

## **Supporting materials**

### **Nucleosome Array Compaction and Aggregation Modulated by CpG Location and Methylation Status**

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- 1. Supporting tables**
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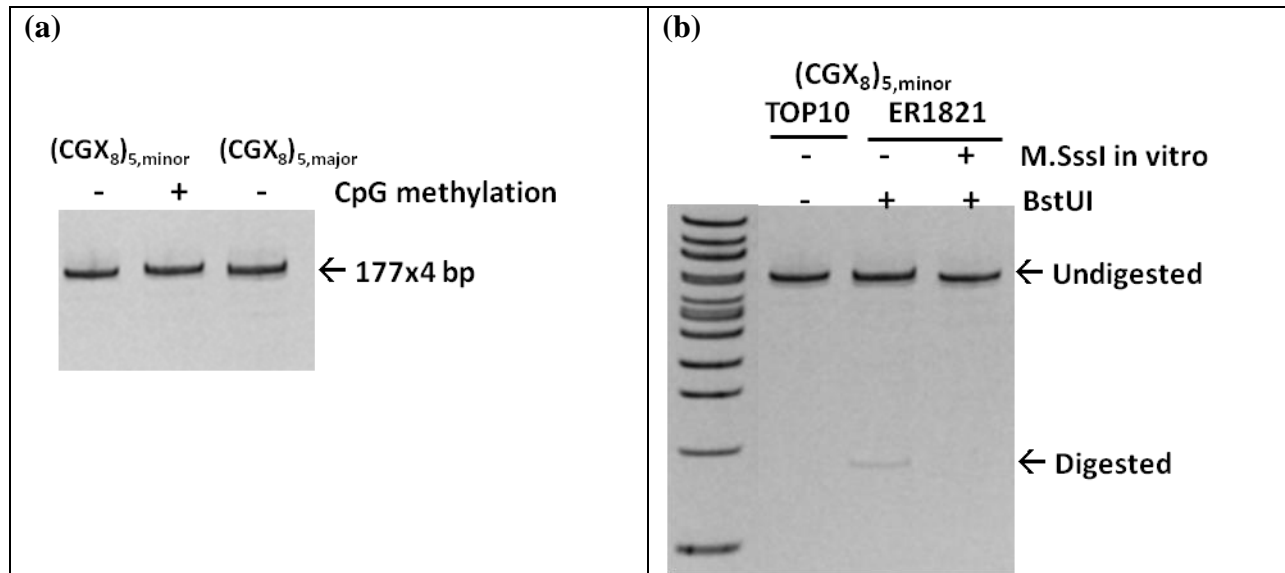
## 1. Supporting tables

**Table S1.** DNA sequences of all four constructs used in this study. Linker DNA sequence is in italics and highlighted. CpG sites are in bold and underlined.

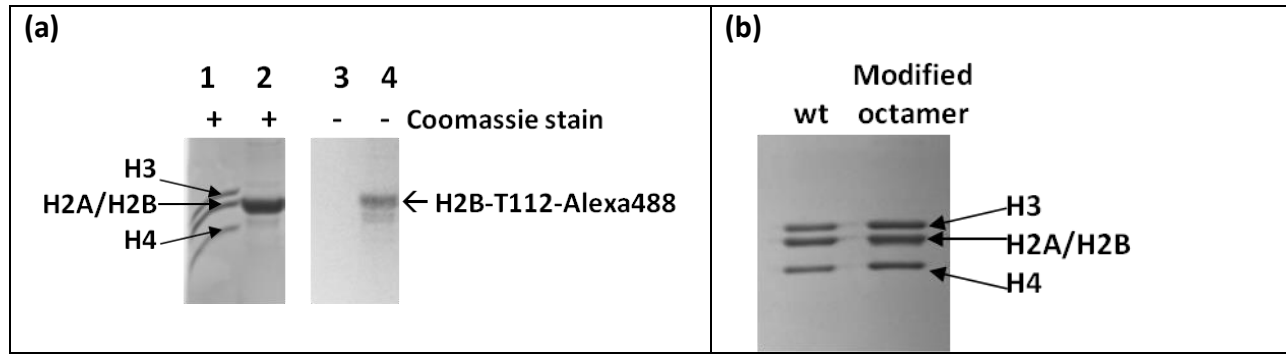
Construct	DNA sequence	Number of CpG sites
Widom-601 (4x177)	<p><i>ATCAGTACTC</i> TGGAGAATCC <b><u>CGGTGCCGAG</u></b> G<b><u>CCG</u></b>CTCAAT  TGGT<b><u>CG</u></b>TAGA CAGCTCTAGC <b><u>ACC</u></b>GCTTAAA <b><u>CGCACGTACG</u></b>  <b><u>CG</u></b>CTGTCCCC <b><u>CGCG</u></b>TTTTAA <b><u>CCG</u></b>CCAAGGG GATTACTCCC  TAGTCTCCAG GCAC<b><u>CG</u></b>TGTCA GATATATACA TCCTGT<b><u>ACTT</u></b>  <b><u>ACGCGGCCGC</u></b> ACAGTACTAC <i>TTAC<b><u>CG</u></b>CCTGG</i> AGAATCC<b><u>CGG</u></b>  TG<b><u>CCG</u></b>AGGCC <b><u>GCT</u></b>CAATTGG <b><u>T<b><u>CG</u></b>TAGACAG</u></b> CTCTAGCACC  <b><u>GCT</u></b>TAAAC<b><u>CGC</u></b> <b><u>ACG</u></b>TAC<b><u>CGCG</u></b> TGTCCCC<b><u>CGC</u></b> <b><u>GTTTTAACCG</u></b>  CCAAGGGGAT TACTCCCTAG TCTCCAGGCA <b><u>CG</u></b>TGTCAGAT  ATATACATCC TGT<b><u>TCTAGAC</u></b> <i>TTAC<b><u>CGCG</u></b>GAGT</i> <b><u>ACTACTTACG</u></b>  <b><u>CGG</u></b>CTGGAGA ATCC<b><u>CGGT</u></b>GC <b><u>CGAGGCCGCT</u></b> CAATTGGT<b><u>CG</u></b>  TAGACAGCTC TAGCAC<b><u>CGCT</u></b> TAAA<b><u>CGCACG</u></b> <b><u>TAC<b><u>CGCG</u></b>CTGT</u></b>  CCCC<b><u>CGCG</u></b>TT TTAAC<b><u>CG</u></b>CCA AGGGGATTAC TCCCTAGTCT  CCAGGCAC<b><u>CGT</u></b> GTCAGATATA TACATCCTGT <b><u>ACTTAC<b><u>CGCG</u></b>G</u></b>  <i>CCAGTACTAC</i> <i>TTAC<b><u>CGCG</u></b>GGC</i> CTGGAGAATC <b><u>CCG</u></b>GTGCC<b><u>GA</u></b>  GGC<b><u>CG</u></b>CTCAA TTGGT<b><u>CG</u></b>TAG ACAGCTCTAG CAC<b><u>CG</u></b>CTTAA  <b><u>ACGCACGTAC</u></b> <b><u>GCG</u></b>CTGTCCC <b><u>CCGCG</u></b>TTTTA <b><u>ACC</u></b>GCCAAGG  GGATTACTCC CTAGTCTCCA GGCAC<b><u>CGT</u></b>GTC AGATATATAC  ATCCTGT<b><u>GCT</u></b> <i>AGCAGTACTA</i> <b><u>CCG</u></b>GT<b><u>GAT</u></b></p>	65
Central dyad (4x177)	<p><i>GATCTTCATG</i> <i>GATATCCCCT</i> GGAGAATCCC <b><u>GGTGCC<b><u>CG</u></b>AGG</u></b>  <b><u>CCG</u></b>CTCAATT GGT<b><u>CG</u></b>TAGAC AGCTCTAGCA <b><u>CCG</u></b>CTTAAAC  <b><u>GCACGTACGC</u></b> <b><u>GCGCGCGCCC</u></b> <b><u>GCG</u></b>TTTTAAC <b><u>CG</u></b>CCAAGGGG  ATTACTCCCT AGTCTCCAGG CAC<b><u>CG</u></b>TGTCAG ATATATACAT  CCTGT<b><u>GCGAT</u></b> <i>AT<b><u>CGAT</u></b>GGAT</i> <i>CTTCATGGAT</i> <i>ATCCCCTGGA</i>  GAATCC<b><u>CGGT</u></b> <b><u>GCC<b><u>CG</u></b>AGGCCG</u></b> CTCAATTGGT <b><u>CG</u></b>TAGACAGC  TCTAGCAC<b><u>CG</u></b> CTTAAAC<b><u>CGCA</u></b> <b><u>CGTAC<b><u>CGCGCG</u></b></u></b> <b><u>CGCGCC<b><u>CGCG</u></b></u></b>  TTTTAAC<b><u>CGC</u></b> CAAGGGGATT ACTCCCTAGT CTCCAGGCAC  <b><u>GT</u></b>GTCAGATA TATACATCCT GT<b><u>GCGATATC</u></b> <b><u>GATGGATCTT</u></b>  <i>CATGGATATC</i> CCCTGGAGAA TCC<b><u>CGGTGCC</u></b> <b><u>GAGGCC<b><u>CG</u></b>CTC</u></b>  AATTGGT<b><u>CGT</u></b> AGACAGCTCT AGCAC<b><u>CGCTT</u></b> AAAC<b><u>CGCACGT</u></b>  <b><u>ACGCGCGCGC</u></b> <b><u>GCC<b><u>CGCG</u></b>TTT</u></b> TAAC<b><u>CG</u></b>CCAA GGGGATTACT  CCCTAGTCTC CAGGCAC<b><u>CGTG</u></b> TCAGATATAT ACATCCTGT<b><u>G</u></b>  <b><u>CGATAT<b><u>CGAT</u></b></u></b> <i>GGATCTTCAT</i> <i>GGATATCCCC</i> TGGAGAATCC  <b><u>CGGTGCC<b><u>CG</u></b>AG</u></b> G<b><u>CCG</u></b>CTCAAT TGGT<b><u>CG</u></b>TAGA CAGCTCTAGC  ACC<b><u>CG</u></b>CTTAAA <b><u>CGCACGTACG</u></b> <b><u>CGCGCGCGCC</u></b> <b><u>CGCG</u></b>TTTTAA  <b><u>CCG</u></b>CCAAGGG GATTACTCCC TAGTCTCCAG GCAC<b><u>CGT</u></b>GTC  GATATATACA TCCTGT<b><u>GCGA</u></b> <i>TAT<b><u>CGATG</u></b></i></p>	72
Major groove (4x177)	<p><i>GATCTTCATG</i> <i>GATATCCCCT</i> GGAGAATCCC <b><u>GGTGCC<b><u>CG</u></b>AGG</u></b>  <b><u>CCG</u></b>CTCAATT GGT<b><u>CG</u></b>TAGAC AGCTCTAGCA <b><u>CCG</u></b>CTTAAAC  <b><u>GCACGTACGC</u></b> <b><u>GCTGTCCCCC</u></b> <b><u>GCG</u></b>TTTTAAC <b><u>CGCGA</u></b>AGGGG</p>	76

	<p>AT<u>CG</u>CTCCCT AG<u>CG</u>TCCAGG CAC<u>CG</u>TGTCAG AT<u>CG</u>GATACAT  CCTGT<u>GCG</u>AT AT<u>CG</u>ATGGAT <i>CTTCATGGAT</i> ATCCCCTGGA  GAATCC<u>CG</u>GT GCC<u>CG</u>AGGCC<u>CG</u> CTCAATTGGT <u>CG</u>TAGACAGC  TCTAGCAC<u>CG</u> CTTAAAC<u>CG</u>CA <u>CG</u>TAC<u>CG</u>CGCT GTCCCC<u>CG</u>CG  TTTTAAC<u>CG</u>C GAAGGGGATC <u>GCT</u>CCCTAGC <u>GT</u>CCAGGCAC  <u>GT</u>GTCAGATC <u>GATA</u>CATCCT GT<u>GCG</u>ATATC <u>GAT</u>GGATCTT  <i>CATGGATATC</i> CCCTGGAGAA TCC<u>CG</u>GTGCC <u>GAG</u>GC<u>CG</u>CTC  AATTGGT<u>CG</u>T AGACAGCTCT AGCAC<u>CG</u>CTT AAA<u>CG</u>CAC<u>CG</u>T  A<u>CG</u>CGCTGTC CCC<u>CG</u>CGTTT TAAC<u>CG</u>CGAA GGGGAT<u>CG</u>CT  CCCTAG<u>CG</u>TC CAGGCAC<u>CG</u>TG TCAGAT<u>CG</u>AT ACATCCTGTG  <u>CG</u>ATAT<u>CG</u>AT <i>GGATCTTCAT</i> <i>GGATATCCCC</i> TGGAGAATCC  <u>CG</u>GTGCC<u>CG</u>AG GCC<u>CG</u>CTCAAT TGGT<u>CG</u>TAGA CAGCTCTAGC  AC<u>CG</u>CTTAAA <u>CG</u>CAC<u>CG</u>TAC<u>CG</u> <u>CG</u>CTGTCCCC <u>CG</u>CGTTTTTAA  <u>CG</u>CGGAAGGG GAT<u>CG</u>CTCCC TAG<u>CG</u>TCCAG GCAC<u>CG</u>TGTCA  GAT<u>CG</u>ATACA TCCTGT<u>GCG</u>A <i>TATCGATG</i></p>	
<p>Minor groove (4x177)</p>	<p><i>GATCTTCATG</i> <i>GATATCCCC</i>TT GGAGAATCCC <u>GGT</u>GCC<u>CG</u>AGG  <u>CG</u>CTCAATT GGT<u>CG</u>TAGAC AGCTCTAGCA <u>CG</u>CTTAAAC  <u>GC</u>AC<u>CG</u>TAC<u>CG</u>C <u>GCT</u>GTCCCCC <u>GCG</u>TTTTT<u>CG</u>C <u>CG</u>CCAAG<u>CG</u>G  ATTACT<u>CG</u>T AGTCTCC<u>CG</u>G CAC<u>CG</u>TGT<u>CG</u>G ATATATACAT  CCTGT<u>GCG</u>AT AT<u>CG</u>ATGGAT <i>CTTCATGGAT</i> ATCCCCTGGA  GAATCC<u>CG</u>GT GCC<u>CG</u>AGGCC<u>CG</u> CTCAATTGGT <u>CG</u>TAGACAGC  TCTAGCAC<u>CG</u> CTTAAAC<u>CG</u>CA <u>CG</u>TAC<u>CG</u>CGCT GTCCCC<u>CG</u>CG  TTTT<u>CG</u>CGC CAAG<u>CG</u>GATT ACT<u>CG</u>TAGT CTCC<u>CG</u>GCAC  <u>GT</u>GTC<u>CG</u>GATA TATACATCCT GT<u>GCG</u>ATATC <u>GAT</u>GGATCTT  <i>CATGGATATC</i> CCCTGGAGAA TCC<u>CG</u>GTGCC <u>GAG</u>GC<u>CG</u>CTC  AATTGGT<u>CG</u>T AGACAGCTCT AGCAC<u>CG</u>CTT AAA<u>CG</u>CAC<u>CG</u>T  A<u>CG</u>CGCTGTC CCC<u>CG</u>CGTTT T<u>CG</u>CGCCAA <u>GCG</u>GATTACT  <u>CG</u>TAGTCTC <u>CG</u>GCAC<u>CG</u>TG T<u>CG</u>GATATAT ACATCCTGTG  <u>CG</u>ATAT<u>CG</u>AT <i>GGATCTTCAT</i> <i>GGATATCCCC</i> TGGAGAATCC  <u>CG</u>GTGCC<u>CG</u>AG GCC<u>CG</u>CTCAAT TGGT<u>CG</u>TAGA CAGCTCTAGC  AC<u>CG</u>CTTAAA <u>CG</u>CAC<u>CG</u>TAC<u>CG</u> <u>CG</u>CTGTCCCC <u>CG</u>CGTTTTT<u>CG</u>  <u>CG</u>CCAAG<u>CG</u> GATTACT<u>CG</u> TAGTCTCC<u>CG</u> GCAC<u>CG</u>TGT<u>CG</u>  GATATATACA TCCTGT<u>GCG</u>A <i>TATCGATG</i></p>	<p>80</p>

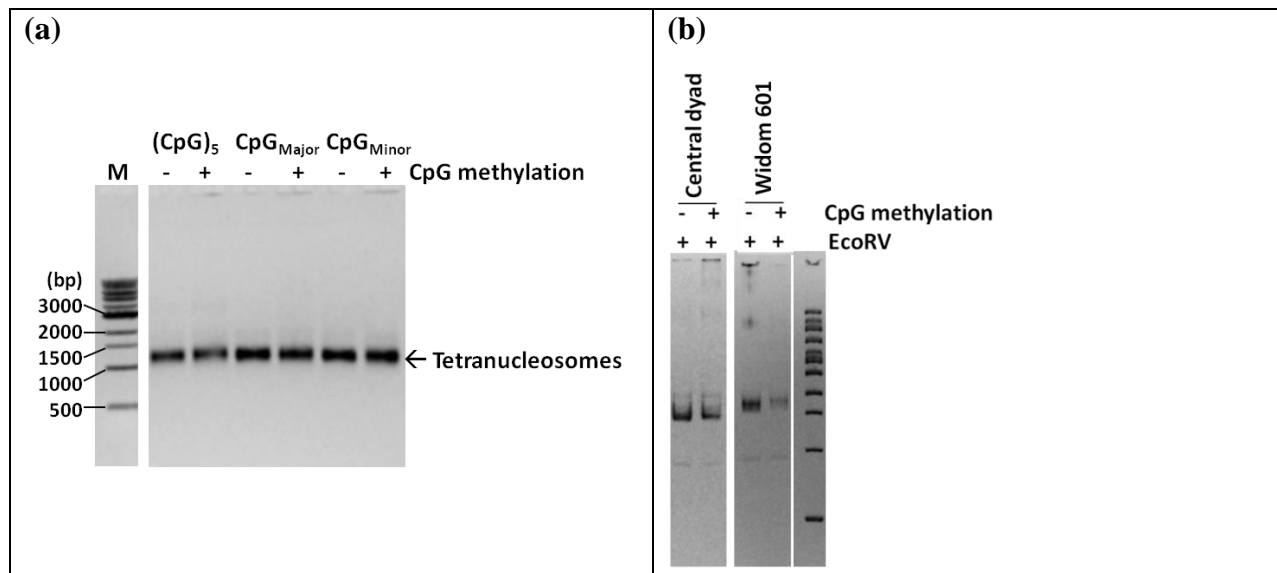
## 2. Supporting figures



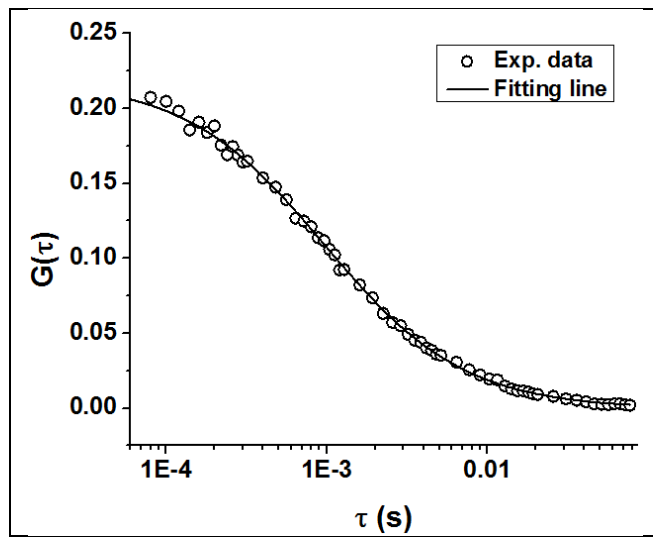
**Figure S1.** (a) Typical 5% PAGE gel of DNA repeats for preparation of tetra-nucleosomes. (b) Typical digestion pattern of DNA fragments with BstUI restriction enzyme examined using a 5% PAGE gel.



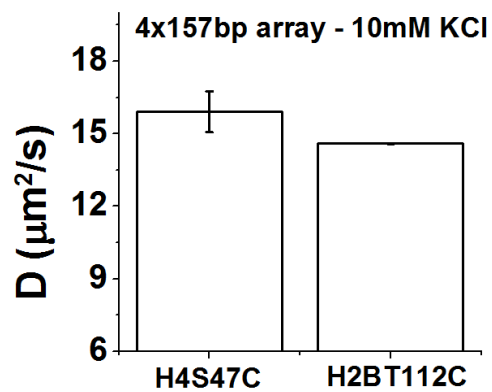
**Figure S2.** (a) Histone H2B proteins labeled with Alexa488 at the T112C position. Lane 1 and 3: wild-type core histone proteins with and without coomassie blue staining. Lane 2 and 4: H2B-T112-Alexa488 with and without coomassie blue staining. It is possible to observe the H2B band without staining due to the presence of Alexa488. (b) 18% SDS-PAGE of the refolded wild-type (wt) and modified histone octamers labeled with Alexa488 at position H2BT112C.



**Figure S3. (a)** Tetra-nucleosome arrays in a 0.8% agarose gel. **(b)** Digestion pattern of the tetra-nucleosome arrays with EcoRV.

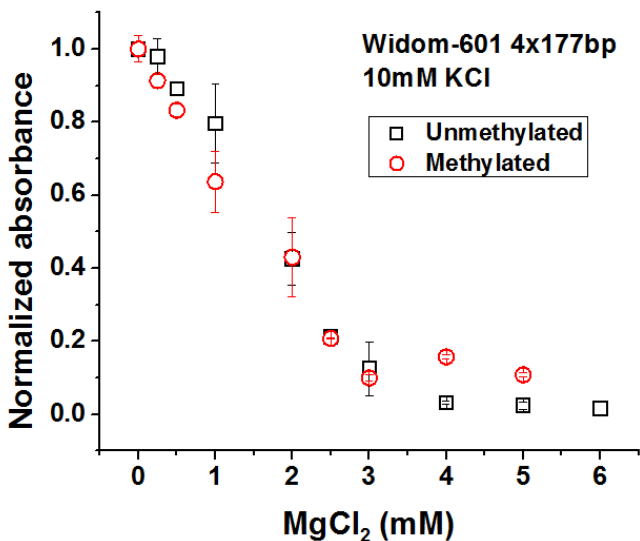


**Figure S4.** Typical autocorrelation curve obtained from FCS experiments.  $D = 13.9\mu\text{m}^2/\text{s}$ ,  $\chi^2 = 0.60$ . Tetra-nucleosome with CpG<sub>Major</sub> pattern at 100mM KCl.



**Figure S5.** Comparison of the diffusivity of Widom-601 tetranucleosome arrays with fluorescent labels at position H4S47C and H2BT112C. The calculated diffusivity is  $15.91 \pm 0.85$  and  $14.59 \pm 0.02$  for the arrays labeled at histone H4 and H2B respectively.





**Figure S6.** Sedimentation assay of Widom-601 tetranucleosome arrays. In this assay, tetra-nucleosome samples with different MgCl<sub>2</sub> concentrations (0-6mM) were incubated at room temperature for 15 min. The samples were then spun at 15000rpm for 15 min at room temperature. The absorbance of the soluble fraction at 256nm was recorded using a spectrophotometer (Cary 100 Bio, Cary Varian). The normalized absorbance was calculated as the absorbance at different MgCl<sub>2</sub> concentrations divided by that of tetra-nucleosomes without MgCl<sub>2</sub>.