# SUPPLEMENTARY TABLES, FIGURES FOR:

# Structural Insights into the Cdt1-Mediated Mcm2-7 Chromatin Loading

Changdong Liu[*], Rentian Wu[*], Zhun Wei[*], Bo Zhou, Jiafeng Wang, Bik K. Tye[†], Chun Liang[‡] and Guang Zhu[‡]

Division of Life Science and State Key Laboratory of Molecular Neuroscience, The Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong, China. [†]Department of Molecular Biology & Genetics, Cornell University, USA

[*]:These authors contributed equally to the work.

[‡]: Corresponding authors

Chun Liang: bccliang@ust.hk; Fax: 852-2358-1552

Guang Zhu: gzhu@ust.hk; Fax: 852-2358-1552

**Figure S1** Validation of hCBD structure with $^{1}H$-$^{15}N$ residual dipolar couplings (RDCs). To validate the three-dimensional structural fold of hCBD in solution, fifty-two experimental $^{1}H$-$^{15}N$ RDCs with hCBD protein weakly aligned in Pf1 were measured for the well-resolved resonances. These RDCs from backbone of hCBD agree well with the values back-calculated from the structure determined based on the NOEs and dihedral angle constraints, with the Q factor being 0.32(44).

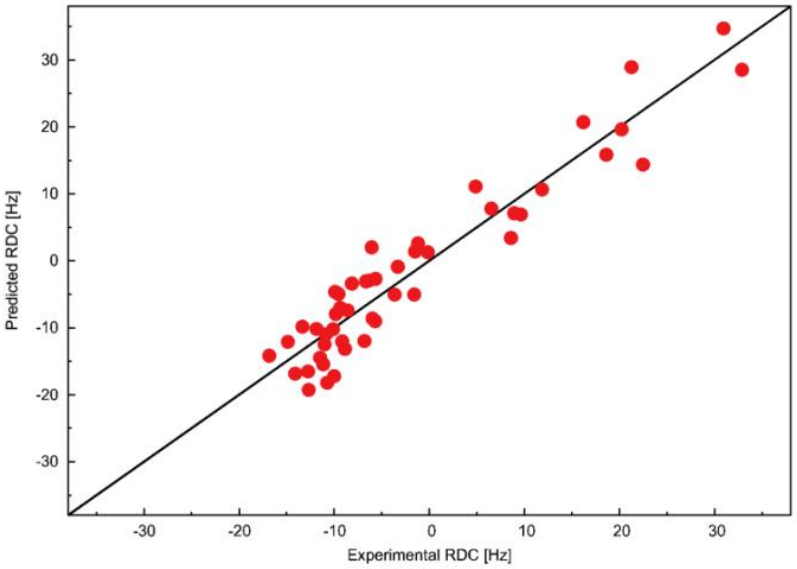
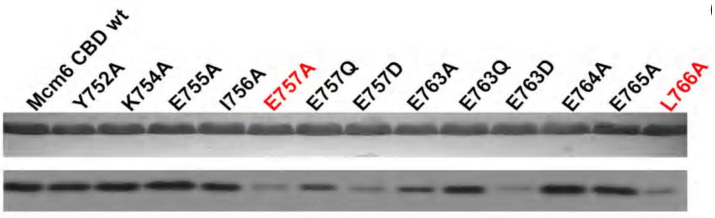
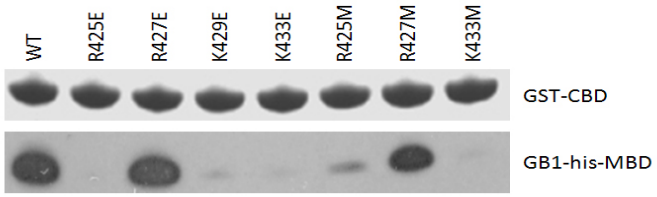


**Figure S2** The effect of mutations in hCBD and hMBD. (A) Similar amounts of human GST-hCBD mutant proteins (upper panel) were used in the GST pull-down assay. The immunoblot with anti-His antibody shows the binding of human GB1-His-hMBD to the wild-type or mutant hCBD (lower panel). Mutants highlighted in red exhibited a significant reduction in binding to hMBD(23). (B) The upper panel shows the relatively equal amounts of GST-CBD used in the GST pull-down assay. The immunoblot with anti-His antibodies shows the binding of GST-CBD to the wild-type (WT) and mutants of GB1-his-MBD (lower panel). (C) SDS-PAGE of the WT and mutants of GB1-his-MBD after Ni-NTA column chromatography. The WT and mutants of GB1-his-MBD are purified to homogeneity by Ni-NTA affinity chromatography.

(A)




(B)




(C)

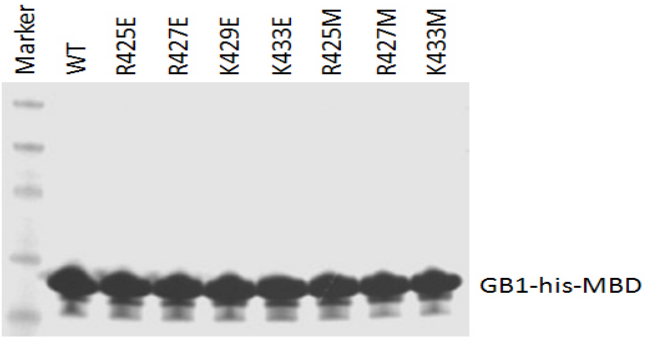


**Figure S3** Alignment of the C-terminal regions of human MCM2-MCM7 sequences.

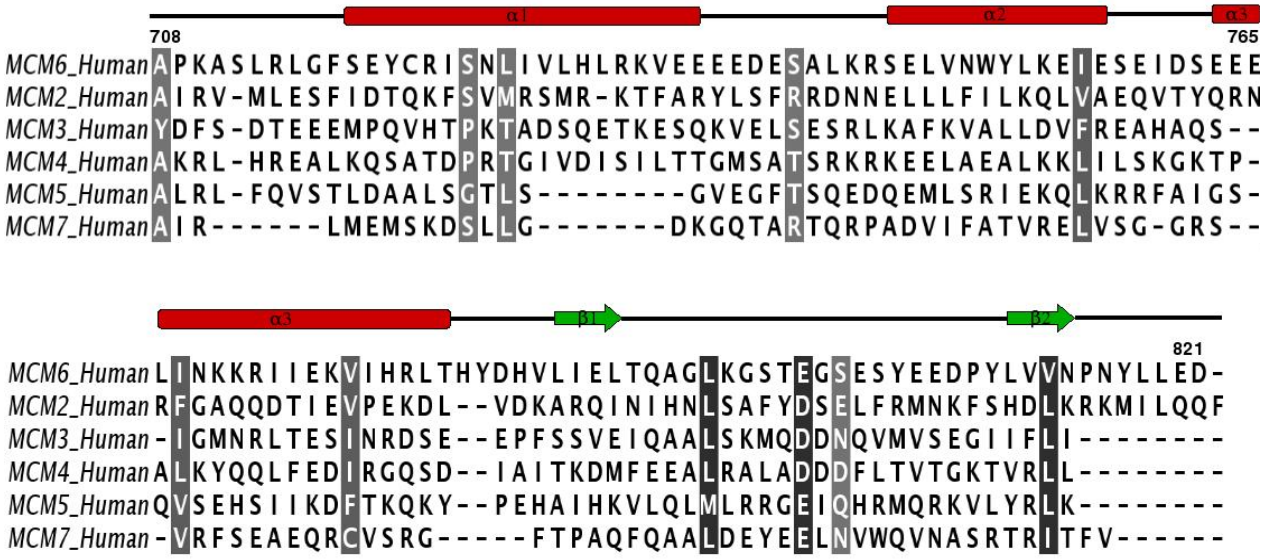


**Table S1** Statistics of the NMR structure of hMBD

| | | |
|---|---|---|
| ***NMR restraints*** | | |
| ***Total Experimental Restraints*** | | ***146*** |
| ***Total NOE Distance Restraints*** | | ***90*** |
| ***Short-range, \|i-j\|<=1*** | | ***68*** |
| ***Medium-range, 1<\|i-j\|<5*** | | ***22*** |
| ***Dihedral restraints*** | | ***56*** |
| ***Statistics for structures*** | | |
| ***Final Energies (kcal/mol)*** | | |
| ***van der Waals*** | ***(kcal/mol)*** | ***-81.4419 ± 9.4536*** |
| ***NOE*** | ***(kcal/mol)*** | ***0.01700 ± 0.0075*** |
| ***Violations*** | | |
| ***Number of NOE violations > 0.5Å*** | | ***0 ± 0*** |
| ***R.m.s. deviation (Å) from experimental distance restraints*** | | ***0.0134 ± 0.0032*** |
| ***Number of dihedral angle constraint violations > 5°*** | | ***0 ± 0*** |
| ***R.m.s. deviation (°) from experimental torsion restraints*** | | ***0.1196 ± 0.1145*** |
| ***Deviations from idealized geometry*** | | |
| ***Bonds (Å)*** | | ***0.00880 ± 0.0004*** |
| ***Angles (°)*** | | ***1.05800 ± 0.0450*** |
| ***Improper (°)*** | | ***1.33720 ± 0.1408*** |
| ***Structural RMSD to the mean coordinate*** | | |
| ***region (residue number)*** | | ***bb/heavy (Å)*** |
| ***411-440*** | | ***4.337/5.341*** |
| ***421-432*** | | ***0.479 / 1.348*** |
| ***Ramachandran plot (% residues)*** | | |
| ***Residues in most favored regions*** | | ***77.6%*** |
| ***Residues in additional allowed regions*** | | ***20.0%*** |
| ***Residues in generously allowed regions*** | | ***2.40%*** |
| ***Residues in disallowed regions*** | | ***0.00%*** |