Supplementary Table 1. DNA Olignucleotides Used in This Study

DNA olignucleotides	DNA sequence [a]
1. NMR Titration	GGGATAT^{m5}CGCGGG CCCTATAGCGCCC
2. EMSA	
13-nt non-methylated DNA	[BioTEG]-GGGATATCGCGGG CCCTATAGCGCCC
13-nt single hemimethylated DNA	[BioTEG]-GGGATATCGCGGG CCCTATAG^{m5}CGCCC
13-nt double hemimethylated DNA	[BioTEG]-GGGATATCGCGGG CCCTATAG^{m5}CG^{m5}CCC
20-nt hemimethylated DNA	[BioTEG]-CA^{m5}CG^{m5}CGA^{m5}CGCA^{m5}CGA^{m5}CG^{m5}CGAA GTGCGCTGCGTGCTGCGCTT
3. Fluorescence Anisotropy	
13-nt non-methylated DNA	[FL]-GGGATATCGCGGG CCCTATAGCGCCC
13-nt single hemimethylated DNA	[FL]-GGGATATCGCGGG CCCTATAG^{m5}CGCCC

[a] ^{m5}C stands for 5-methyl cytosine.

Supplementary Figure Legends

Figure S1. Structure-based sequence alignment of the SRA domain family. Absolutely conserved residues are color-coded in red, highly conserved residues are in blue; First four proteins accession numbers are Q96T88, CAI13293, Q8VDF2, Q7TMI3. Residues that exhibit major NMR resonance perturbations are underlined, residues subject to mutagenesis analysis are highlighted in bold, and residues constituting the positively charged patch on the protein surface are indicated by stars.

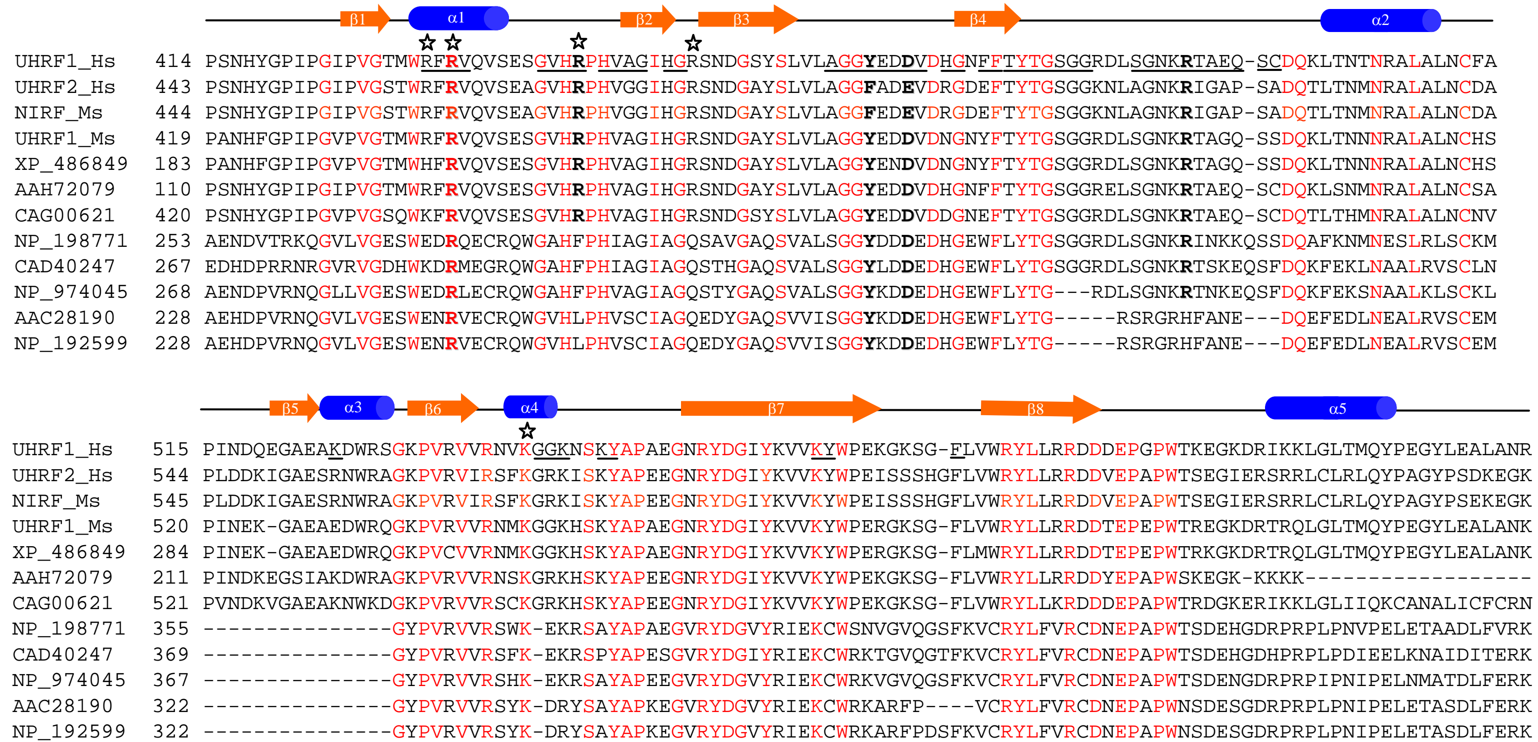
Figure S2. Comparison of the structural fold of human UHRF1 SRA domain to that of OB fold proteins Cdc13 (PDB code 1s40), RPA (1fgu), trbp111 (1pxf) and full methyl, non-methyl DNA binding protein MeCP2 (3c2I) and CXXC domain (2j2s).

Figure S3. NMR backbone assignment of the SRA domain. (**a**) HSQC spectrum of triple labeled SRA domain. (**b**) Sequential assignment for protein residues R491-N495, which are missing in the crystal structure, based on the resonance connectivity found in the 3D HNCACB and HNCOCACB spectra. The solid lines correlate the connectivity in the protein sequence

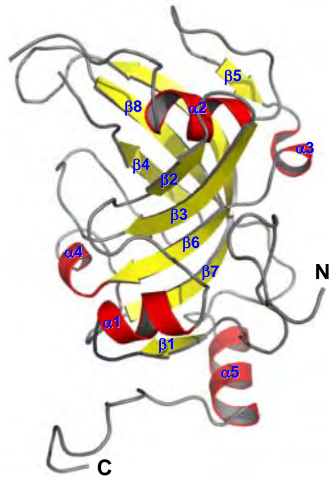
Figure S4. Mutational analysis of SRA domain binding to hemimethyalted DNA. (**a**) ^{1}H 1D NMR spectra of the wild-type SRA domain (upper panel) and the D469G mutant (lower panel). The similar NMR resonance patterns in the methyl group region of 0-2 ppm are seen between the two proteins indicating that D469G mutation does not cause any significant perturbation to the protein structure. The NMR spectra were collected at

293K with protein samples of ~ 0.2 mM in a 50 mM sodium phosphate buffer of pH 6.5 containing 50 mM sodium chloride. (**b**) Comparison of the wild-type SRA domain or the D469G mutant binding to hemimethylated and non-methylated DNA, as assessed in a fluorescence anisotropy assay. Binding of a 5'-fluorescein-tagged 13-nt hemimethylated or non-methylated DNA (5 nM) to the wild type (black and magenta, respectively), or to the D469G mutant SRA domain (gray and blue, respectively) is shown as a function of protein concentration (from 5 nM to 0.5 mM). The DNA sequences are listed in Supplementary Table 1.

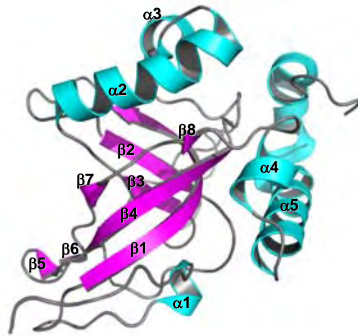
Supplementary Figure 1



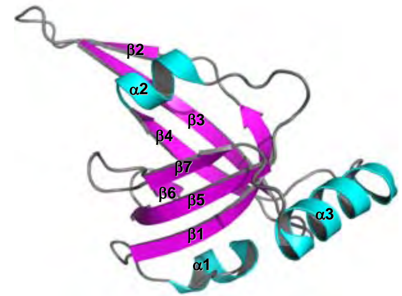
Supplementary Figure 2



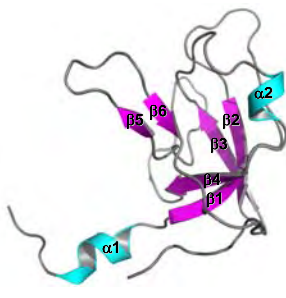
SRA domain (hemimethyl CG binding)



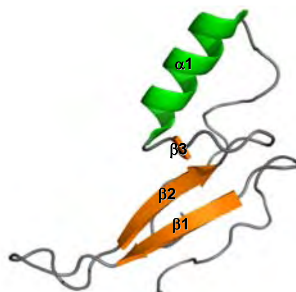
Cdc13 (ssDNA binding)



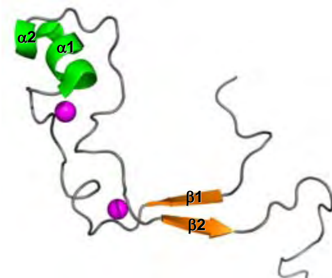
RPA (ssDNA binding)



trbp111(t-RNA binding)



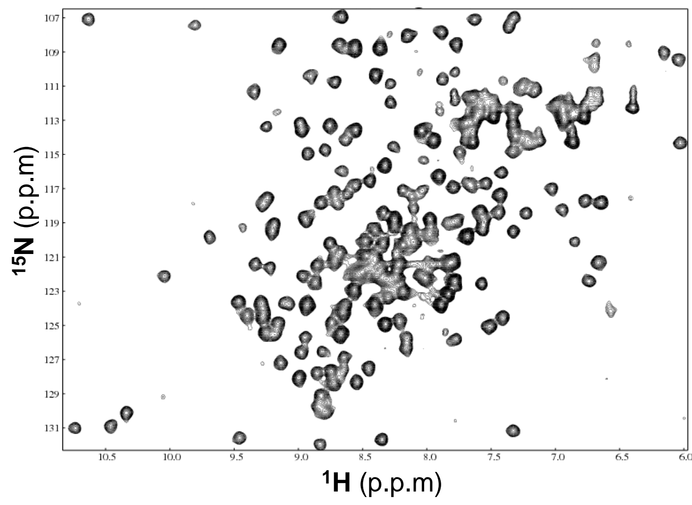
MeCP2 (symmetric methyl CG binding)



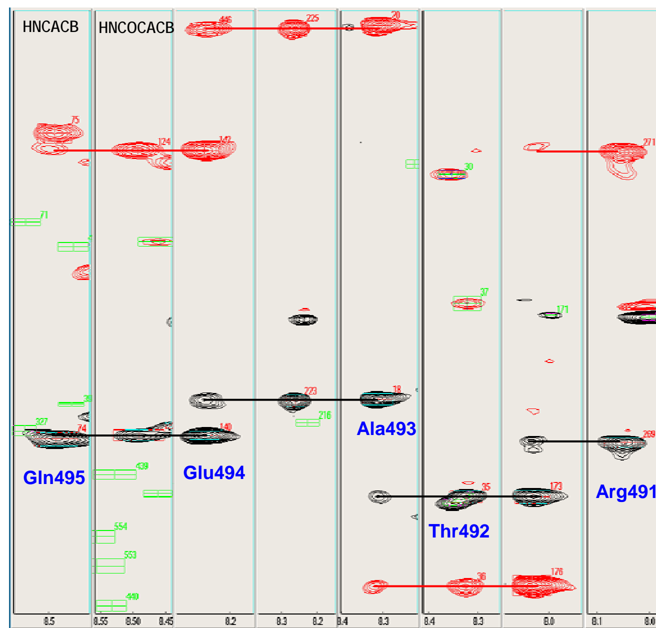
CXXC domain (non-methyl CG binding)

Supplementary Figure 3

a



b



Supplementary Figure 4