Supporting Information

Structure Determination of DNA Methylation Lesions N1-meA and N3-meC in Duplex DNA Using A Cross-Linked Protein–DNA System

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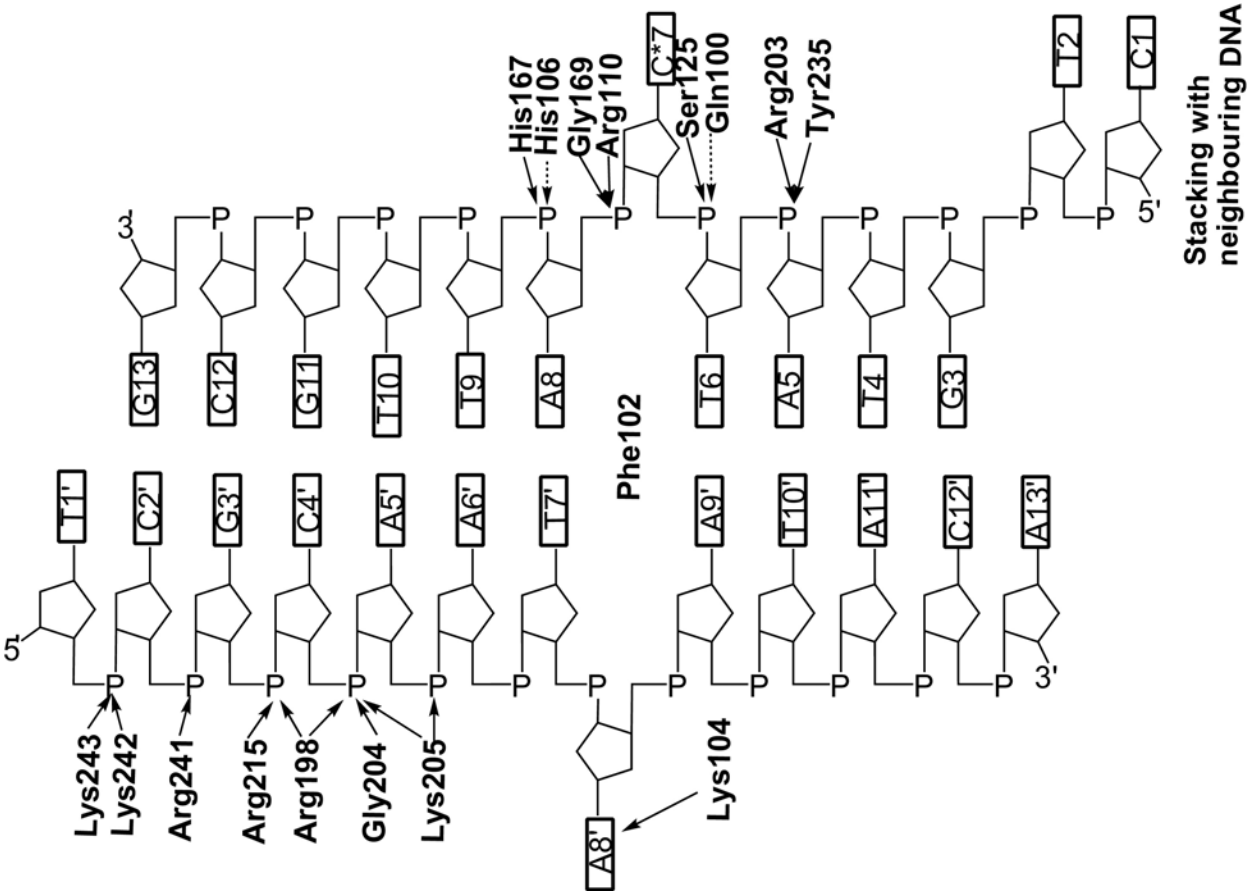
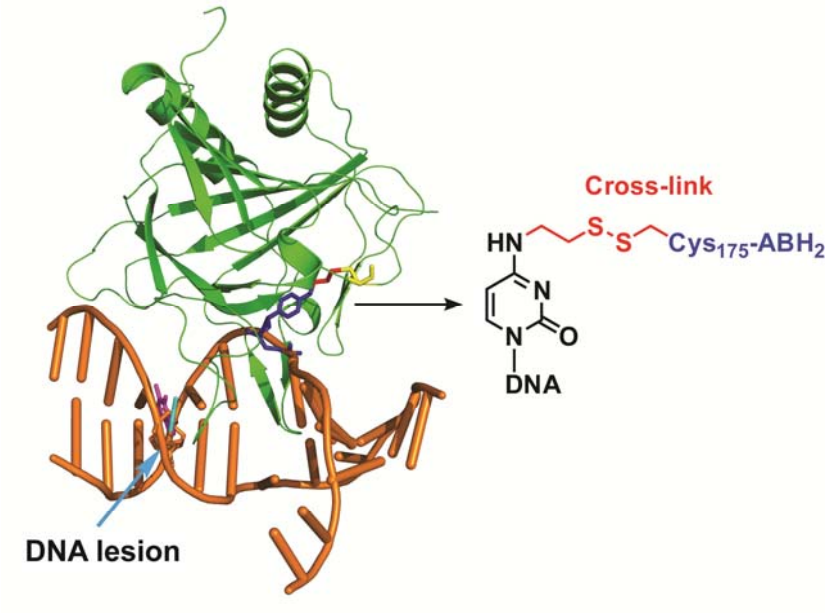
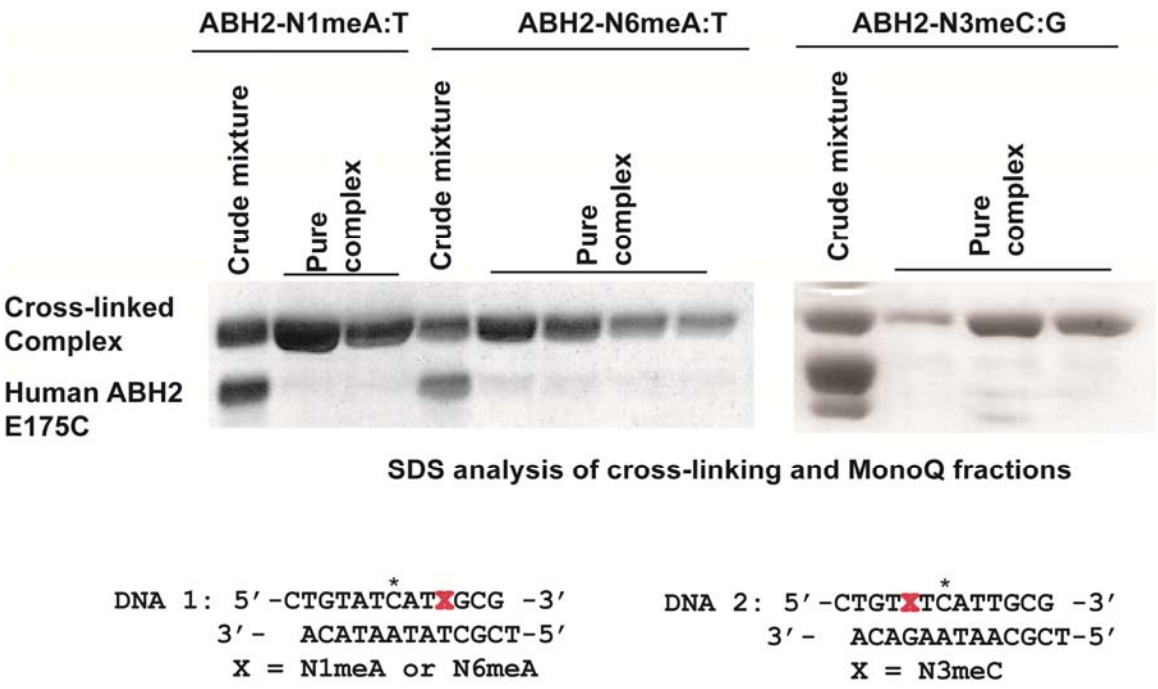


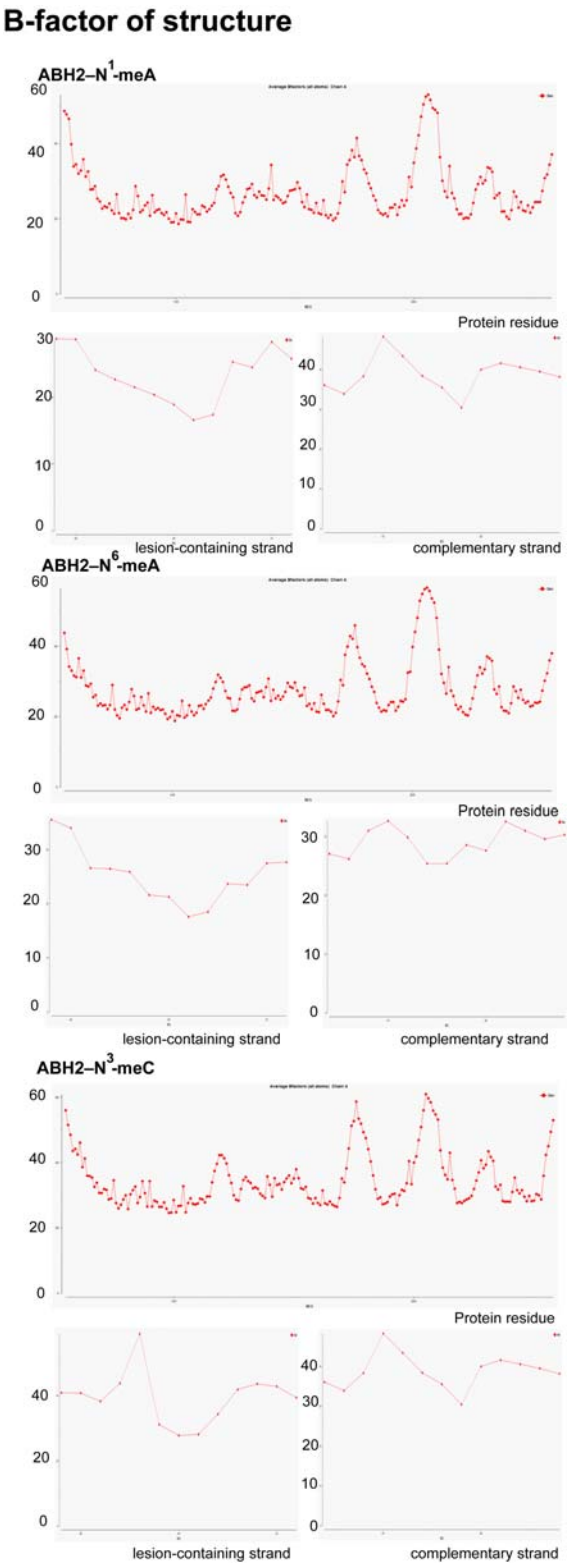
Supplementary Figure 1. Schematic diagram showing the interactions between ABH2 and dsDNA in structure 3BTX. Solid arrows stand for direct interaction and dashed arrows for water mediated contacts.



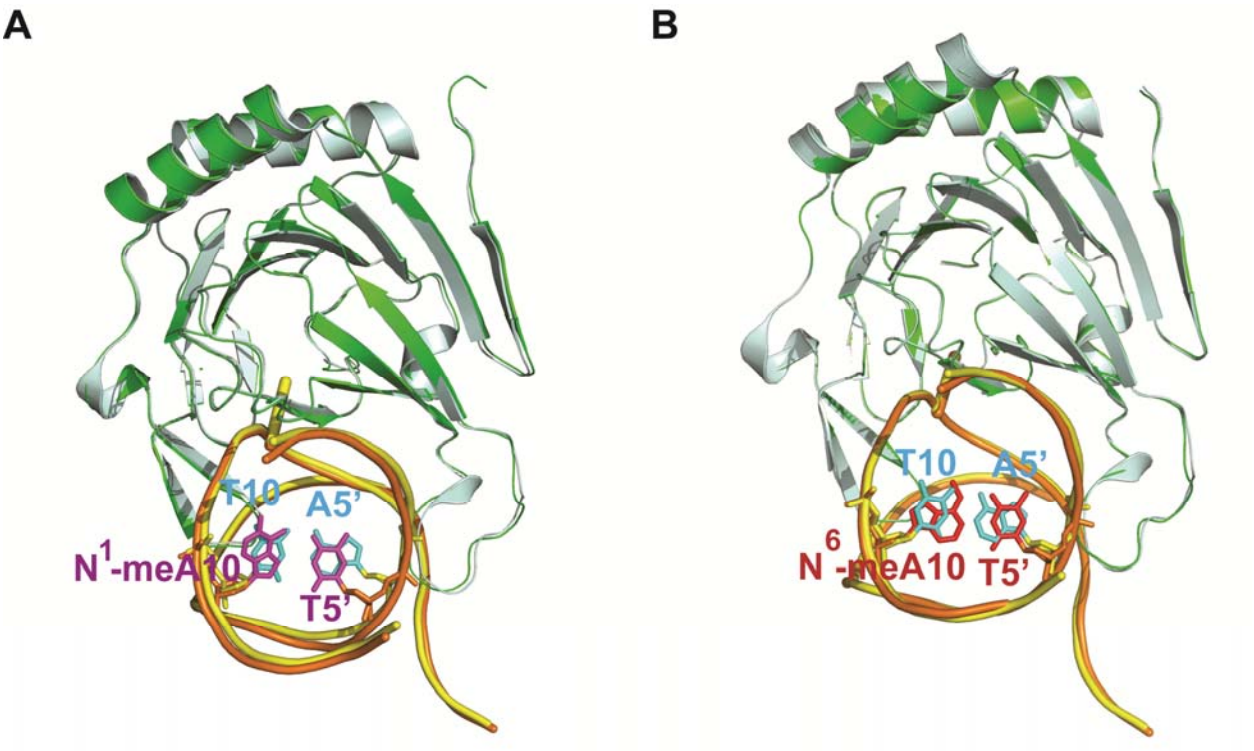
Supplementary Figure 2. A cross-linked ABH2–dsDNA complex for structural determination of modified bases in duplex DNA. Cartoon of the complex with ABH2 (in green, from ABH2–N^1-meA/T complex), dsDNA (in orange), and N^1-meA:T' (in magenta and cyan). C* is shown in blue, 2-carbon linker in red, and Cys175 in yellow.



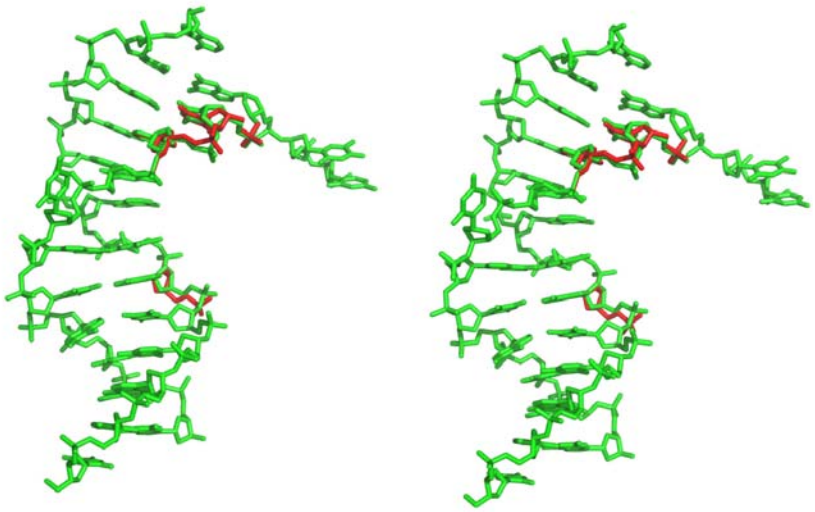
Supplementary Figure 3: Active site cross-linking for the ABH2–dsDNA complex. Coomassie stained non-reducing SDS PAGE gel analysis of the protein-DNA cross-linking and complex purification. The reaction was run with 12 uM protein and 10 uM dsDNA at 14 °C overnight. The high mobility band is free protein and the low mobility band is the protein-DNA complex. C* indicates a 2-carbon linker cystamine modification on the N4 position.



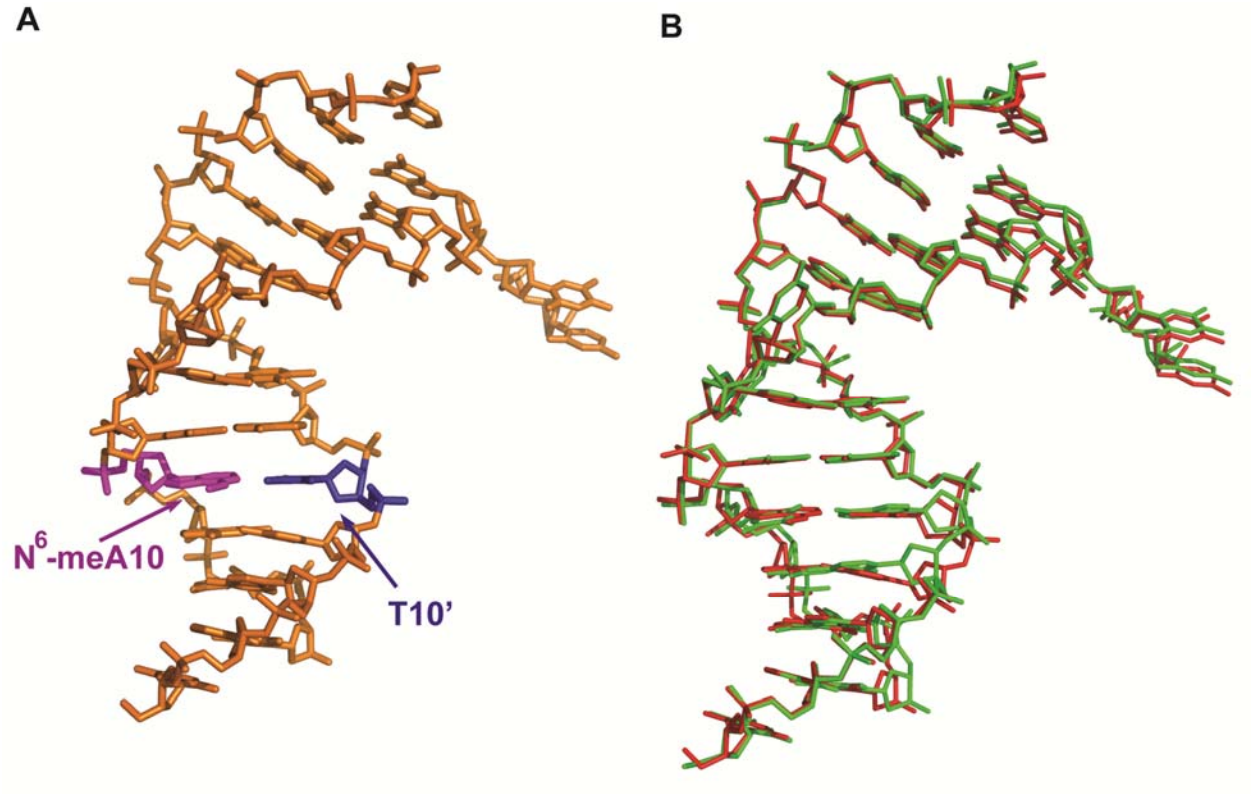
Supplementary Figure 4: B-factor of the protein and DNA.



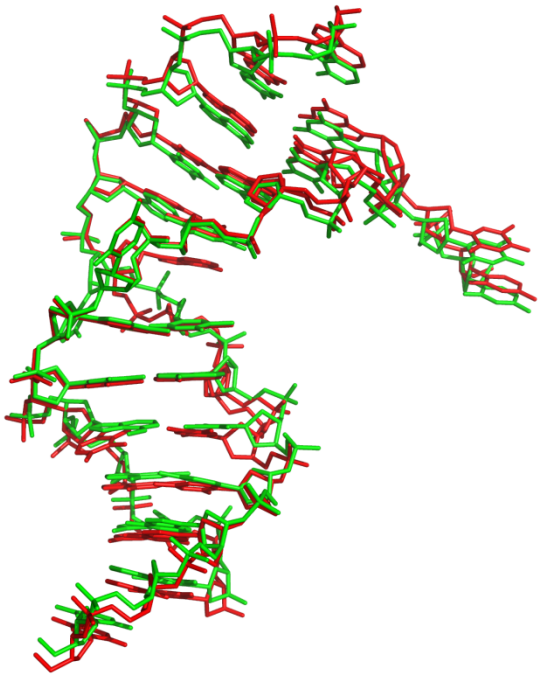
Supplementary Figure 5: Superposition of the N^1-meA-containing, N^6-meA-containing, and the unlesioned ABH2–dsDNA complex (3BTX) structure. (**A**) Cartoon of ABH2–N^1-meA complex (protein in green, DNA in orange, N^1-meA:T in magenta) superposed to unlesioned 3BTX (protein in pale cyan, DNA in yellow, T:A pair in cyan) with a r.m.s.d of 0.49 Å. (**B**) Cartoon of ABH2–N^6-meA complex (protein in green, DNA in orange, N^6-meA:T in red) superposed to unlesioned 3BTX (protein in pale cyan, DNA in yellow, T:A pair in cyan) with a r.m.s.d of 0.21 Å.



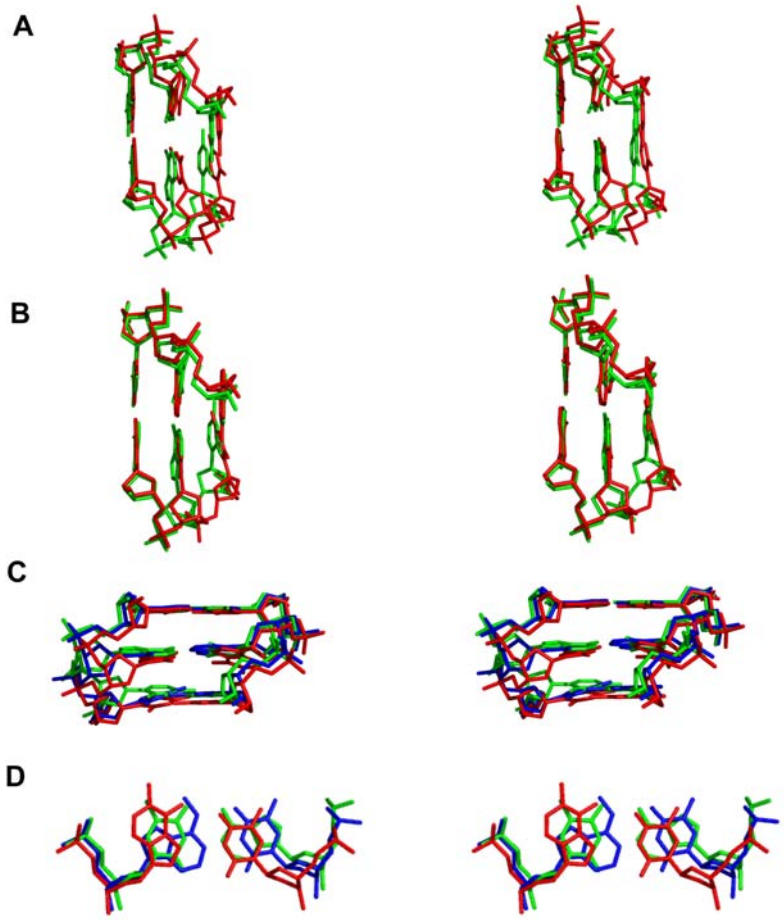
Supplementary Figure 6. Stereo view of two slightly different conformations of N^1-meA-containing DNA. Only minor changes of the backbone occurs, but the base pairs remain almost unaffected.



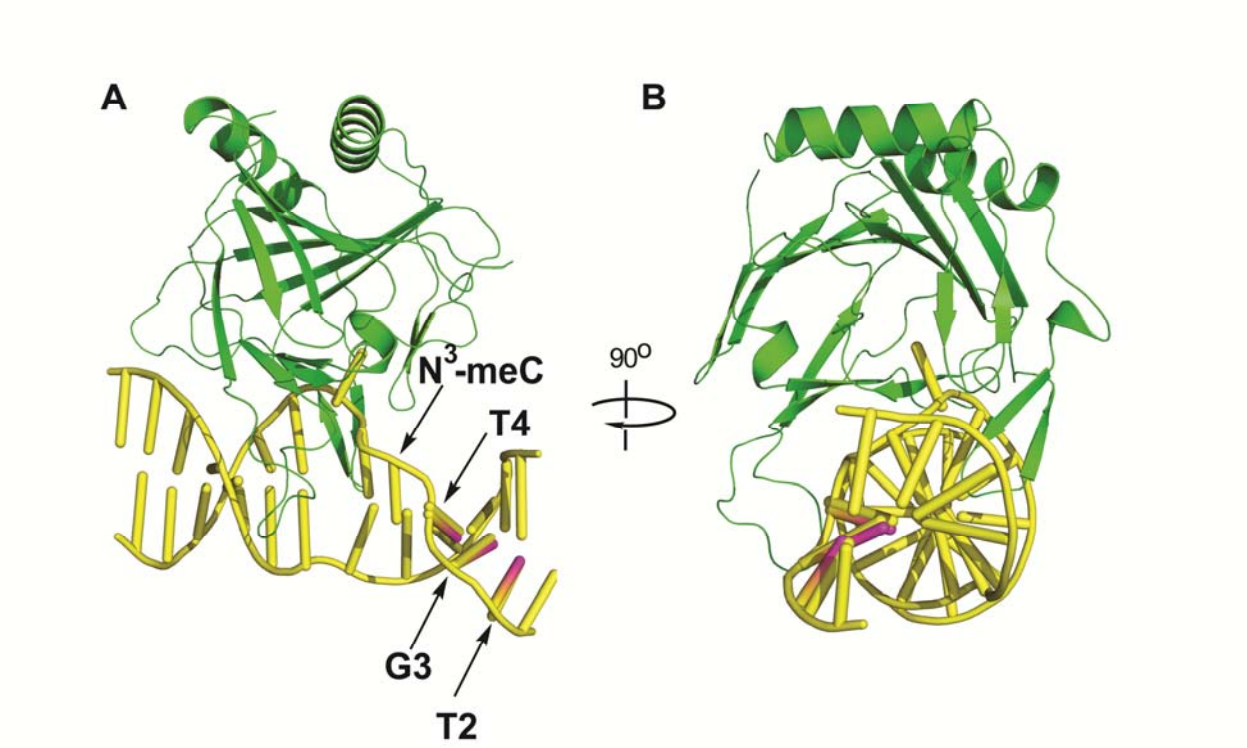
Supplementary Figure 7. Stick diagram of the DNA1 structure containing N^6-meA:T. (**A**) Duplex DNA structure of DNA1 with N^6-meA:T. N^6-meA10 in magenta, opposite T10' in cyan. dsDNA 1: 5'-CTGTATC*AT(^{6me}A)GCG-3' paired with 5'-TCGCTATAATACA-3'**.** (**B**) DNA structure of DNA1 with N^6-meA:T aligned with the unlesioned DNA in structure 3BTX. DNA containing N^6-meA in red, unlesioned DNA in green. dsDNA in 3BTX: 5'-CTGTATC*ATT GCG-3' paired with 5'-TCGCAATAATACA-3'**.**



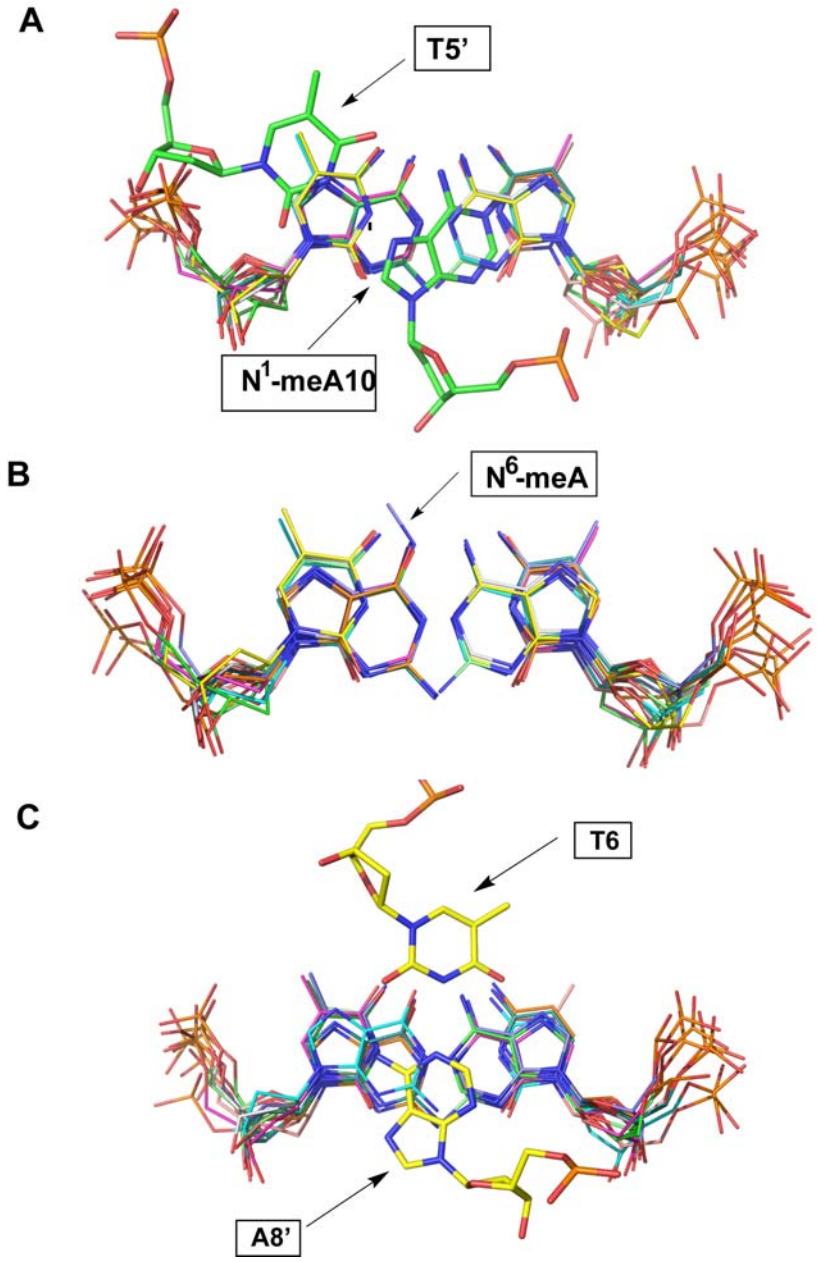
Supplementary Figure 8. DNA structure of DNA1 containing N^1-meA:T aligned with the one containing N^6-meA:T. DNA containing N^1-meA in red, the one with N^6-meA in green. dsDNA 1: 5'-CTGTATC*AT(meA)GCG-3' paired with 5'-TCGCTATAATACA-3'.



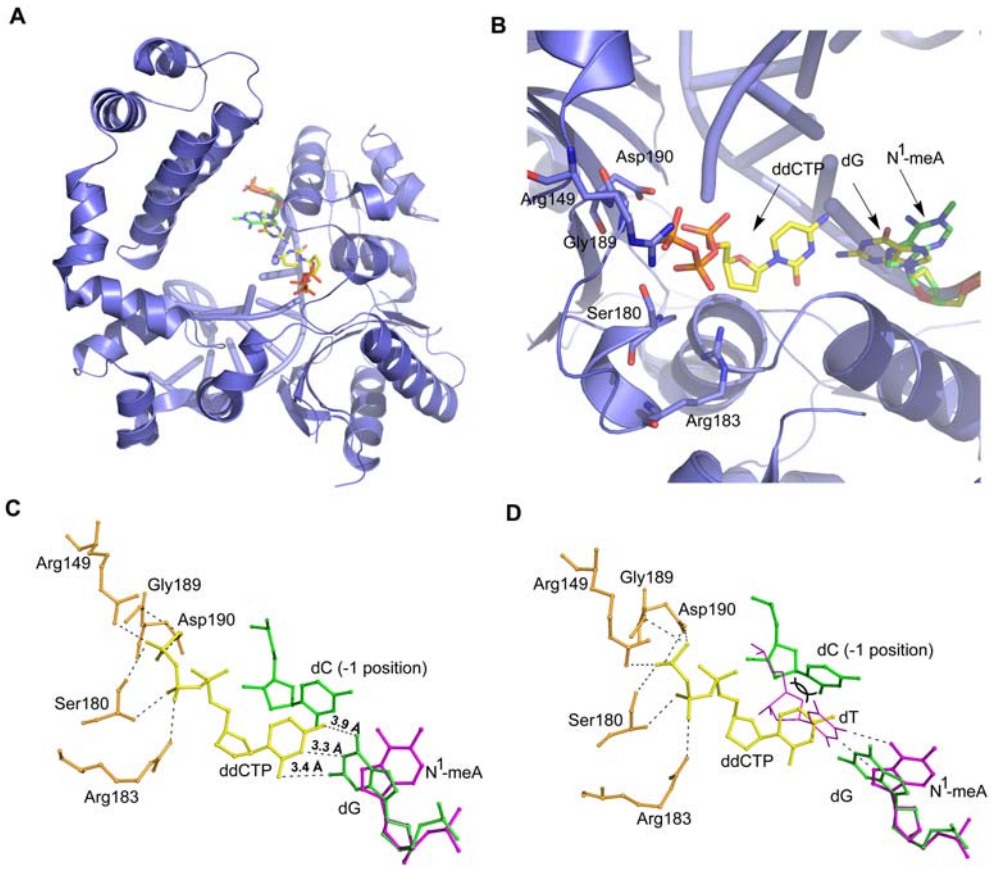
Supplementary Figure 9. Superposition of the N^1-meA:T, N^6-meA:T, and the T:A (3BTX) structure. (**A**) Stereo view of N^1-meA:T (red) and the T:A (green) pairs, as well as the two flanking C:G and A:T base-pairs of each structure. (**B**) Stereo view of N^6-meA:T (red) and the A:T (green) pairs, as well as the two flanking C:G and T:A base-pairs of each structure. (**C**) Overlay of N^1-meA:T(red), N^6-meA:T (blue), and T:A (green) base-pairs. (**D**) Stereo view of the N^1-meA:T, N^6-meA:T and T:A pairs from top showing the greater base-pair shear of the N^1-meA:T pairs, as well as different displacement of the bases into the major and minor groove respectively. Colors are represented same as in C.



Supplementary Figure 10. While N^3-meC and the opposite G are well positioned in the duplex, a few bases neighboring to N^3-meC adopt two slight different conformations shown in the figure. (**A**) Cartoon diagram of ABH2–N^3-meC/G complex. The protein is shown in green, dsDNA in yellow. Bases (T4-T2) neighboring to N^3-meC in conformation A are shown in yellow with conformation B in magenta. (**B**) Same structure as in **A**, rotated 90° to left to show a side view of this complex structure.



Supplementary Figure 11. Base-pairs overlay of N^1-meA-containing DNA structure, N^6-meA and N^3-meC one. (**A**) Stick view of base-pairs of N^1-meA-containing DNA structure from top, with N^1-meA:T dislocated from other Watson-Crick base-pairs' position. (**B**) Stick view of base-pairs of N^6-meA-containing DNA structure, with N^6-meA:T pair well-ordered. (**C**) Stick view of base-pairs of N^3-meC-containing DNA structure. N^3-meC doesn't form base pair with the opposite G, while the Wobble base-pair T6:A8' shows a dramatically displacement.



Supplementary Figure 12. Modeling the N^1-meA lesion in the crystal structure of rat DNA polymerase β (PDB ID 2BPG) (1). (**A**) Cartoon of the N^1-meA (green) in the crystal structure of rat DNA polymerase β, template and ddCTP (yellow) (PDB ID 2BPG). (**B**) An enlarged view of the active site with the backbone of the N^1-meA superimposed to dG on the template. Same color coding as in **A**. (**C**) Stick representation of the N^1-meA in the active site of 2BPG, illustrating the *syn* conformation of N^1-meA compared with dG. The protein residues are colored as orange, ddCTP in yellow, the flanking dC and the complementary dG of ddCTP in green, and the N^1-meA in magenta. (**D**) Modeling the N^1-meA:T Hoogsteen base pair in the active site. The color scheme is same as in **C**, with dT in magenta. This figure illustrated the clash of incoming dTTP to the neighboring dC as well as the loss of interactions to the active site residues when it forms Hoogsteen base pair with the *syn*-conformation N^1-meA. These unfavorable factors will block

DNA primer extension, and thus N^1-meA cannot be bypassed by polymerase β. Although polymerase β is not the primary DNA replicative enzyme, it may still give hints on how N^1-meA is blocked by the DNA replicative polymerase.

References

1. Pelletier, H., Sawaya, M. R., Kumar, A., Wilson, S. H., Kraut, J. (1994) Structure of ternary complexes of rat DNA polymerase β, a DNA template-primer, and ddCTP. Science, 264, 1891-1903.
2. Nair, D. T., Iohnson, R. E., Prakash, S., Prakash, L., and Aggarwal, A. K. (2004) Replication by human DNA polymerase-l occurs by Hoogsteen base-pairing. Nature, 430, 377-380.