Gopalakrishnan *et al*., Online Supplemental Material

Figure Legends, Figures, and Tables

Supplemental Figure S1: DNMT3B co-IPs with CENP-C during the G1 phase of the cell cycle demonstrating that the interaction is not restricted to M phase. Untransfected HeLa cells were synchronized by double thymidine block at the G1/S boundary and nuclear extract was prepared and used for the indicated co-IPs.

Supplemental Figure S2: Efficiency and specificity of the DNMT3B and CENP-C siRNAs at reducing target RNA and protein levels. HCT116 cells were transfected with either CENP-C or DNMT3B siRNA Smartpools from Dharmacon as described in Materials and Methods. RNA and whole cell extract was prepared from each knock down or mock transfected cells. (**A**) Quantitative RT-PCR analysis for CENP-C (light gray bars) and DNMT3B transcripts (dark gray bars). Values are the average of three PCR reactions from two independent knock down transfections, relative to amplification of GAPDH. (**B**) Levels of CENP-C and DNMT3B protein following siRNA knock down by western blotting. Results of a GAPDH siRNA knock down are also shown as a control (middle lanes). KD – knock down.

Supplemental Figure S3: Bisulfite genomic sequencing (BGS) analysis of the alpha satellite repeat in HCT116 cells (**A**) mock transfected, (**B**) transfected with CENP-C siRNA, or (**C**) transfected with DNMT3B siRNA. Following knock down, genomic DNA

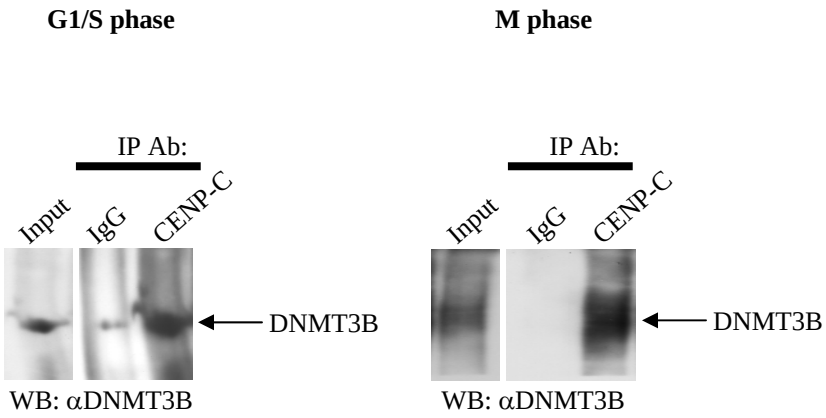
was used for BGS analysis. The region analyzed is shown as a bar at the top with the seven CpG sites within the analyzed region indicated with tick marks. Below this, the BGS data is summarized for each sequenced clone (each row of circles). Open circle – unmethylated CpG, closed circle – methylated CpG. The percent methylation for all analyzed clones and CpG sites is also given. The location of the CpG sites present in the CENP-B box (binding site) are also shown. These data are summarized in Figure 5 in the main text.

Supplemental Figure S4: BGS analysis of the satellite 2 repeat in HCT116 cells (**A**) mock transfected, (**B**) transfected with CENP-C siRNA, or (**C**) transfected with DNMT3B siRNA. The region analyzed is shown as a bar at the top with the 23 CpG sites within the analyzed region indicated with tick marks. Methylation data is presented as described in Fig. S3. These data are summarized in Figure 5 in the main text.

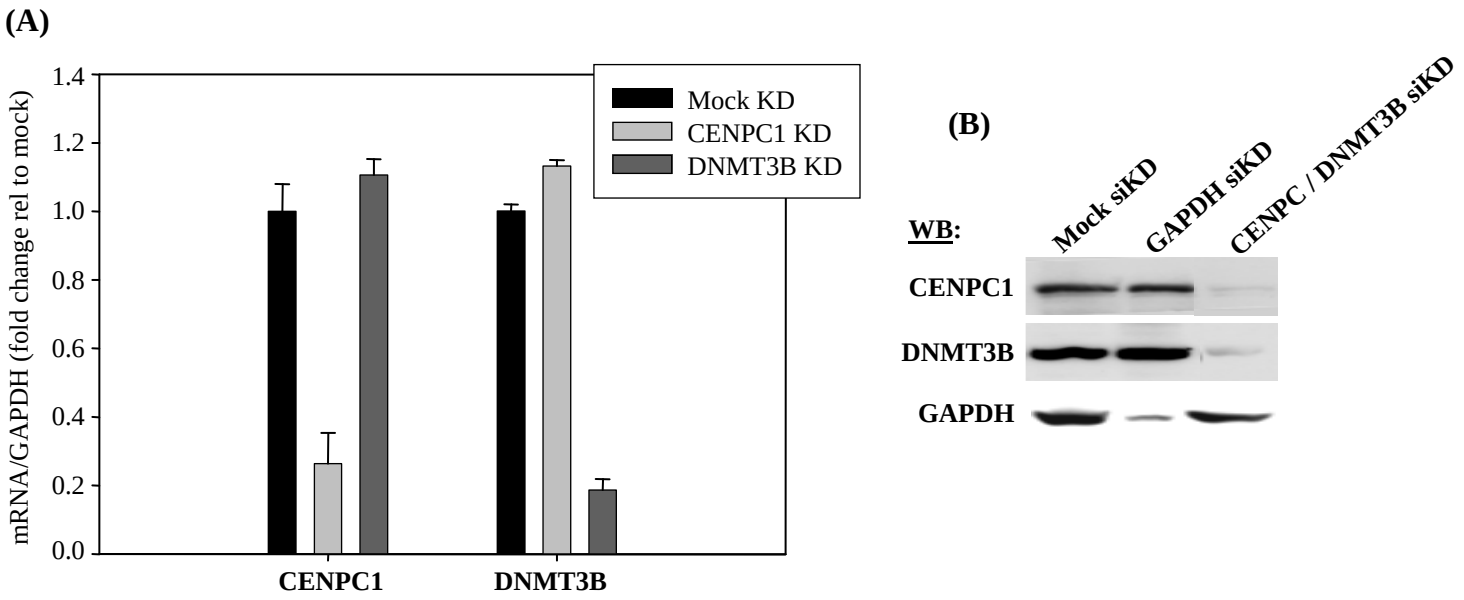
Supplemental Figure S5: CENP-C binding is reduced in the complete absence of DNMT3B. Chromatin immunoprecipitation (ChIP) was used to determine CENP-C binding in HCT116 DNMT3B knockout cells (3BKO, gray bars) relative to parental HCT116 cells (black bars). Following ChIP, levels of (**A**) alpha satellite and (**B**) satellite 2 DNA enrichment were determined by quantitative PCR as described in the main text. ChIP with a DNMT3B antibody and normal rabbit IgG were used as controls for non-specific background binding.

Supplemental Figure S6: SiRNA knock down of CENP-C has no effect on epigenetic marks at two single copy genes. As described in the main text, chromatin was prepared from mock or CENP-C/DNMT3B siRNA-transfected HCT116 cells and used for ChIP with the indicated antibodies. (**A**) ChIP analysis for the *WIF1* gene, which is known to hypermethylated and transcriptionally silenced in HCT116 cells. WIF1 ChIP primers are located at -198 (F) to -115 (R) relative to the transcription start site. (**B**) ChIP analysis of the *GAPDH* gene, a constitutively active housekeeping gene. GAPDH ChIP primers are located at -310 (F) to -180 (R) relative to the transcription start site. Results are shown as the average of triplicate PCR reactions from two independent siRNA knock downs and the error bar is the standard deviation. CENP-C and DNMT3B ChIP results are graphed separately at the right since their binding was lower that that of the histone marks (left panel).

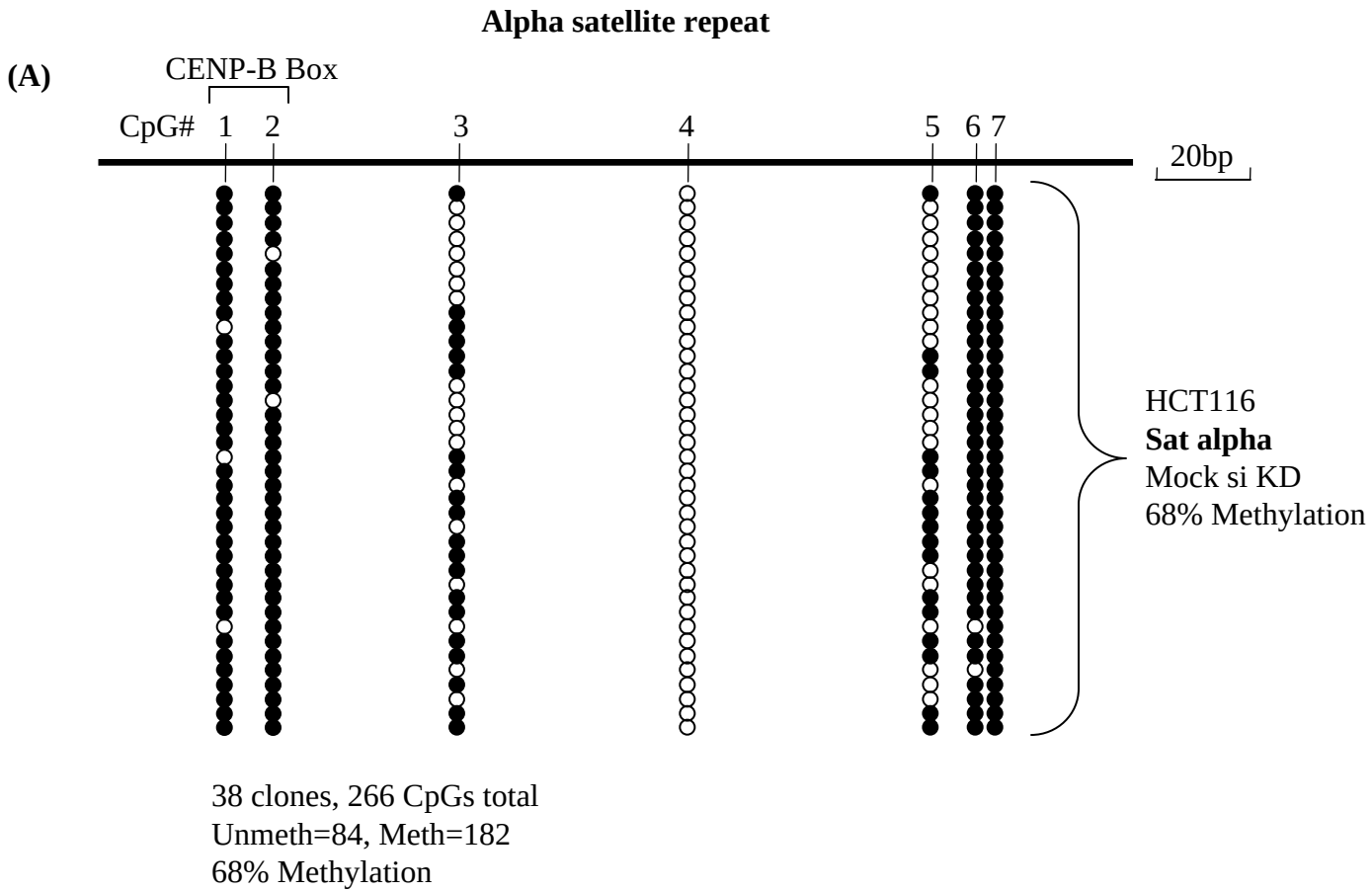
Gopalakrishnan *et al.*, Online Supplemental Data



Gopalakrishnan *et al.*, Supplemental Fig. S1

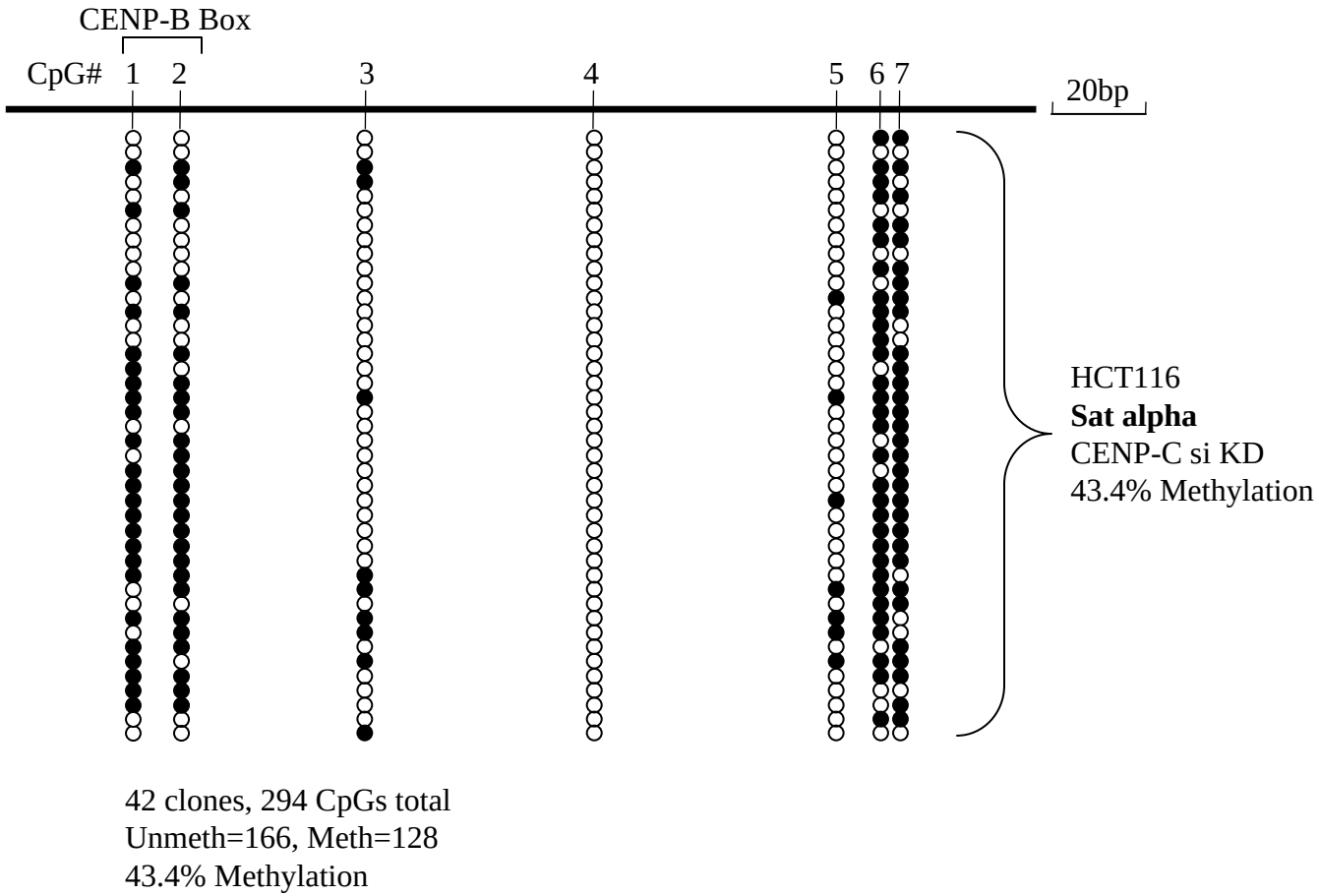


Gopalakrishnan *et al.*, Supplemental Fig. S2


Alpha satellite repeat
(A)
CENP-B Box
CpG# 1 2 3 4 5 6 7
20bp
HCT116
Sat alpha
Mock si KD
68% Methylation
38 clones, 266 CpGs total
Unmeth=84, Meth=182
68% Methylation

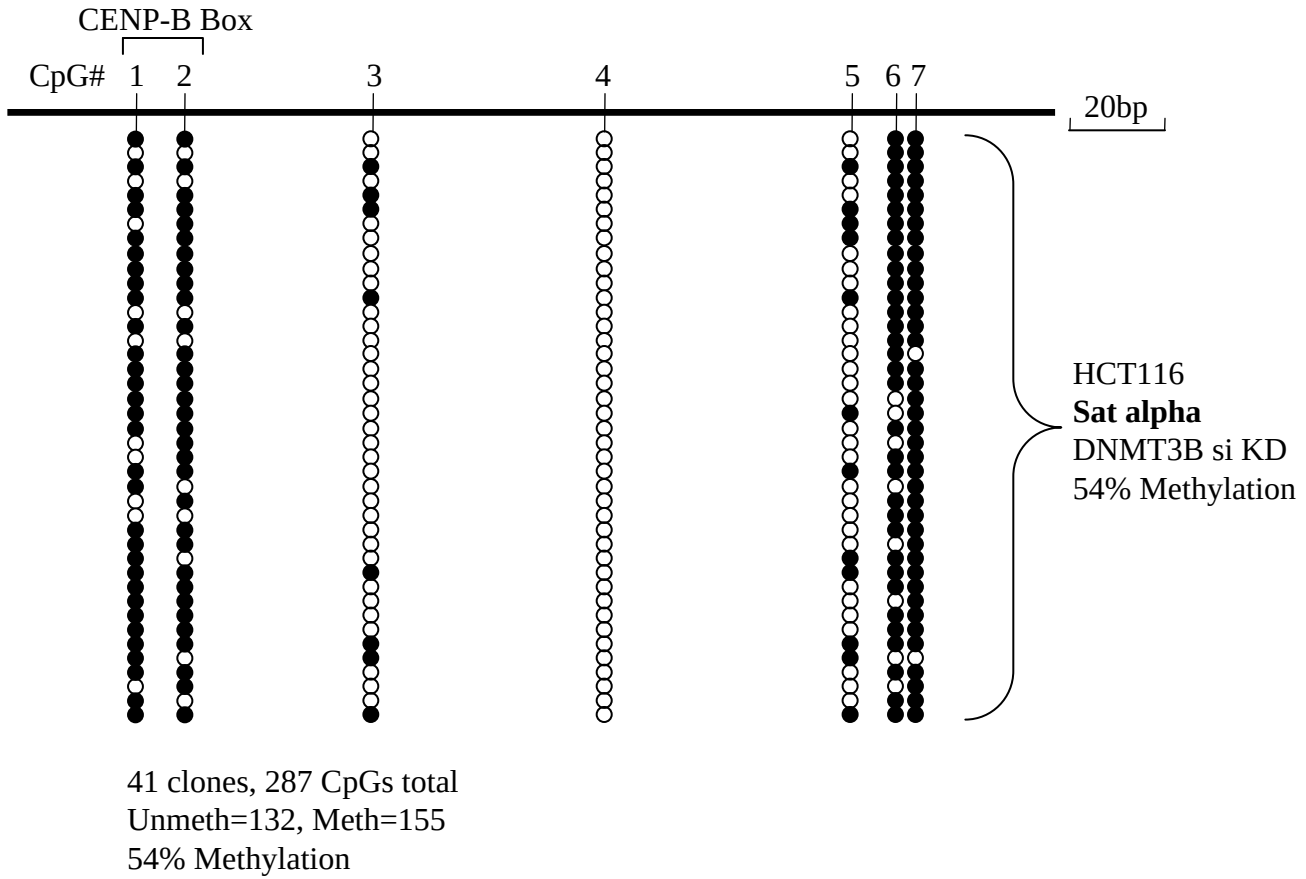
Alpha satellite repeat

(B)



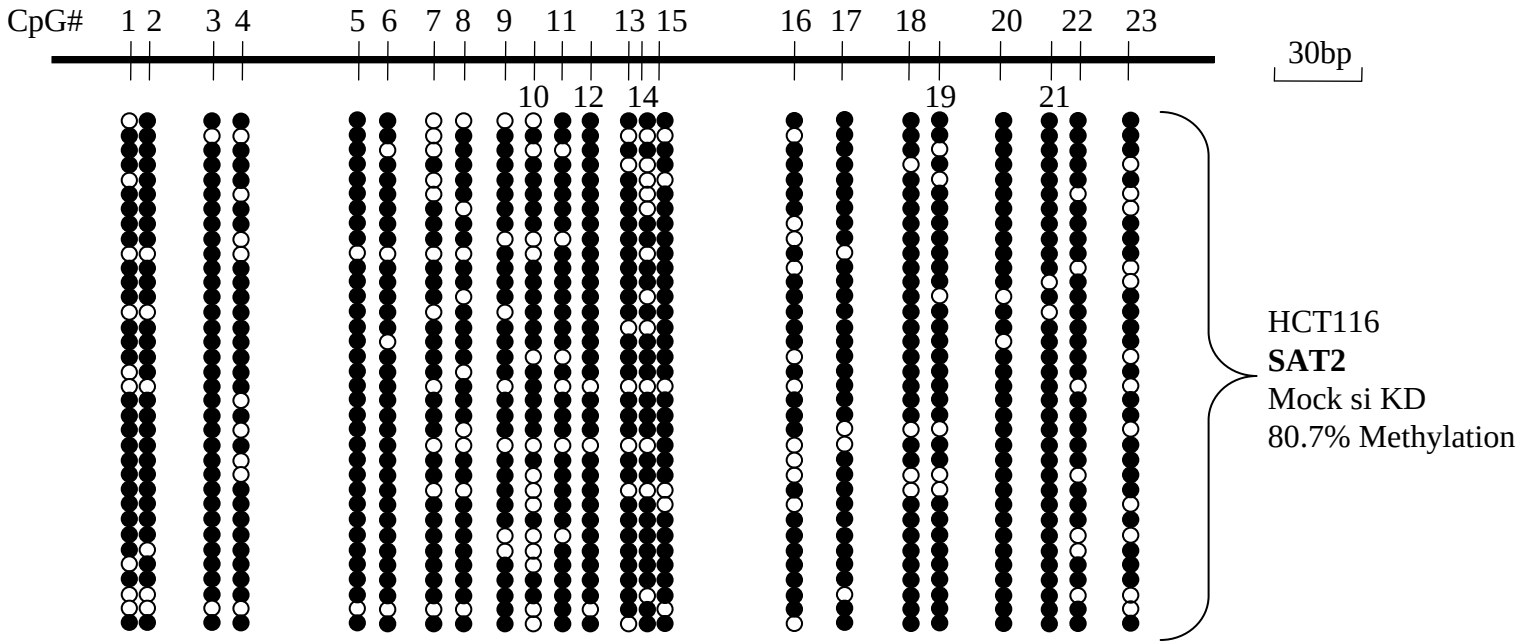
Alpha satellite repeat

(C)



Satellite 2 repeat

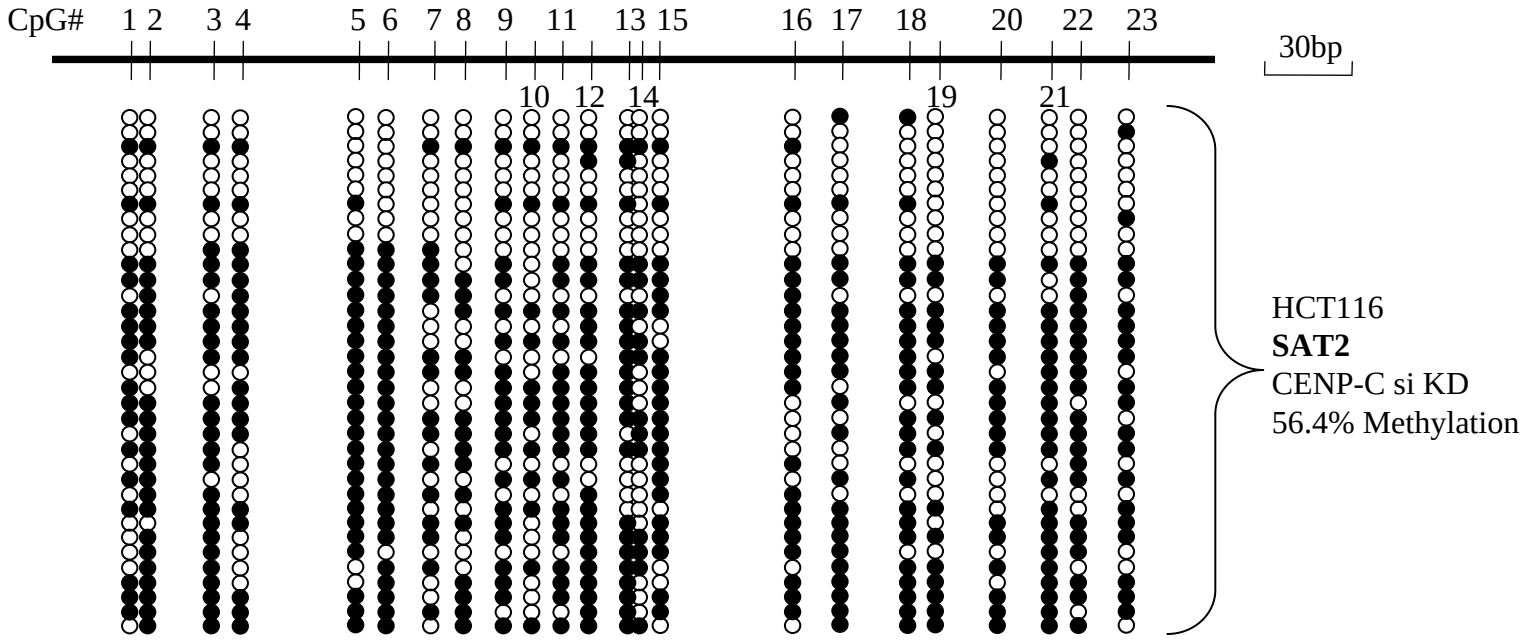
(A)



35 clones, 805 CpGs total
Unmeth=155, Meth=650
80.7% Methylation

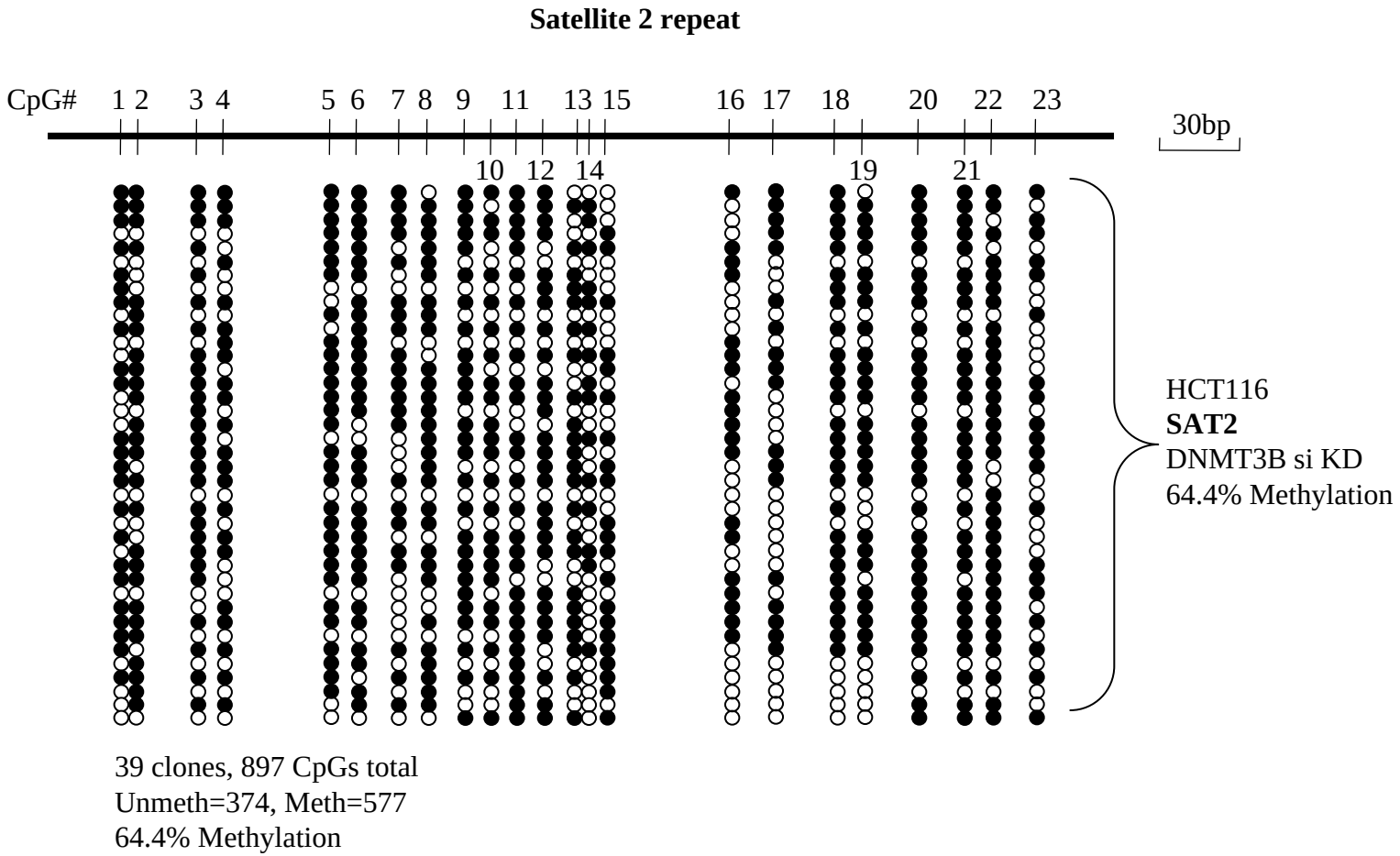
Satellite 2 repeat

(B)

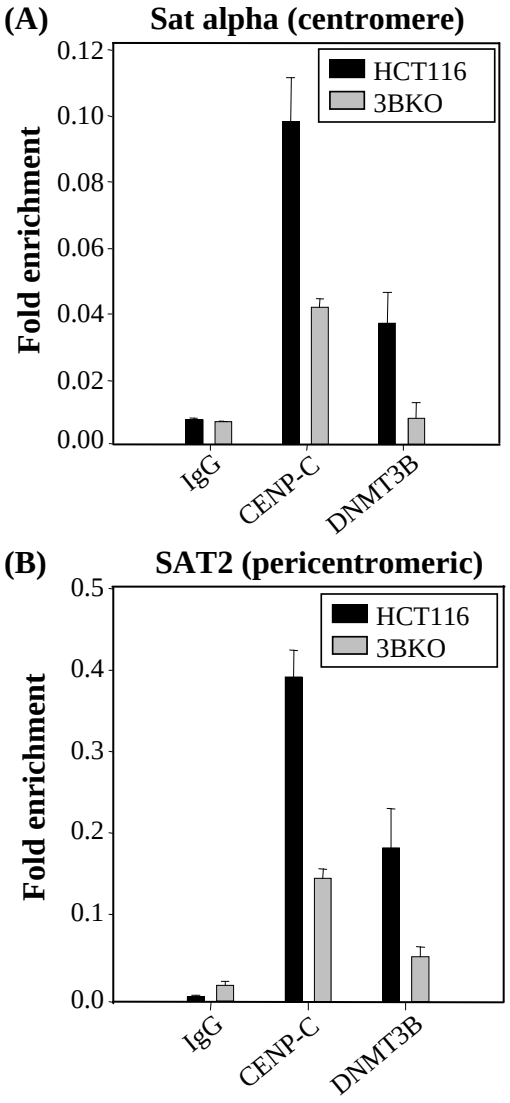


35 clones, 805 CpGs total
Unmeth=370, Meth=435
56.4% Methylation

(C)



Gopalakrishnan *et al*., Supplemental Fig. S4 cont.



Gopalakrishnan *et al.*, Supplemental Fig. S5

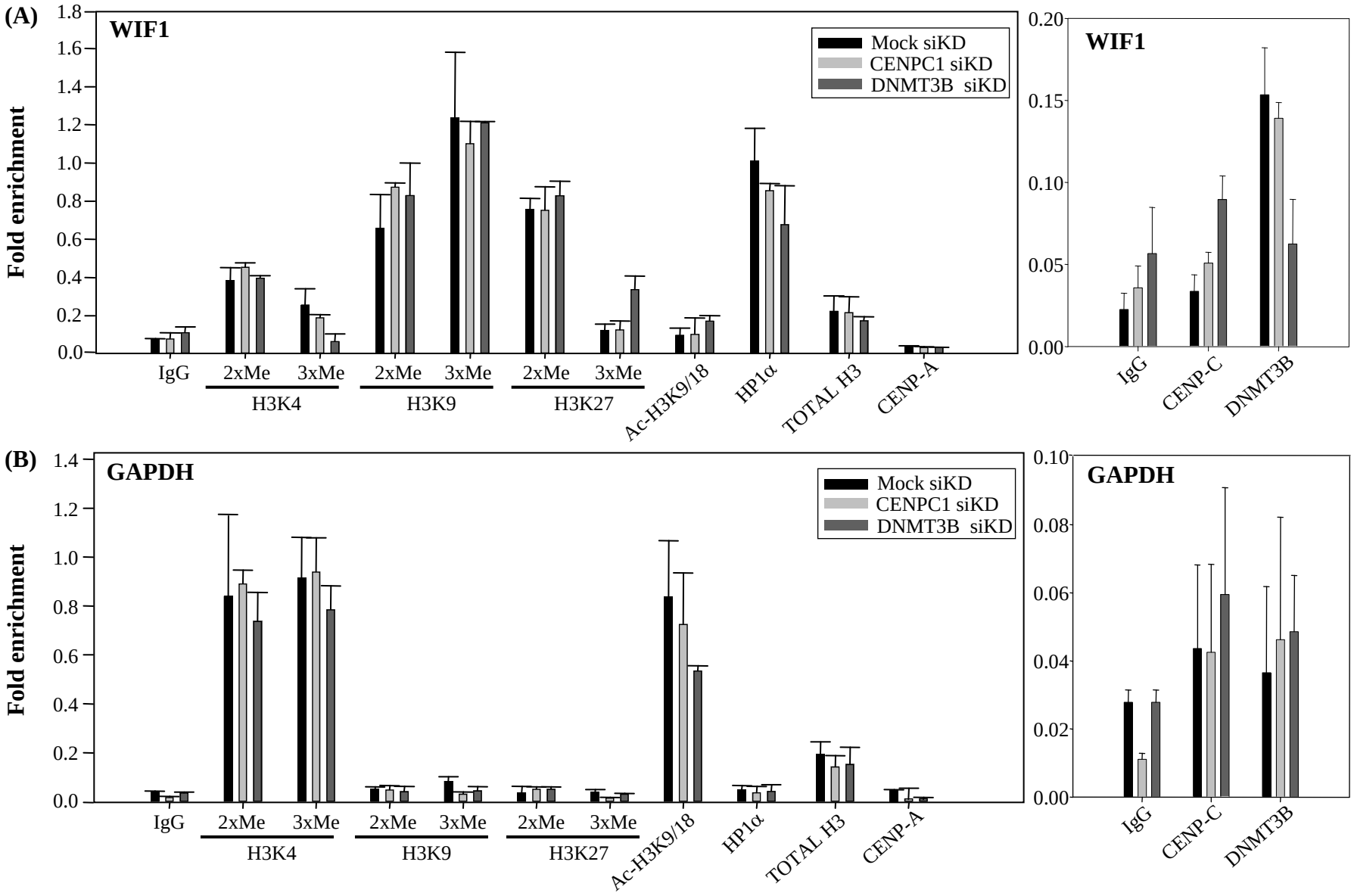


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Table S1: Antibodies used in this study for ChIP and western blotting (WB).

Antibody	Manufacturer	Dilution/amount
Normal rabbit IgG	Pierce	10μg (ChIP)
DNMT3B	Novus Biologicals	15μg (ChIP)/1:1000 WB
Ac-H3K9/K18	Upstate	10μg (ChIP)
2XMeH3K4	Upstate	10μg (ChIP)
3XMeH3K4	Abcam	10μg (ChIP)
2XMeH3K9	Abcam	10μg (ChIP)
3XMeH3K9	Abcam	10μg (ChIP)
2XMeH3K27	Abcam	5μg (ChIP)
3XMeH3K27	Abcam	10μg (ChIP)
CENP-C	Abcam	15μg (ChIP)/ 1:500 (WB)
CENP-A	Abcam	5μg (ChIP)
Mouse anti-FLAG M2	Sigma	1:1000 WB
Rat anti-HA	Roche	1:1000 WB
HP1α	Affinity BioReagents, custom #25	15 μl (ChIP)

Table S2: Primers used in this study.

Primers	Sequence	Used for:
Satellite alpha	5'- TCATTCCCACAAACTGCGTTG -3' (F)	RT-PCR/ChIP
	5'- TCCAACGAAGGCCACAAGA – 3' (R)	
Satellite 2	5'- CTGCACTACCTGAAGAGGAC- 3' (F)	RT-PCR/ChIP
	5'- GATGGTTCAACACTCTTACA- 3' (R)	
WIF1	5'- AGCCCTTCCCGCTCTTCTGTT – 3' (F)	ChIP
	5'- CGGCAGAGACGTAAGACTGGCAAA -3' (R)	
GAPDH	5' – TCGTTCCCAAAGTCCTCCTGTTTC -3' (F)	ChIP
	5'- TCCGCAGCCGCCTGGTTC- 3' (R)	
DNMT3B	5'- GCTCTTACCTTACCATCG -3' (F)	RT-PCR
	5'- TGAACTGTCTCCATCTCC 3' (R)	
CENP-C1	5' –CCAAAGAACAGAATCCATCAC (F)	RT-PCR
	5'- TACTCCACTAATCACGAATCC – 3' (R)	
Satellite alpha	5'- GGATATGTGGATAGTTTTGAAG-3' (F)	BGS
	5'-TTCCTTTTTCACCATAAACCTC-3'(R)	
Satellite 2	5'- GAATTATTGAATAGAATTGAATGG-3' (F)	BGS
	5'- TAAATAATAACTCCTTTCATTT-3' (R)	