SUPPLEMENTARY MATERIALS

Supplementary Figure Legends

Figure S1. (**A**) ^{15}N HSQC spectra show ubiquitin binds to DNMT1_351 domain (aa: 351-1616), as illustrated by the severe line broadening effect caused by the addition of DNMT1_351 domain. (**B**) Reciprocal NMR titration confirms the interaction of ubiquitin with DNMT1 RFTS domain (aa: 351-600). Superimposition of ^{15}N HSQC spectra showing a large group of RFTS amide resonances have chemical shift perturbations or line broadening effect upon addition of ubiquitin. (**C**) Structure superimposition of UHRF1 N-terminal UBL domain (PDB: 2FAZ) and ubiquitin (PDB: 1UBQ) gives an RMSD 0.52 Å. (d) ^{15}N HSQC spectra show UHRF1 UBL binds to DNMT1_351 domain (aa: 351-1616.) (e) Reciprocal NMR titration confirms DNMT1 RFTS domain binds to UHRF1 UBL.

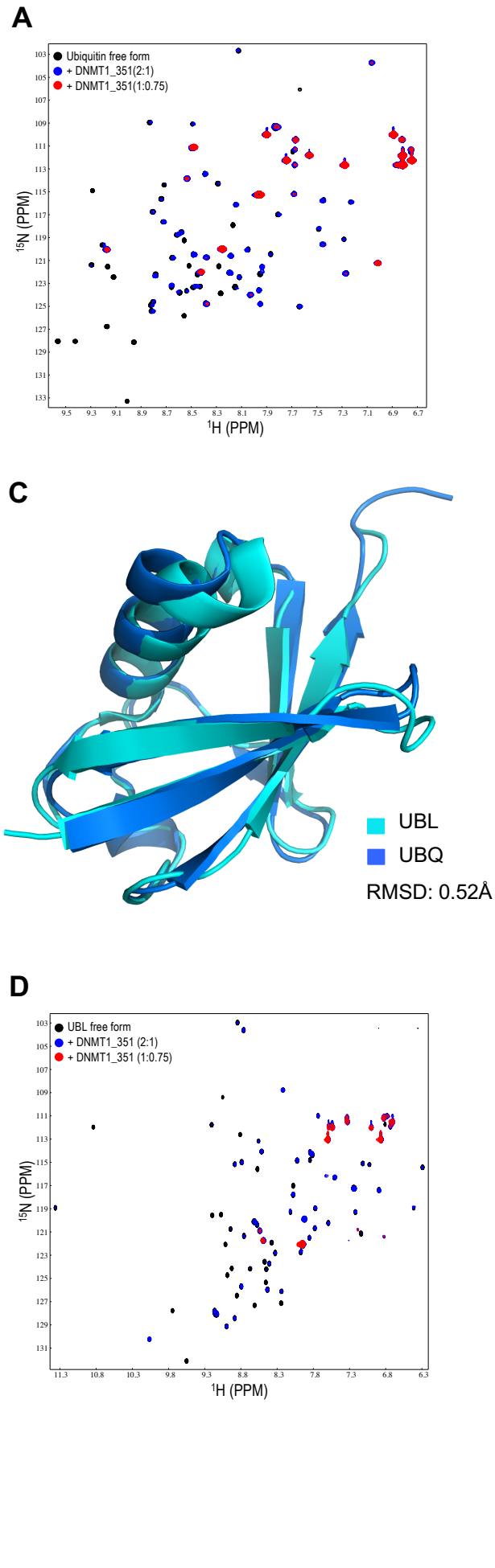
Figure S2. (**A**) DNMT1 RFTS domain and ubiquitin form a complex with 1:2 stoichiometry, as confirmed by SDS-PAGE gel analysis of the crystal of the RFTS/ubiquitin complex. This result is consistent with the structural data and ITC titration results. (**B**) The RFTS long loop encompassing residues 386-404, which is invisible in previous reported structures (PDB: 4WXX or 3AV4, in the figure the human DNMT1 structure with PDB code 4WXX was selected for the comparison and colored in gray) but now well-defined in our structure, is sandwiched by two ubiquitin molecules as highlighted in red rectangular box. (**C**) Surface representations of the RFTS/ubiquitin complex with structural domains colored with the same scheme as shown in Figure 2a. (**D**) Electrostatic surface potential representation of ubiquitin in complex with cartoon model of DNMT1 RFTS domain. (**E**) Electrostatic surface potential representation of DNMT1 RFTS in complex with cartoon model of ubiquitin molecules.

Figure S3. Structural superimposition of the α-helical bundle (aa: 500-589) of human DNMT1 RFTS domain (PDB: 4WXX, colored in gray) with that of RFTS-ubiquitin complex (color in green/marine/cyan) gives an RMSD of 0.95 Å. However, the relative orientation of β-barrel has shifted obviously due to the bending of α-helix (aa: 493-518),

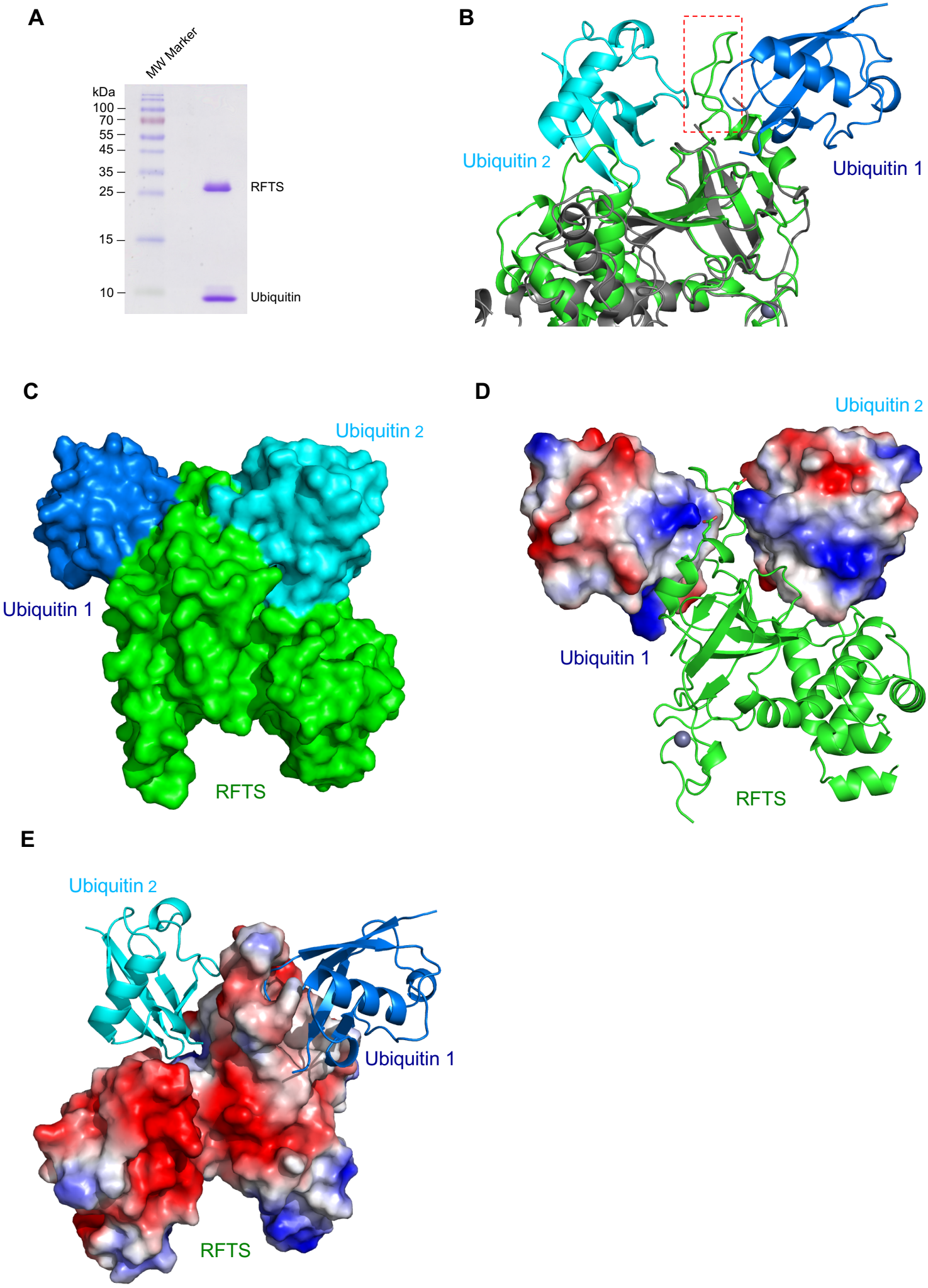
suggesting RFTS domain undergoes conformational changes upon ubiquitin binding.

Figure S4. **Relative quantification of total 5mC levels in the genomic DNA of mouse ES cells by ELISA-based methylated DNA quantification kit.** (**A**) The relative 5-methylcytosine levels in the genomic DNA of wild-type ES cells, DNMT1$^{-/-}$ ES cells or DNMT1$^{-/-}$ ES cells stably expressing DNMT1 wild-type, E384A, E397A, E384A/E397A or Y339G point mutant respectively. (**B**) The relative 5-methylcytosine levels in the genomic DNA of wild-type control or UHRF1$^{-/-}$ mouse ES cells or UHRF1$^{-/-}$ ES cells stably expressing UHRF1 wild-type, UHRF1-ΔUBL or UHRF1-ΔRING truncated mutants respectively. Data are represented as mean ± SEM. Significant changes compared with wild type control are indicated as asterisk (p-value <0.05), calculated by two-paired t-test.

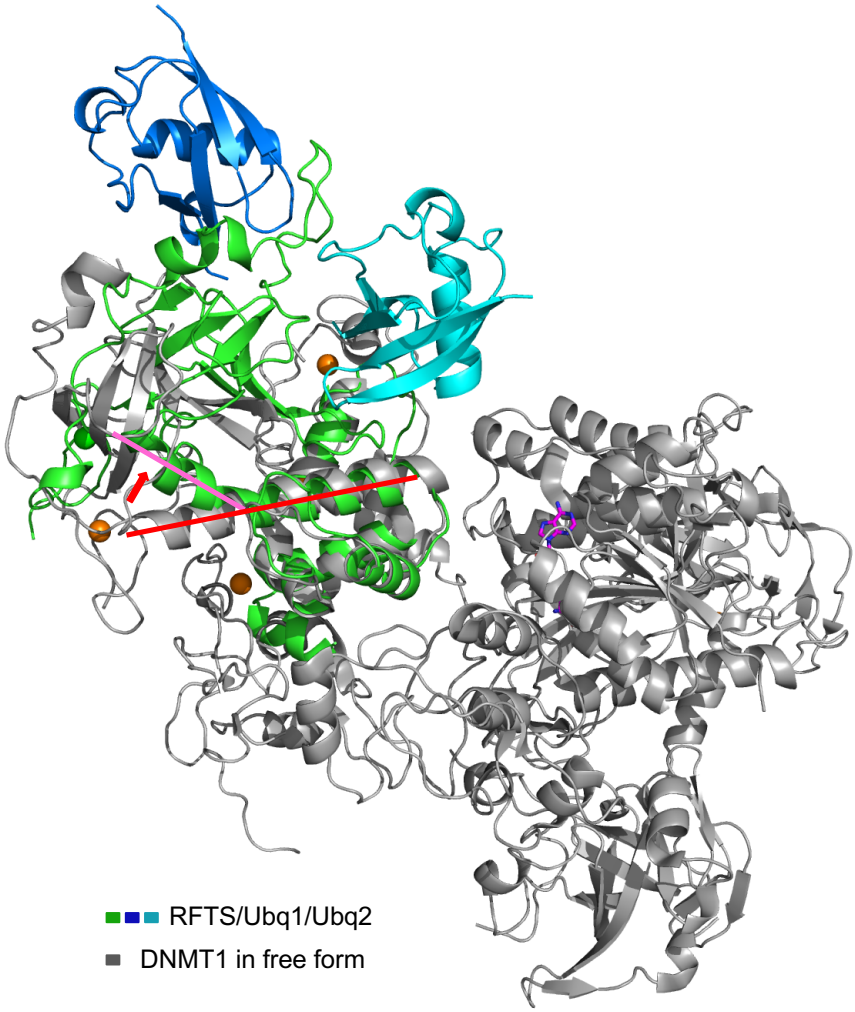
Supplementary Figure 1



Supplementary Figure 2

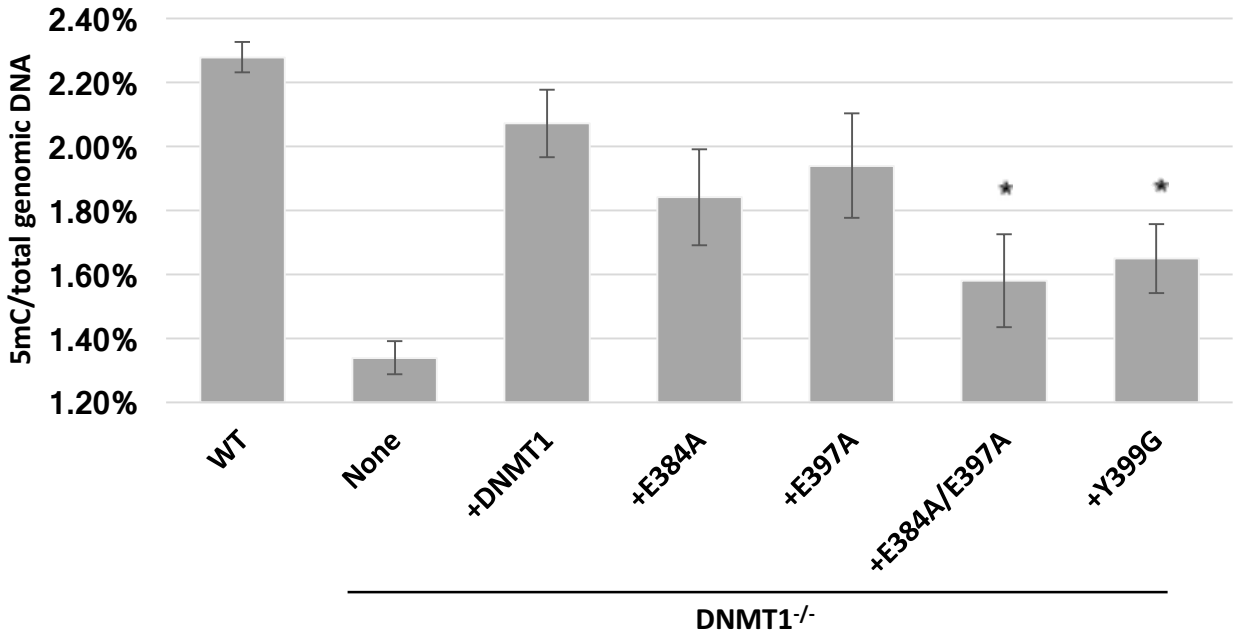


Supplementary Figure 3



Supplementary Figure 4

A



B