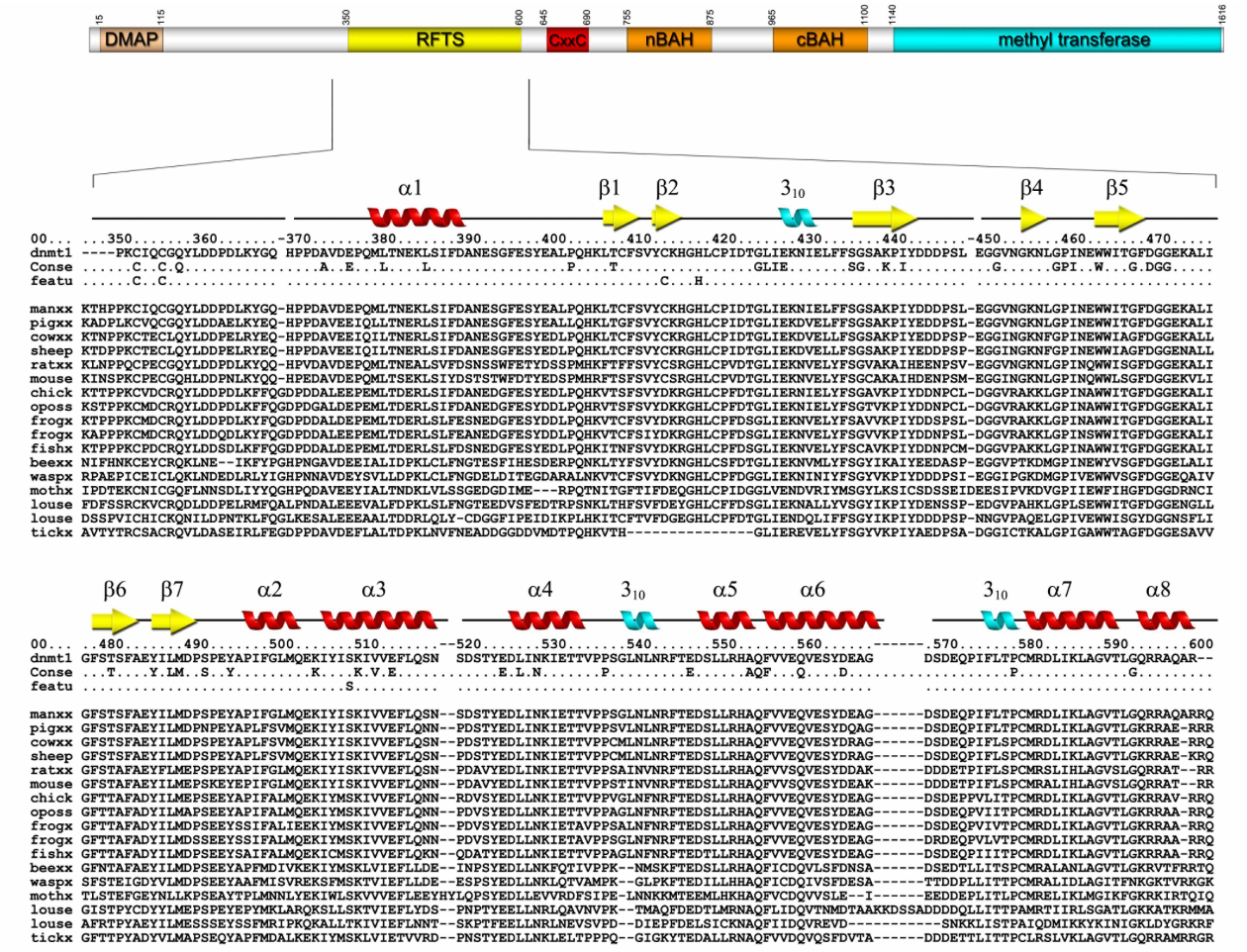

Fig. S1: Sequence alignment of the RFTS domain. Sequence alignment and secondary structure of Dnmt1 RFTS domain. Conse: strictly-conserved residues, featur: features. The number row corresponds to the human sequence. Ortholog sequences are aligned.

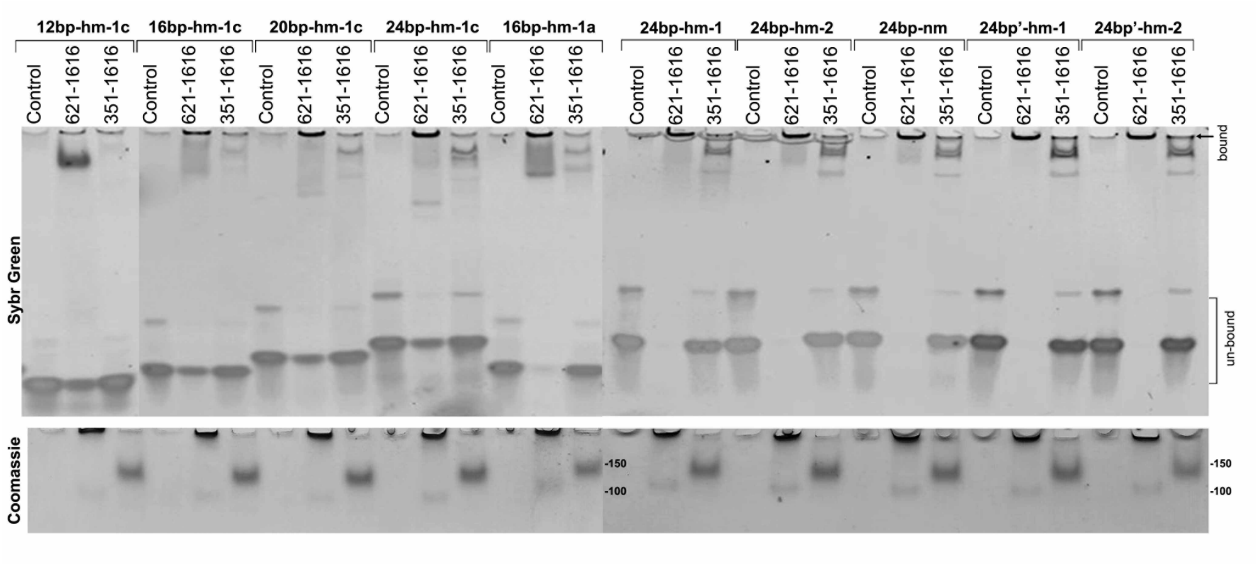

Fig. S2: RFTS-mediated inhibition of DNA binding is independent of DNA sequence, length, methylation status or CpG content. EMSA analysis of DNA oligonucleotides (100 ng) of different lengths, sequences and methylation states with 5 μM of protein. The sequences and nomenclature of all oligonucleotides are described in Table 1. The upper panel shows DNA by Sybr Green staining and the lower panel shows the presence of Dnmt1 protein in the same gel by Coomassie staining. The gel shows the strong DNA-binding activity of the Dnmt1 construct 621-1616; binding is impaired in the RFTS-containing 351-1616 construct.

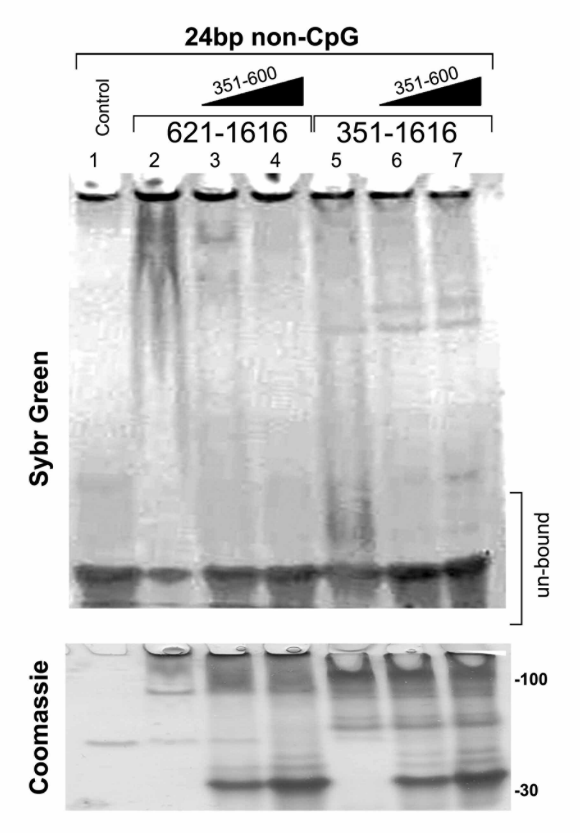

Fig. S3: *In trans* RFTS inhibition of binding of non-CpG containing DNA. The RFTS domain (2.5 – 5 μM) was added *in trans* to assays containing 5 μM of RFTS-lacking (621-1616) or RFTS-containing (351-1616) Dnmt1 and 100 ng of non-CpG oligonucleotide. The upper panel shows DNA by Sybr Green staining and the lower panel shows the presence of Dnmt1 protein in the same gel by Coomassie staining. The RFTS domain inhibits DNA binding to the RFTS-lacking protein, which allows the DNA to migrate rapidly. No binding was detected with the 351-1616 protein construct.

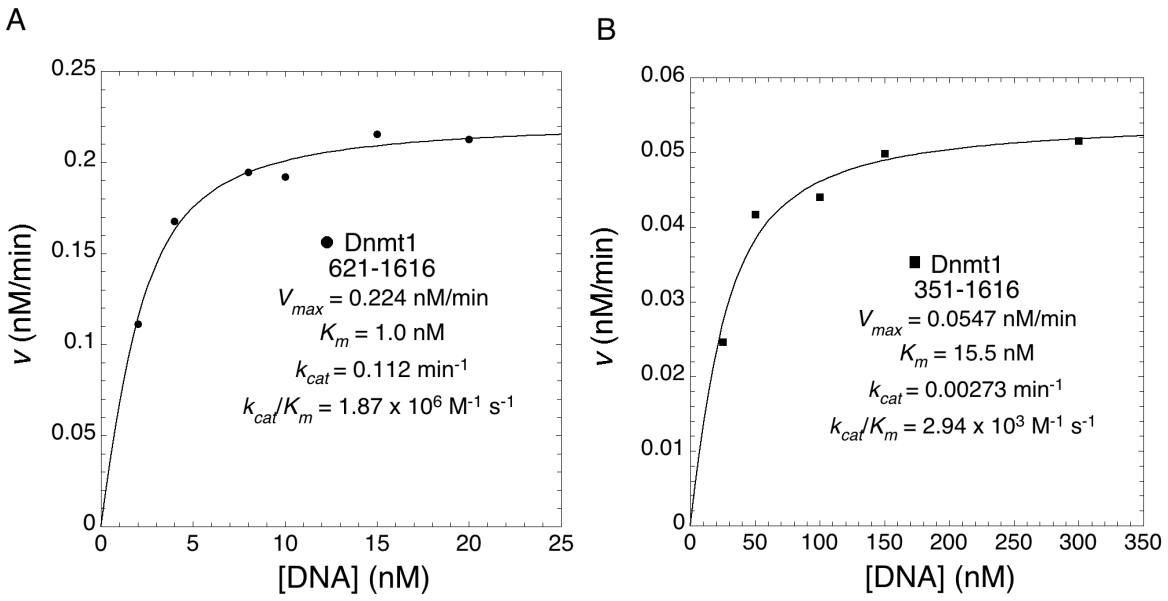

Fig S4: Kinetics of RFTS-lacking (621-1616) and RFTS-containing (351-1616) Dnmt1. DNA substrate-dependent methytransfer rates for 2 nM of RFTS-lacking (A) and 20 nM of RFTS-containing (B) Dnmt1 measured at 1 mM SAM. The initial velocity data was fitted to the quadratic velocity equation for tight-binding substrates. The RFTS domain confers a ~40-fold reduction in k_{cat} and a ~15-fold increase in K_m for the DNA substrate.