Supplementary Data

Structural insights into CpG-specific DNA methylation by human DNA methyltransferase DNMT3B

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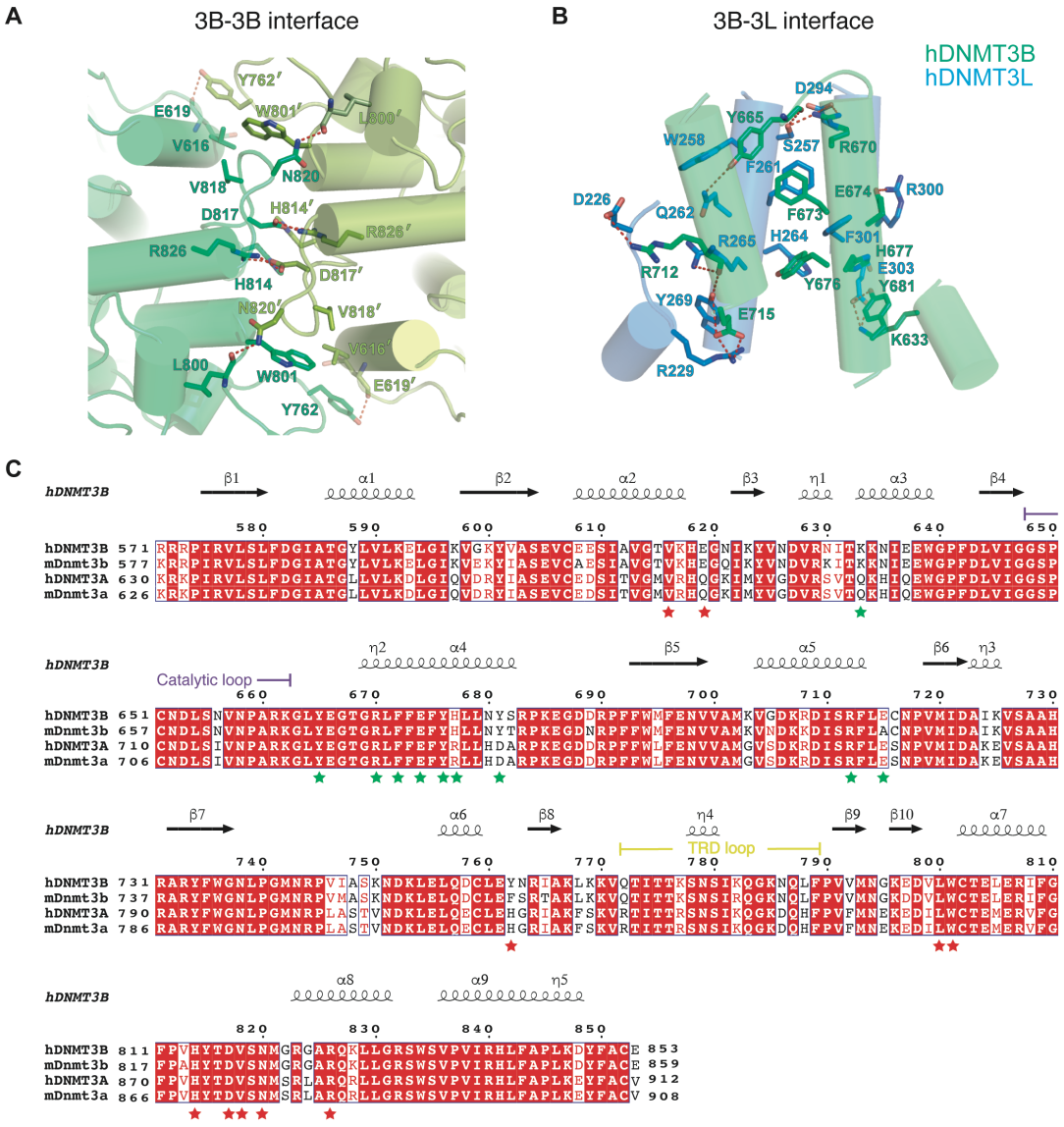
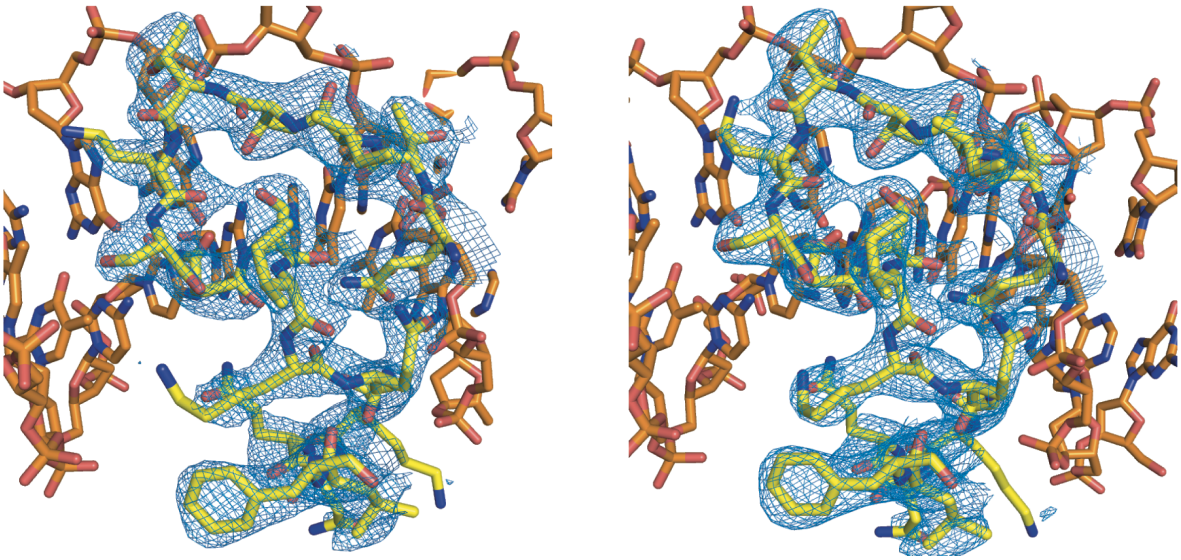
Supplementary Table S1.
Oligonucleotides and DNA substrates used in crystallization and activity assays.

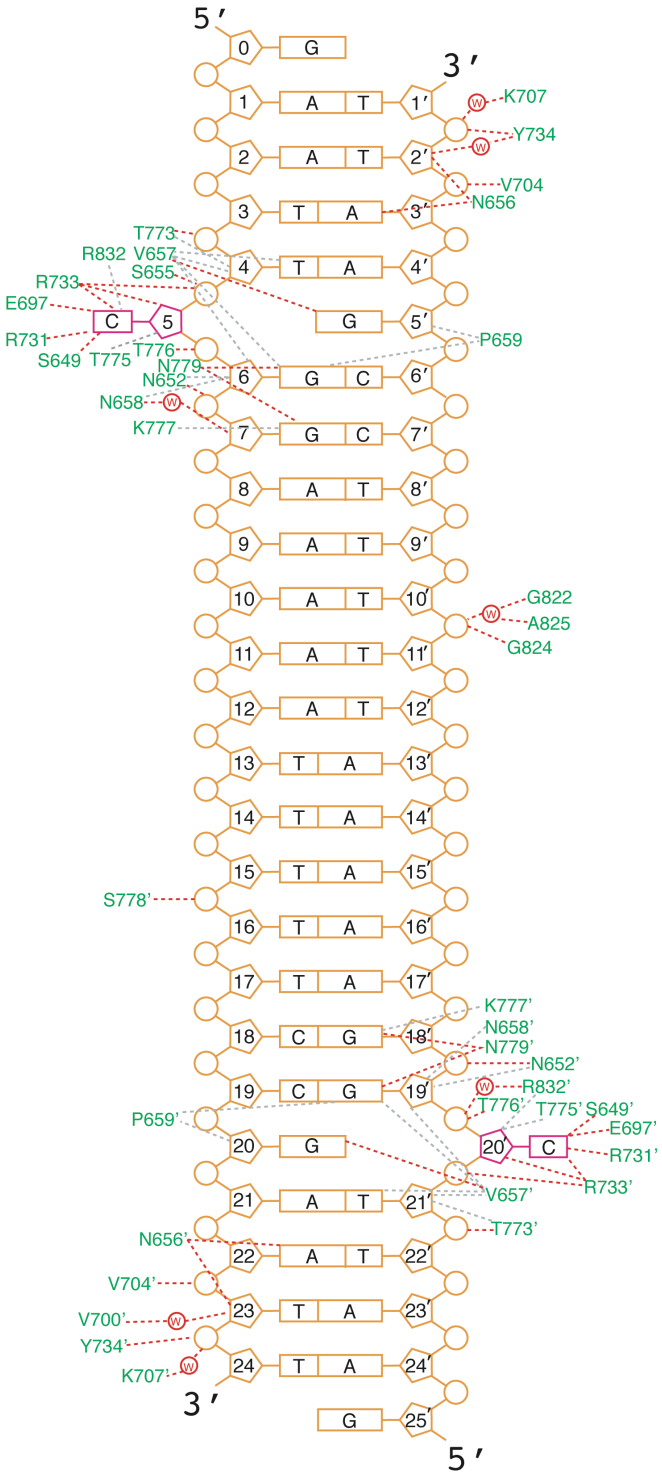
Experiment	Name	Sequence (5'-to-3')
Crystallization	CpGpG	GAATTCGGAAAAATTTTTCCGAATT
	CpGpT	GCATGCGTTCTAATTAGAACGCATG
MTase-Glo assay	CG	AATTCGAAAAAATTTTTTCGAATT
	CA	AATTCAAAAAAATTTTTTTGAATT
	CT	AATTCTAAAAAATTTTTTTAGAATT
	CGG	AATTCGGAAAAATTTTTCCGAATT
	CGA	AATTCGAAAAAATTTTTTCGAATT
	CGT	AATTCGTAAAAATTTTTTACGAATT
	CG-2	Forward: AAAACGAAAAAAAAAAAAAAAAAA Reverse: TTTTTTTTTTTTTTTTTTCGTTTT
	CG-3	Forward: AAAACGAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAA Reverse: TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT TTTTTCGTTTT
Methylation-coupled restriction enzyme cleavage assay	494-bp DNA substrate	GGCCGTTCTTCTGGATGTTTGAGAATGTTGTAGCC ATGAAGGTTGGCGACAAGAGGGACATCTCACGG TTCCTGGAGTGTAATCCAGTGATGATTGATGCCA TCAAAGTTTCTGCTGCTCACAGGGCCCGATACTT CTGGGGCAACCTACCCGGGATGAACAGGCCCGTG ATAGCATCAAAGAATGATAAACTCGAGCTGCAG GACTGCTTGGAATACAATAGGATAGCCAAGTTAA AGAAAGTACAGACAATAACCACCAAGTCGAACT CGATCAAACAGGGGAAAAACCAACTTTTCCCTGT TGTCATGAATGGCAAAGAAGATGTTTTGTGGTGC ACTGAGCTCGAAAGGATCTTTGGCTTTCCTGTGC ACTACACAGACGTGTCCAACATGGGCCGTGGTGC CCGCCAGAAGCTGCTGGGAAGGTCCTGGAGCGTG CCTGTCATCCGACACCTCTTCGCCCCTCTGAAGG ACTACTTTGCATGTGAATAG
Fluorescence polarization assay	3'-FAM-labeled CG-3	Forward: AAAACGAAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAA-FAM Reverse: TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT TTTTTCGTTTT



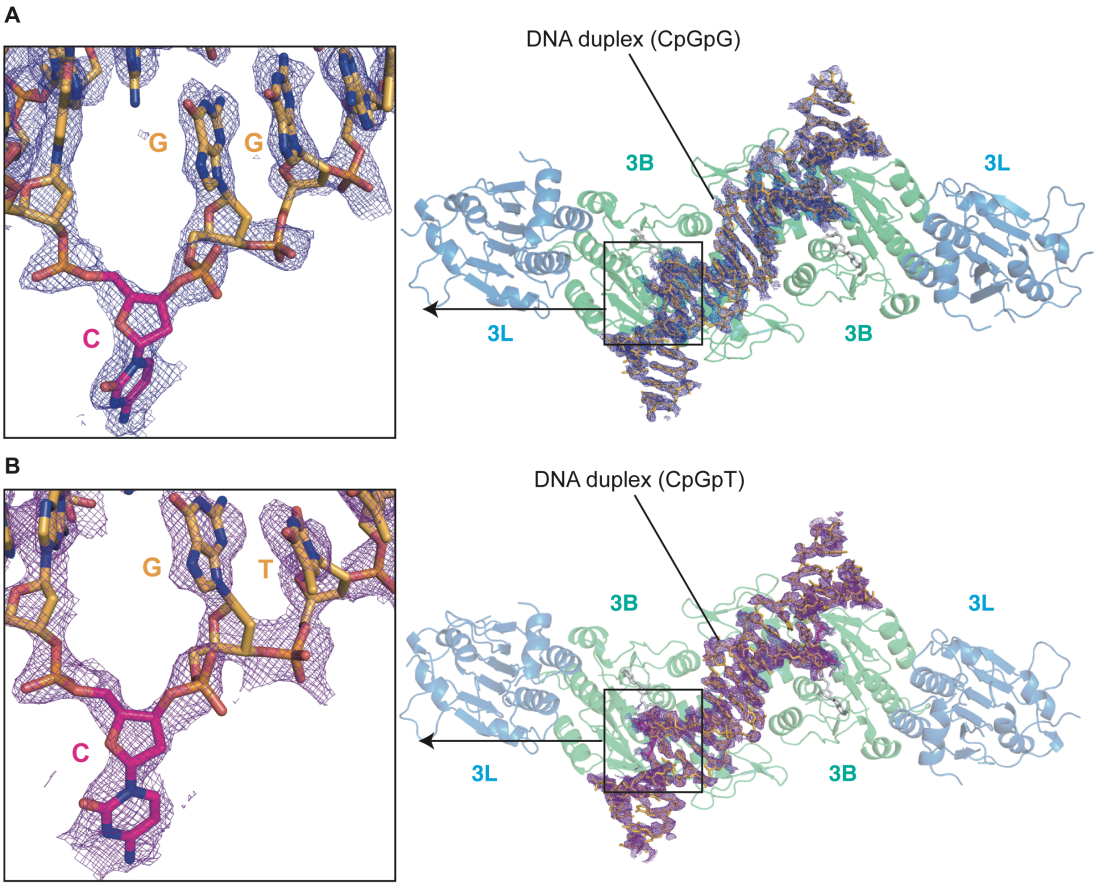
Supplementary Figure S1. Conserved residues are located in the DNMT3B-3L interfaces. (A-B) Amino acids located in the 3B-3B and 3B-3L interfaces are shown by the stick models, in green for those of DNMT3B and in blue for those of DNMT3L. **(C)** Sequence alignment of the methyltransferase catalytic domain of human hDNMT3B, mouse mDnmt3b, human hDNMT3A and mouse mDnmt3a. Secondary structures of DNMT3B derived from the crystal structure of the DNMT3B-3L-DNA (CpGpG) complex (PDB entry: 6KDA) are shown above the sequences. The conserved residues located in the 3B-3B and 3B-3L interfaces are marked with green and red stars below the sequences, respectively.



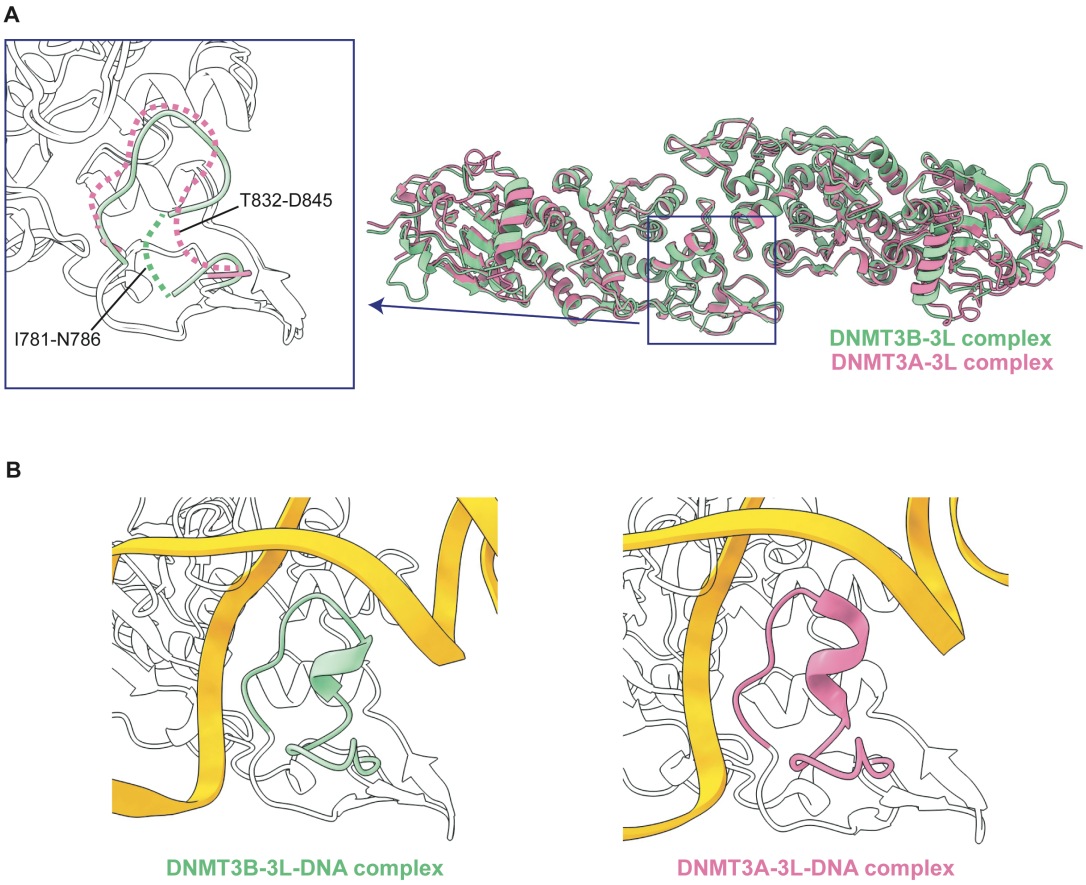
Supplementary Figure S2. TRD loops of DNMT3B interacts at the major groove of DNA duplex in the crystal structure of DNMT3B-3L-DNA (CpGpG). The electron density maps (2Fo-Fc) of TRD loops are contoured at 1σ level. The average B-factor of TRD loops is 53.03 $Å^2$



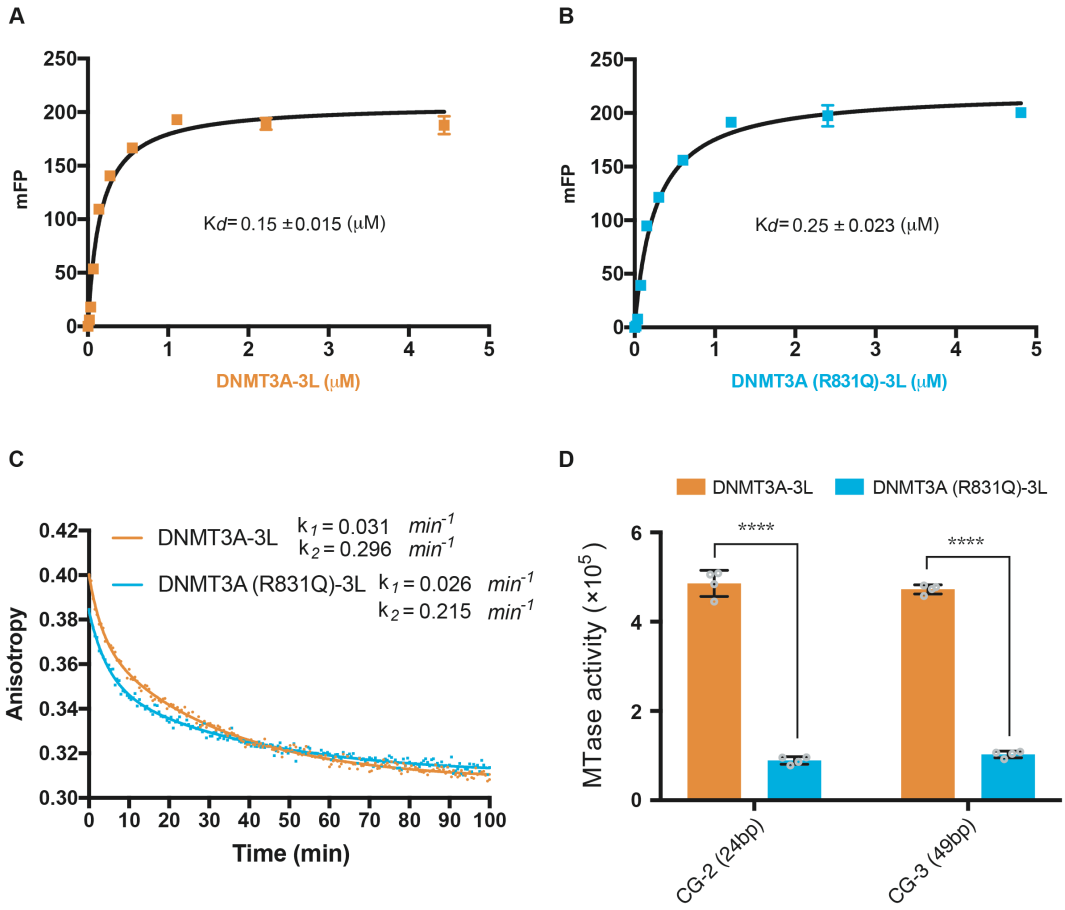
Supplementary Figure S3. Schematic diagram of the interactions between DNMT3B and DNA. The hydrogen bonds and van der Waals contacts are shown as red and gray dashed lines, respectively. Water molecules are shown as the letter "w" enclosed in a red circle.



Supplementary Figure S4. Electron density for the DNA in the crystal structures of DNMT3B-3L-DNA (CpGpG) and DNMT3B-3L-DNA (CpGpT) complexes. **(A-B)** The DNA duplexes (CpGpG) and (CpGpT) are shown as orange stick models. Cytosines of CpGpG and CpGpT sites are colored magenta. Zoomed-in views (left) of the CpGpG and CpGpT sites are shown with a feature-enhanced map contoured at 1.8 σ level.



Supplementary Figure S5. The stable TRD loop contributes to the processivity of DNMT3B. (A) Superimposition of the DNA-free form of the catalytic domains of DNMT3A (pink) and DNMT3B (green) reveals that the TRD loop is completely disordered in DNMT3A but less flexible in DNMT3B (only six residues 781-786 are disordered). **(B)** Cartoon representations of TRD loops of DNMT3B (green, the left panel) and DNMT3A (pink, the right panel) bound with DNA duplex (orange).



Supplementary Figure S6. DNA binding and methylation activity of human DNMT3A-3L and DNMT3A (R831Q)-3L. **(A-B)** Dissociation constants (K_d) of DNMT3A-3L and DNMT3A (R831Q)-3L complexes upon binding with the 49-bp FAM-labeled DNA (CG-3) were measured by fluorescence polarization (n=3). R831 in DNMT3A directly involved in DNA interactions and thus the R831Q mutant had a decreased DNA binding activity. **(C)** Dissociation rate constants (k_1 and k_2) for DNMT3A-3L and DNMT3A (R831Q)-3L complexes were determined by measuring the decreasing signal of fluorescence anisotropy of FAM-labeled GC-3 using unlabeled DNA as competitors. **(D)** Methylation activities of DNMT3A-3L and DNMT3A (R831Q)-3L for DNA of different lengths were measured by methyltransferase-Glo assays (in Luminescence, RLU, n=4; error bars denote SD; **** represents P<0.0001). Statistical significance (P values) was determined by two-tailed Student's t-test.