Supplemental Figures

Figure S1

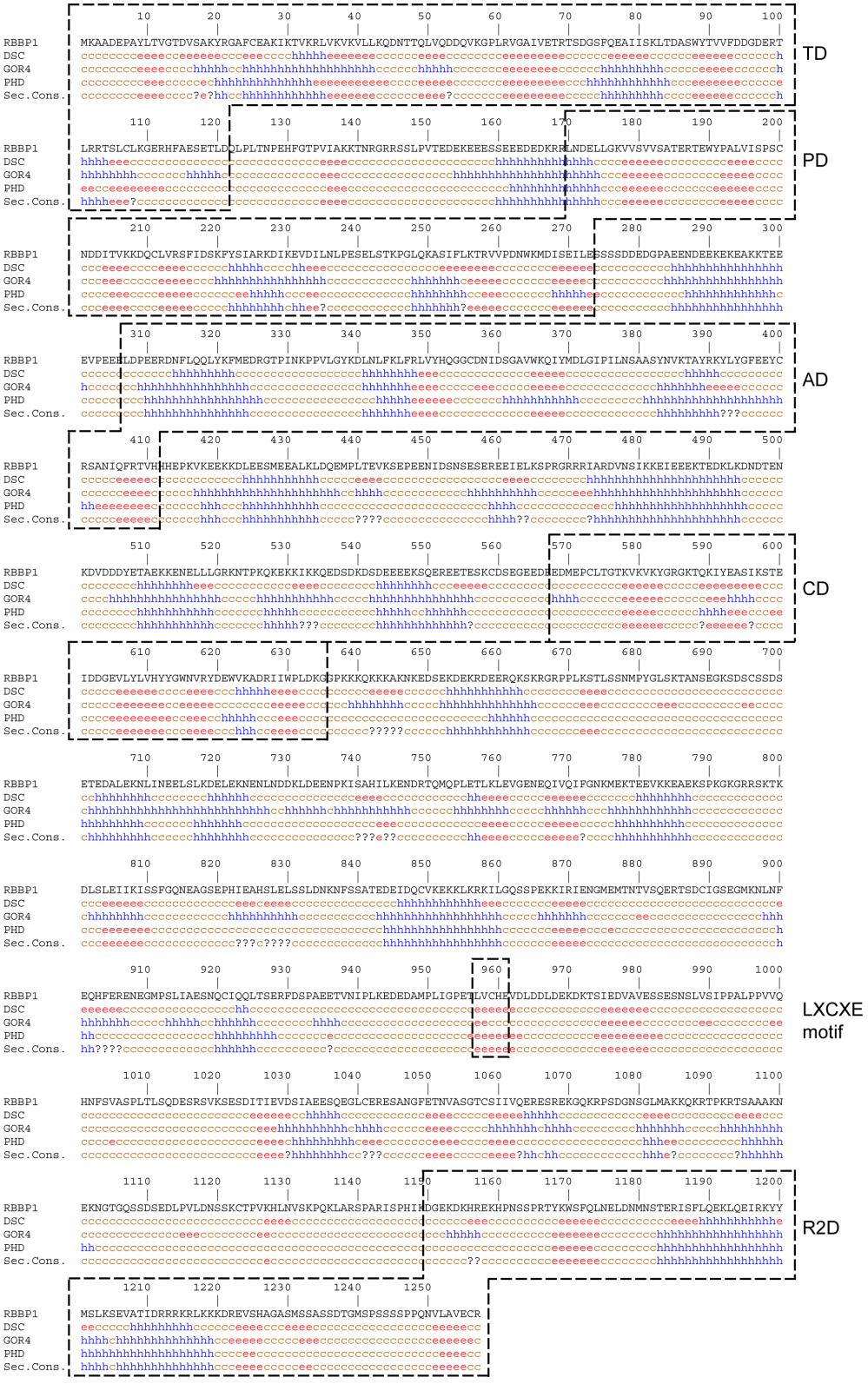

Figure S1. The consensus secondary structure prediction of RBBP1 using the NPS@ server [1]. Combining this prediction and the following two figures, five ordered domains were defined and are indicated by dashed lines. The LXCXE motif, which is the pRb binding site, is also indicated.

Figure S2

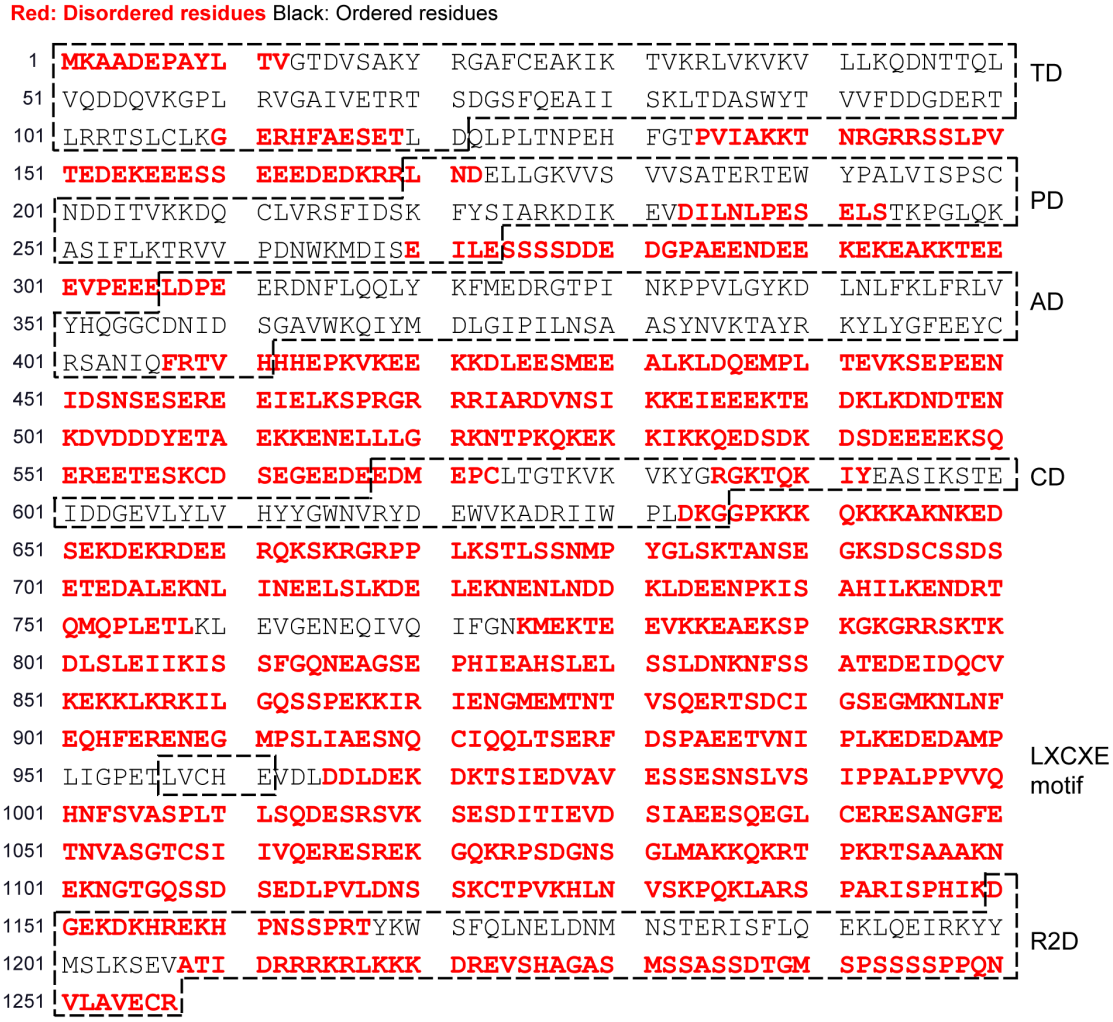

Figure S2. The result of protein disorder prediction of RBBP1 using the PrDOS server [2].

Figure S3

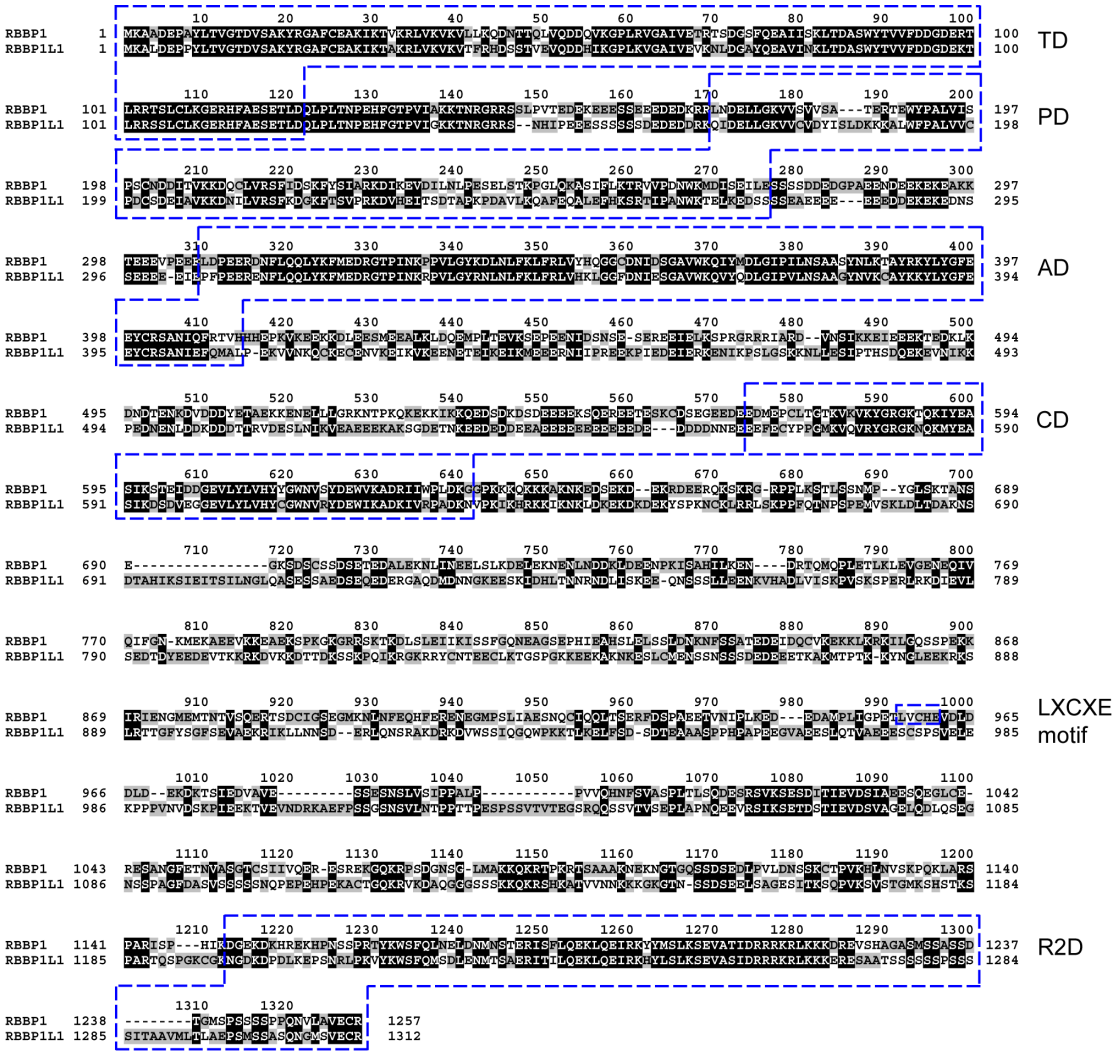

Figure S3. Sequence alignment of RBBP1 and RBBP1L1. The sequence identity between RBBP1 and RBBP1L1 of the domains is 80% for TD, 44% for PD, 78% for AD, 65% for CD, 61% for R2D, and the identity of other regions is less than 29%.

Figure S4.

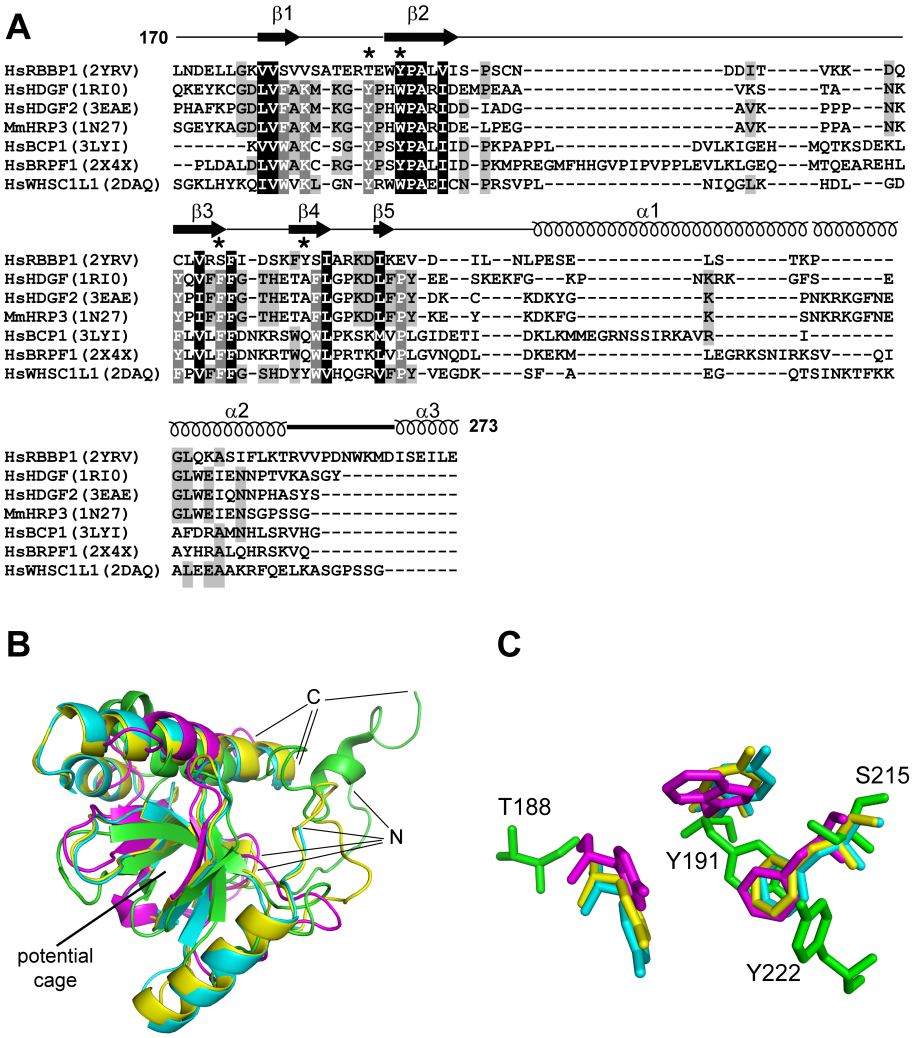

Figure S4. Structural comparison of RBBP1 PD with other PWWP domains. PWWP domains were named originally after the Pro-Trp-Trp-Pro motif in the domain, but the motif is not conserved in all PWWP domain proteins [3], such as the Brpf1 PWWP domain [4]. The residues corresponding to the motif Pro-Trp-Trp-Pro in PWWP domains are Glu^{189}-Trp^{190}-Tyr^{191}-Pro^{192} in the RBBP1 PD. (A) Structure-based sequence alignment of RBBP1 PD with other PWWP domains for which structures are available. The structure-based alignments were made using SSM [5]. The PDB accession codes used in the alignment are shown in parentheses. Residues forming an aromatic cage and structurally-corresponding residues are indicated by stars. (B) Superimposition of PDs of RBBP1 (green, PDB 2YRV), Hrp3 (magenta, PDB 1N27), Bcp1 (cyan, PDB 3LYI) Brpf1 (yellow, PDB 2X4X). (C) The residues forming aromatic cages and structurally-corresponding residues in the structure superimposition. The colors are same as those in (B). Two of the three residues involved in forming the aromatic cages in other PWWP domains are not conserved in the RBBP1 PD. However, an additional aromatic residue Tyr^{222} is present around the expected position of the aromatic cage in the RBBP1 PD. Therefore, whether or not the RBBP1 PD is able to recognize methylated histone tails could not be determined from inspection of the structure alone.

Figure S5.

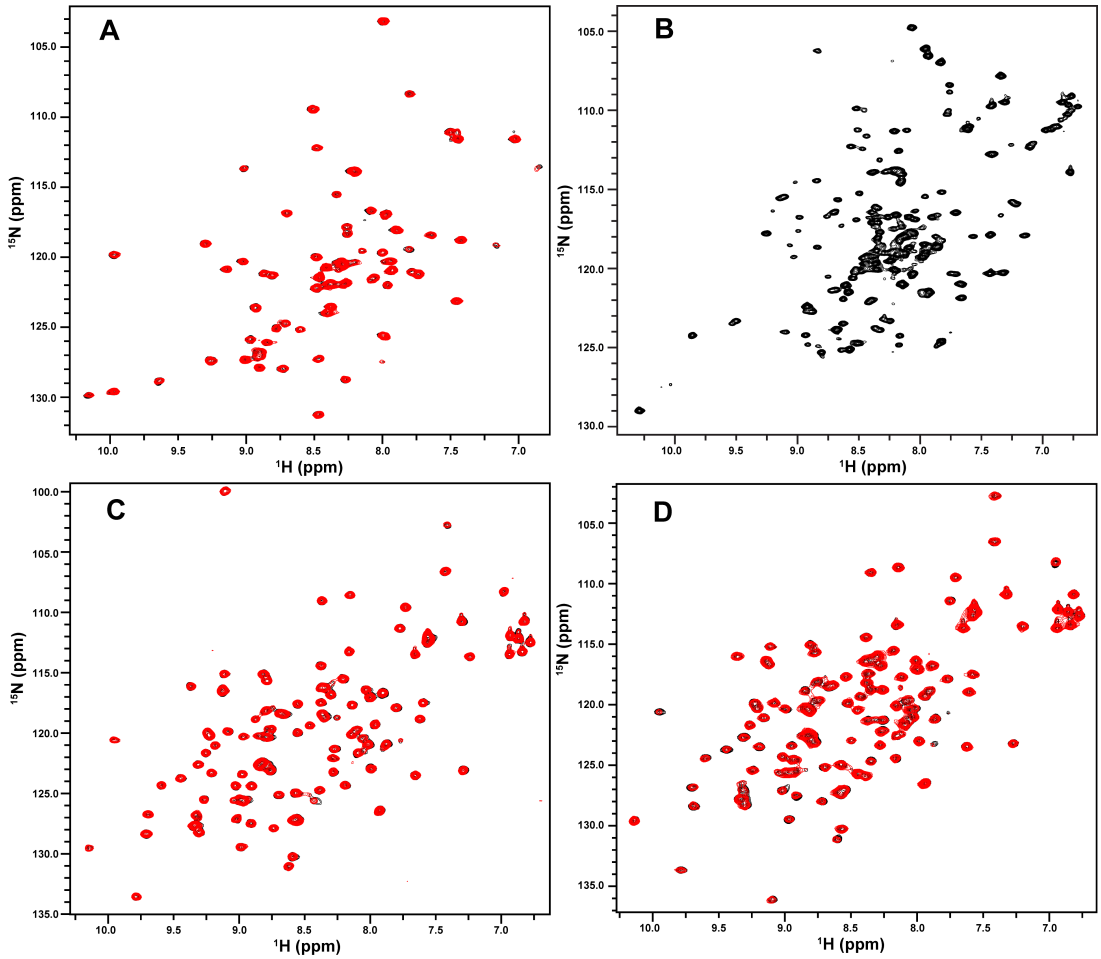

Figure S5. RBBP1 CD, PD and TD are independent domains and do not interact with each other. (A) ^{1}H-^{15}N HSQC spectra of RBBP1 CD in the absence (black) and presence (red) of RBP1 PD. (B) ^{1}H-^{15}N HSQC spectrum of RBBP1 PD. (C) ^{1}H-^{15}N HSQC spectra of RBBP1 TD in the absence (black) and presence (red) of RBBP1 CD. (D) ^{1}H-^{15}N HSQC spectra of RBBP1 TD in the absence (black) and presence (red) of RBBP1 PD.

Figure S6

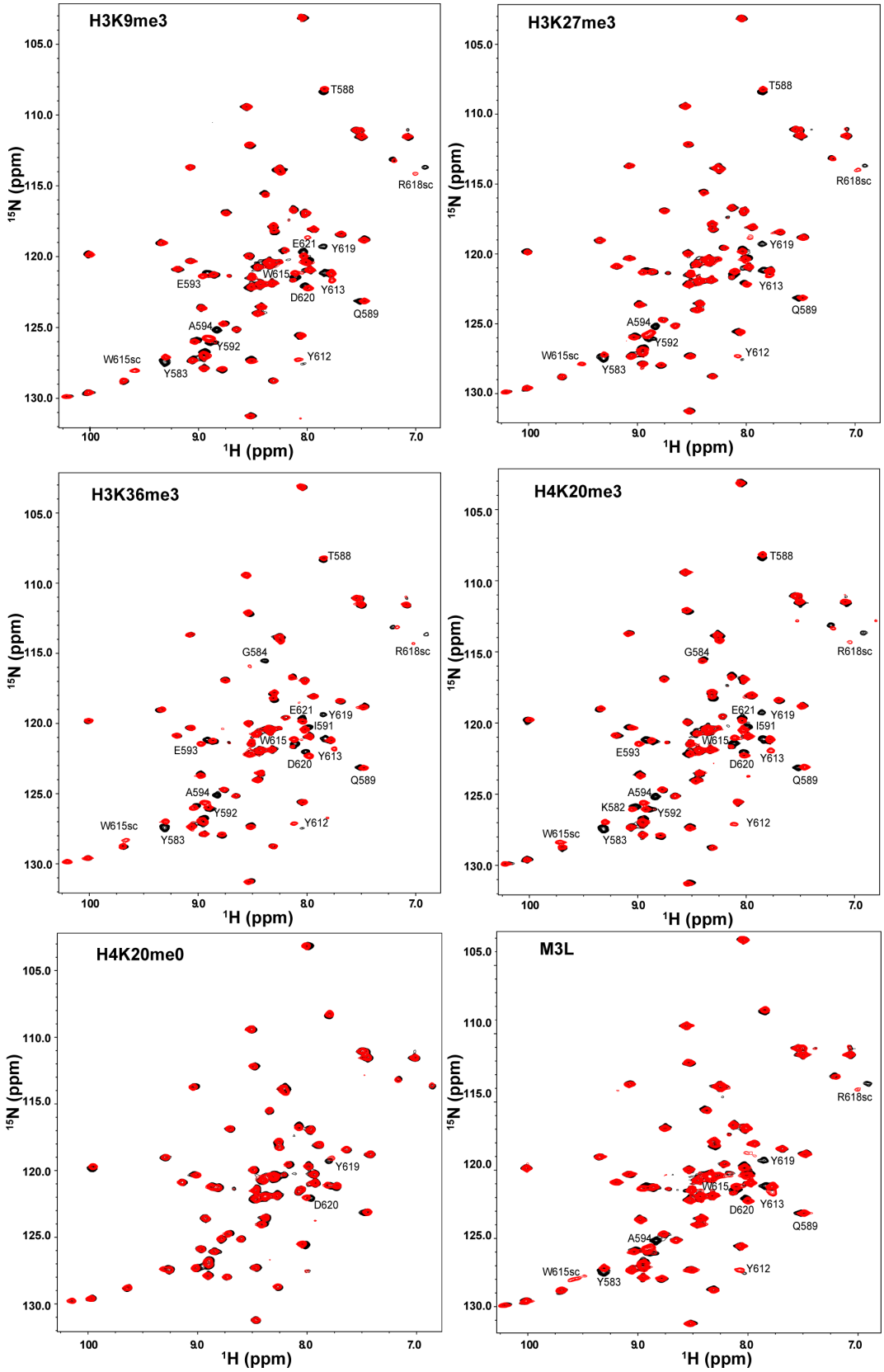

Figure S6. The overlaid ^{1}H-^{15}N HSQC spectra of RBBP1 CD in the titration with H3K9me3 (1:12), H3K27me3 (1:12), H3K36me3 (1:12), H4K20me3 (1:12), M3L (1:12) and H4K20me0 (1:18).

Figure S7.

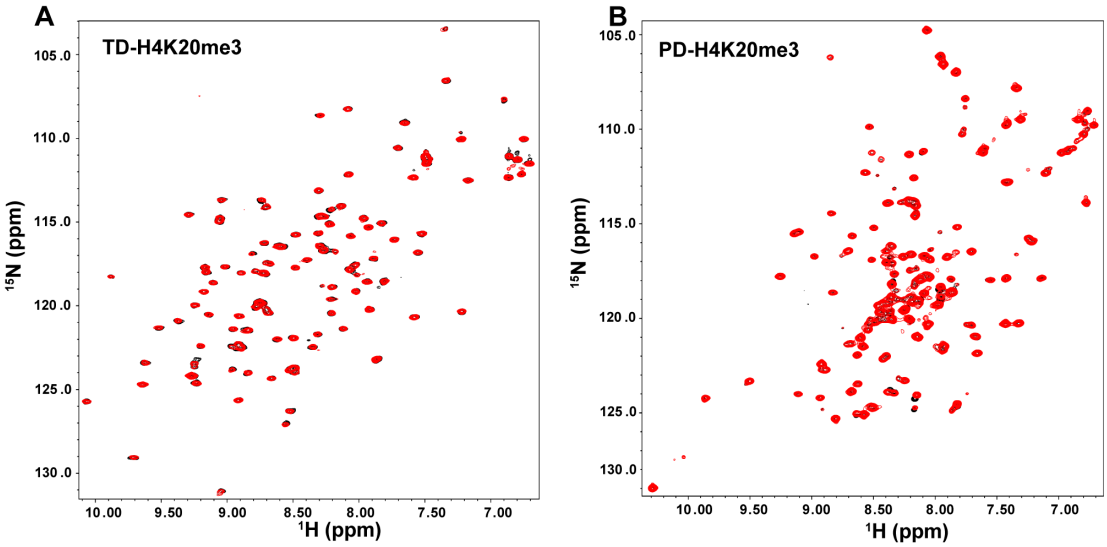

Figure S7. The overlaid ^{1}H-^{15}N HSQC spectra of RBBP1 TD (A) and PD (B) in the titration with H4K20me3 (1:7 for TD and 1:6 for PD). The spectra of RBBP1 TD and PD titrated with other peptides were the same and are not shown.

Figure S8.

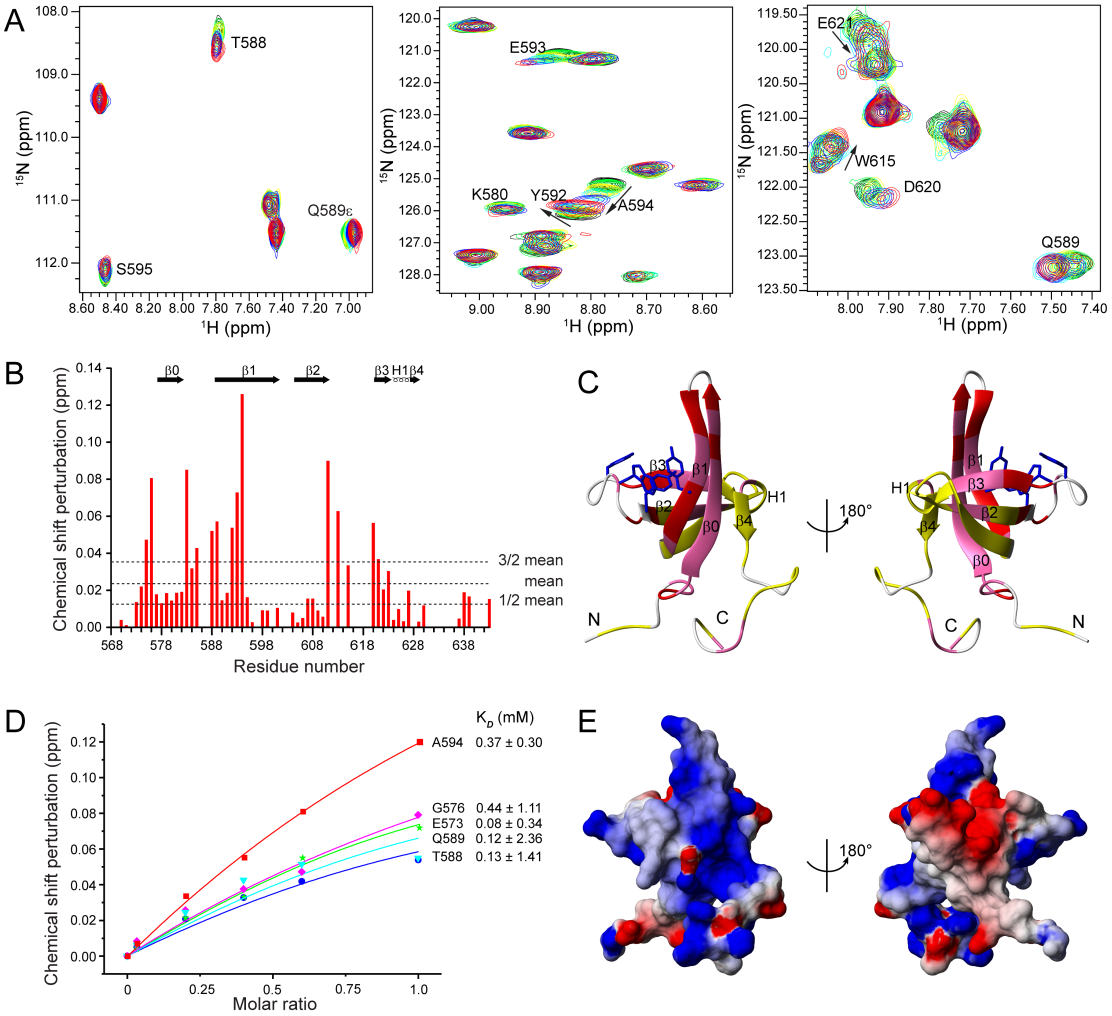

Figure S8. The interaction of RBBP1 CD and dsDNA. (A) Regions of ^{1}H-^{15}N HSQC spectra of RBBP1 CD titrated with dsDNA. (B) Bar diagram of chemical shift perturbations versus residue number at a molar ratio 1:1 of RBBP1 CD to dsDNA. (C) Mapping of chemical shift perturbations (CSP) onto the RBBP1 CD structure. The residues with a CSP value more than 1.5 times the mean CSP value are shown in red; those with a CSP value between 1.5 and 0.5 times the mean are shown in pink; and those with a CSP value less than 0.5 times the mean are shown yellow. The residues that form the aromatic cage are shown as blue sticks. (D) K_D values for binding of RBBP1 CD to dsDNA. (E) Electrostatic potential surface of RBBP1 CD. The orientations are the same as those in (C). The positively and negatively charged surfaces are in blue and red, respectively.

Figure S9.

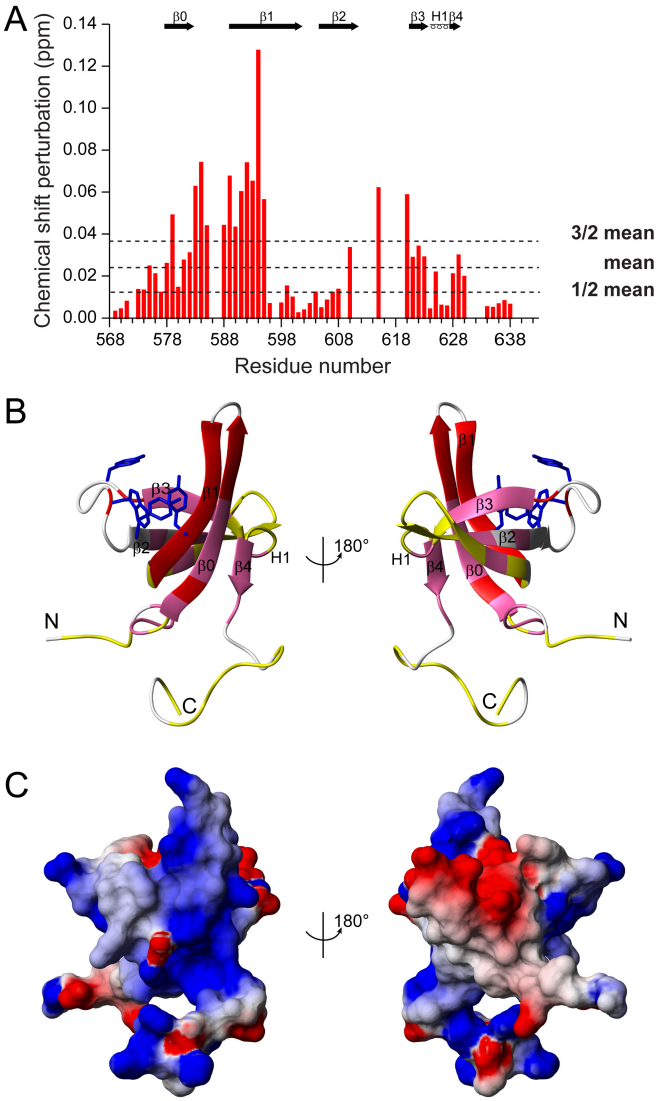

Figure S9. The interaction of the RBBP1 CD with H4K20me3 in the presence of dsDNA. (A) Bar diagram of chemical shift perturbations versus residue number at a molar ratio 1:5 of RBBP1 CD (in the presence of dsDNA at a 1:1 molar ratio to the RBBP1 CD) to H4K20me3. (B) Mapping of chemical shift perturbations (CSP) to RBBP1 CD structure. The residues with a CSP value more than 1.5 times the mean CSP value are shown in red; those with a CSP value between 1.5 and 0.5 times the mean are shown in pink; and those with a CSP value less than 0.5 times the mean are shown yellow. The residues that form the aromatic cage are shown as blue sticks. (C) Electrostatic potential surface of RBBP1 CD. The orientations are the same as those in (B). The positively and negatively charged surfaces are in blue and red, respectively.

References

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