SUPPLEMENTARY FIGURES

Figure S1

DNaseI analysis revealed that the structural properties of $(CpG)_3$ are altered by point mutation and cytosine methylation. A restriction fragment of the globin promoter, labelled at the 5'-end of the antisense strand (star in (B)), was mildly digested with DNaseI in methylated (M) and mock-methylated (-) form. Panel (A) shows a magnified region from this antisense strand analysis at the mutation site. Symbols (circle, diamond, triangle) indicate nuclease cleavage within particular sequence contexts; these are also marked for Trip8A because the equivalent Trip2 pattern is less clear. The black arrowheads indicate DNaseI cleavage sites enhanced by methylation of $(CpG)_3$ (LE, lane (M) versus lane (-)). Methylation of the mutant sequences enhanced nuclease cleavage at CpGs remaining from $(CpG)_3$, although this was not always evident for the weakly-cleaved downstream CpG (small arrowhead). The novel CpG shared by Trip8A, Trip9B and Trip9C (the white arrowhead with asterisk marks cleavage on the 5' side of this cytosine) was associated with a methylation-sensitive compression that made the identification of particular cleavage sites in these digests difficult. Arrowheads alongside Trip9B attempt to identify wild-type-equivalent (black) and novel (white) nuclease cleavage sites at the $(CpG)_4$ sequence of this mutant. See Figure 4, main paper, for full legend.

Figure S2

Difference plots for the DNaseI analyses of the Watson (Fig. 4, main paper) and Crick (Fig. S1) strands at the $(CpG)_3$ mutation site. The profiles were generated by subtracting the scan of the unmethylated wild-type DNaseI digest from the scan of each unmethylated and methylated mutant. The difference plot for the methylated wild-type (red) is presented at the top of all columns for the purposes of comparison. The plots have been ordered to reflect their influence upon nucleosome positioning at site 5A (see Table 1, main paper). Thus,

methylated Trip3 and Trip25 are placed at the top and labelled (-) to reflect notable inhibition of positioning at 5A, whereas unmethylated Trip1 and Trip23 are at the bottom and labelled (+++) to reflect their lack of influence.

Figure S3

Flexibility plots constructed according to Packer et al (37) that reveal the effect of point mutation upon the predicted flexibility of $(CpG)_3$. Predicted flexibility (lower values reflect higher flexibility) is plotted against the mid-point of a tetranucleotide window moved through the sequence at 1 bp steps. The wild-type and derivative sequences are shown below each panel.