DNA methylation and chromatin organization in insects: insights from the ant *Camponotus floridanus*

Karl M. Glastad, Brendan G. Hunt, Michael A. D. Goodisman

Supplementary tables and figures:

Table S1: Percentages of genes that were significantly methylated and also marked by a given hPTM, as well as over- or under-representation of hPTM enrichment among methylated genes as determined by a Fisher's exact test (all Fisher's exact test P values << 0.0001).

	% methylated that are also marked by hPTM	% marked by hPTM that are also methylated	Fisher's exact test significant direction
H3	81.1	70.8	over
H3K4me3	80.6	79	over
H3K4me1	27.2	80	over
H3K27me3	1.2	23.4	under
H3K27ac	85.8	69.5	over
H3K36me3	79.6	79.6	over
H3K9me3	1.5	3.3	under
H3K9ac	67.3	42.1	over
PolII	49.5	66.1	over

Table S2: Spearman's rank correlations between fractional DNA methylation and histone modification normalized tag enrichment at genic features ("TSS-proximal" represents a 2kb length-normalized gene measure 500bp upstream of TSS to 1.5kb downstream of gene start). $P < 0.0001$ for all listed correlations.

	TSS-proximal	Exon	Intron
H3	0.599	0.307	0.185
H3K4me3	0.621	0.417	0.290
H3K4me1	0.158	0.361	0.182
H3K27me3	0.162	-0.086	-0.143
H3K27ac	0.596	0.376	0.349
H3K36me3	0.617	0.459	0.202
H3K9me3	-0.564	-0.448	-0.319
H3K9ac	0.272	-0.153	-0.052
PolII	0.464	0.121	0.134

Table S3: Numbers of HMRs associated with specific gene features. Genic: intersecting any gene annotation (gene set model or valid cufflinks transcript). Proximal: falling within 2kb either up- (5') or downstream (3') of any gene annotation. Non-genic: HMRs not falling within 2kb of a gene annotation. Non-genic HMRs were further divided into those which showed experimental evidence of expression (>4 RNA-sequencing reads mapped to HMR) despite the lack of a gene annotation, and those without (without RNA-seq).

Feature	**HMR count**
Genic	6927
exonic	*4447*
Intronic	*2480*
Proximal	433
5'	*100*
3'	*333*
Non-genic	22
with RNA-seq	*8*
without RNA-seq	*14*
Total	**7382**

Table S4. Association tests between a genomic region's differential methylation status (whether it is a DMR or unchanging methylated region) and differential ChIP enrichment (differentially enriched between castes or not) for the 8 factors assessed in this study among windows with sufficient DNA methylation and differential enrichment data.

	DMR status	% nonDiffChip	% DiffChip	N	P value
H3K27ac	nonDMR	48.44	51.56	5850	NS
	DMR	47.98	52.02	2728	
H3K27me3	nonDMR	60	40	597	NS
	DMR	55.88	44.12	300	
H3K36me3	nonDMR	29.65	70.35	5501	<0.0001
	DMR	25.61	74.39	2722	
H3K4me1	nonDMR	85.9	14.1	2128	NS
	DMR	86.22	13.78	849	
H3K4me3	nonDMR	43.9	56.1	5917	<0.0001
	DMR	38.63	61.37	3219	
H3K9ac	nonDMR	69.69	30.38	2946	0.0031
	DMR	65.21	34.79	1602	
H3K9me3	nonDMR	58.11	41.89	101	NS
	DMR	55.56	44.44	85	
PolII	nonDMR	47.31	52.69	4170	NS
	DMR	48.15	51.85	1942	

NS, non-significant

Table S5: Comparisons between differentially methylated regions and overlapping differentially enriched ChIP calls (using standard data from 'consolidatedCIs' file). P value from likelihood ratio test. Percentages reflect the percent membership a given row shows in the associated column.

Differential ChIP enrichment call		Differential methylation call			P value
		Male	**Worker**	**NA**	
H3K27ac	% Male	46.23	54.3	50.78	0.0004
	% Worker	53.77	45.7	48.22	
	N	928	1606	4525	
H3K27me3	% Male	95.52	92.47	92.33	NS
	% Worker	4.48	7.53	7.67	
	N	67	93	313	
H3K36me3	% Male	88.25	89.5	87.4	0.0068
	% Worker	11.75	10.5	12.6	
	N	2196	3112	7981	
H3K4me1	% Male	30.77	32.65	31.21	NS
	% Worker	69.23	67.35	68.79	
	N	91	147	487	
H3K4me3	% Male	78.45	84.97	78.71	<0.0001
	% Worker	21.55	15.03	21.29	
	N	815	1942	5077	
H3K9ac	% Male	90.97	80.85	83.1	0.0145
	% Worker	9.03	19.15	16.9	
	N	144	329	728	
H3K9me3	% Male	40.48	26.83	33.33	NS
	% Worker	59.52	73.17	66.67	
	N	42	41	87	
PolII	% Male	54.5	63.2	59.1	0.0010
	% Worker	45.5	36.8	40.9	
	N	556	1333	3579	

NS, non-significant

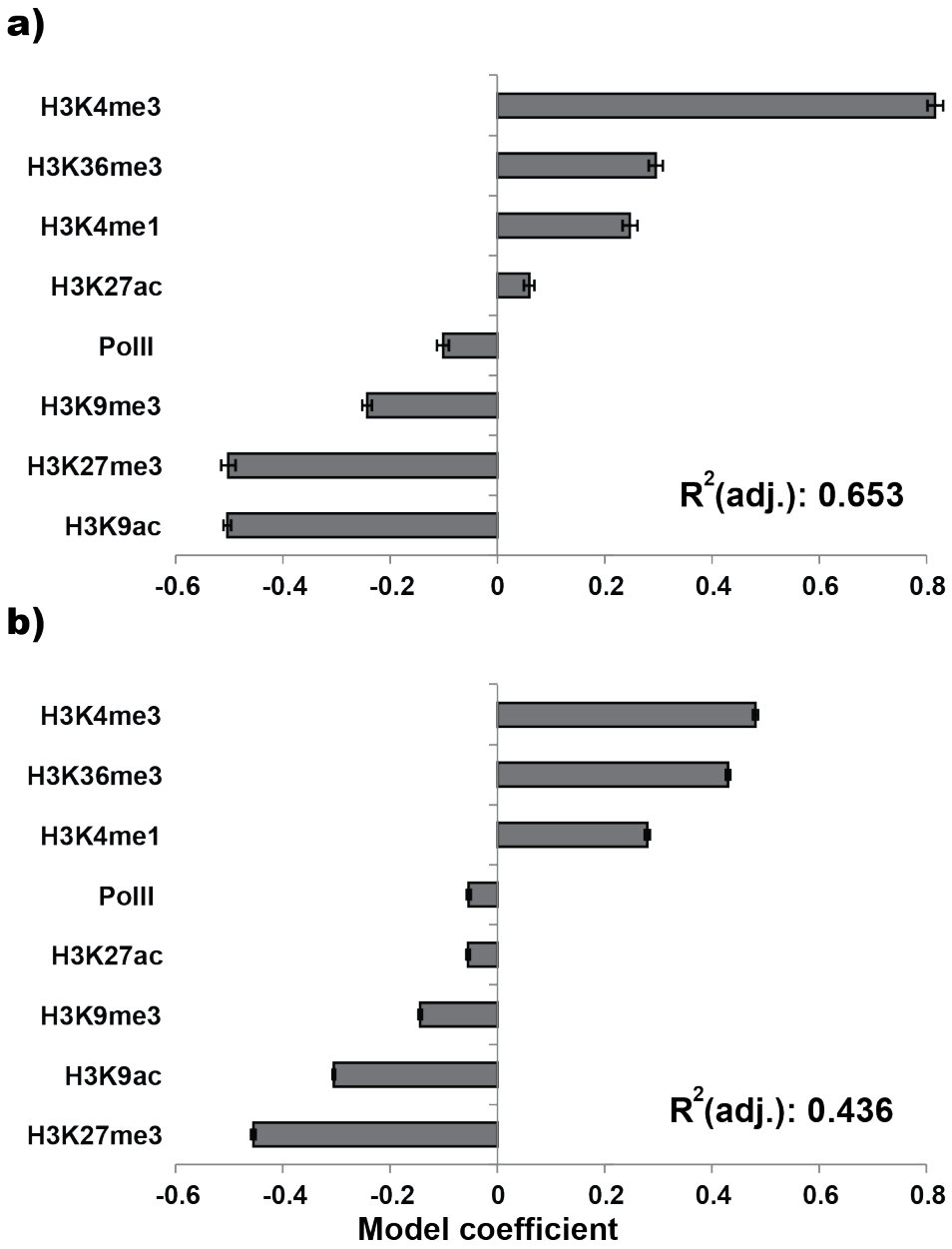

Figure S1: hPTM levels explain DNA methylation variation. Model coefficients for multiple regression of hPTM enrichment levels against DNA methylation levels within the same feature for a) CDS, and b) exons+introns (as distinct features) as the dependent variable. Magnitude of bars represent estimated model coefficients. Interaction terms not included. Error bars represent standard error. R^2 values given represent adjusted R^2 for full model fit.

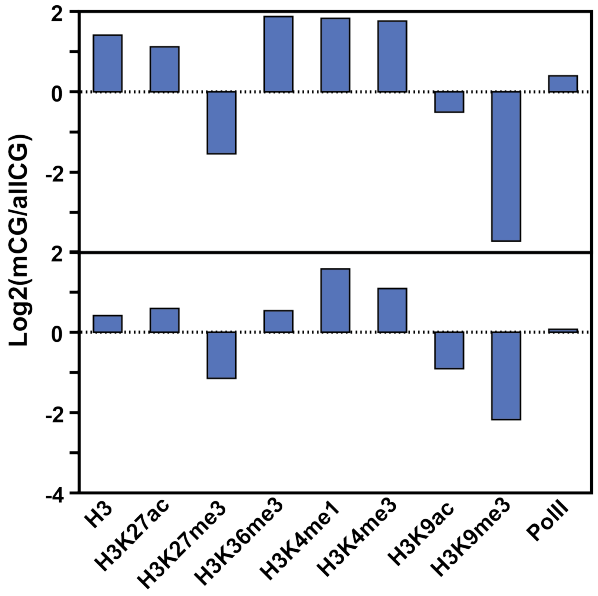

Figure S2: Methylated CpGs are strongly over- or under-represented among regions significantly enriched for different hPTMs. For both a) within all gene bodies, as well as b) within only methylated gene bodies the log2-transformed ratio of the proportion of methylated CpGs (mCGs) falling within the given hPTM-enriched regions to that of of all CpGs (allCGs) falling within the same regions is given.

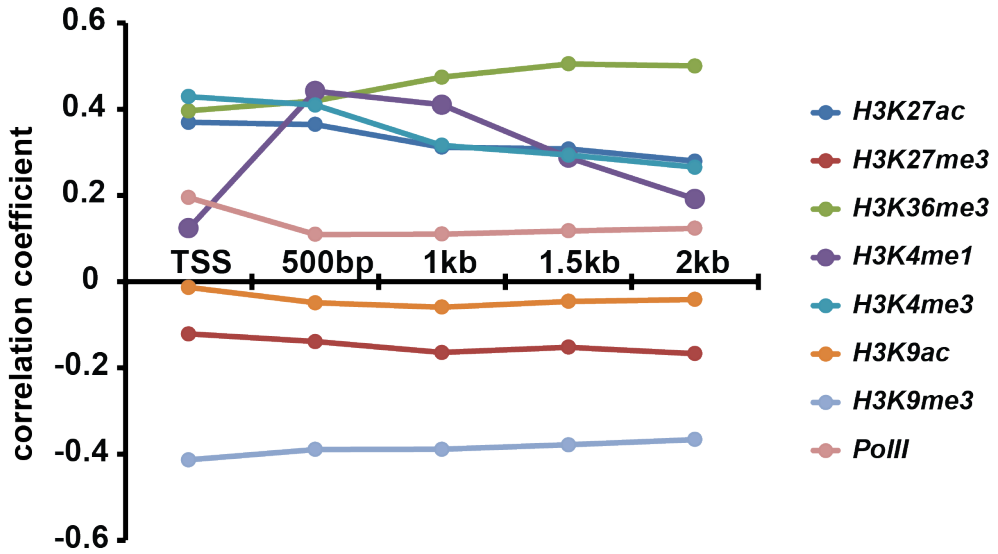

Figure S3. DNA methylation is correlated with hPTM enrichment at a fine spatial scale within genes. The correlation coefficients for spearman's rank correlations between DNA methylation and hPTM enrichment for each hPTM for 500bp windows downstream of gene TSSs are shown. Each point represents the correlation between DNA fractional methylation and the given hPTM tag fold enrichment within a 500bp window starting the given distance (x axis) from the TSS (eg TSS=0-500bp from start of TSS). Only genes longer than 2.5kb were used. All correlations are significant ($P<0.05$).

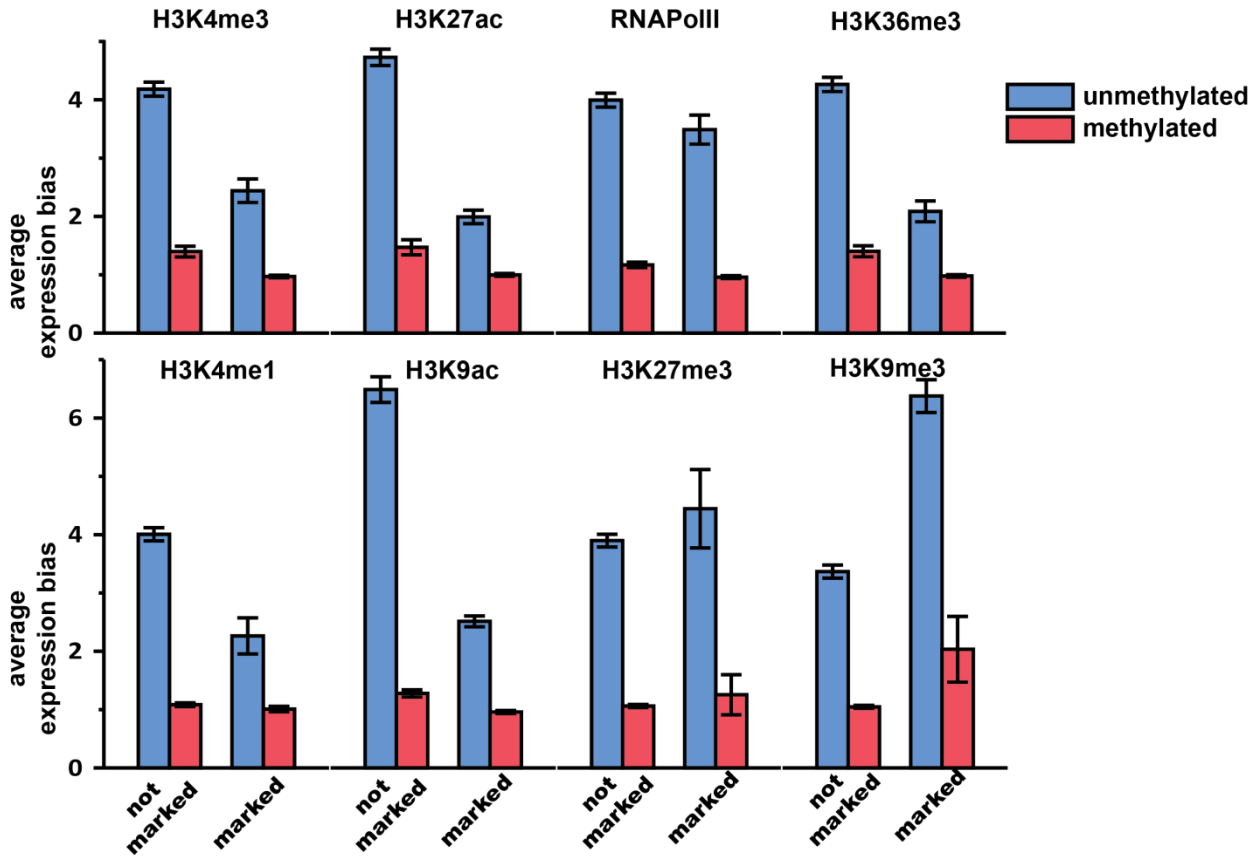

Figure S4: Levels of expression bias (average of absolute $\log_2$(FPKM) ratios for 3 comparisons) of genes associated with histone modifications. Methylated genes exhibit consistently lower levels of expression bias relative to unmethylated genes.

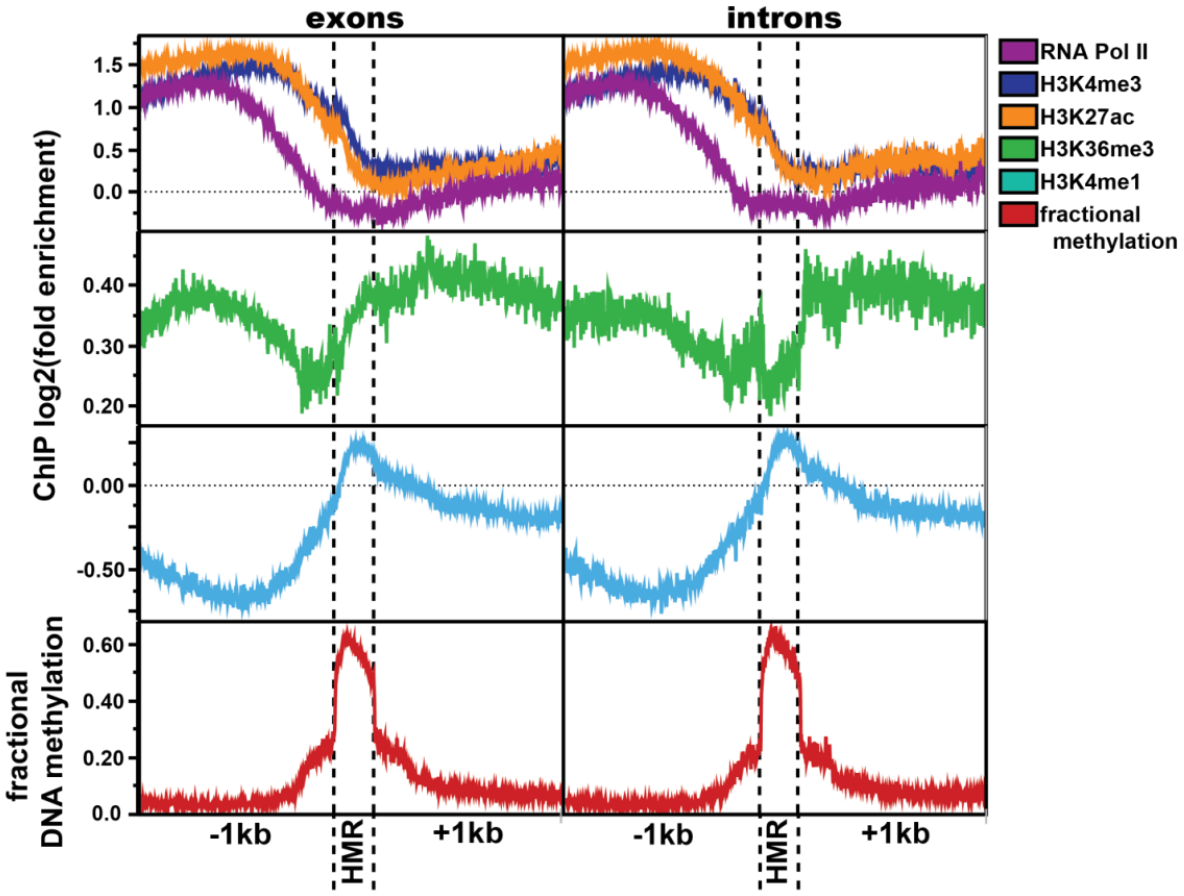

Figure S5: Chip profiles as they relate to highly methylated regions (HMRs) localized to exons and introns. Shown ChIP measures correspond to those in Figure 3 of the main text.

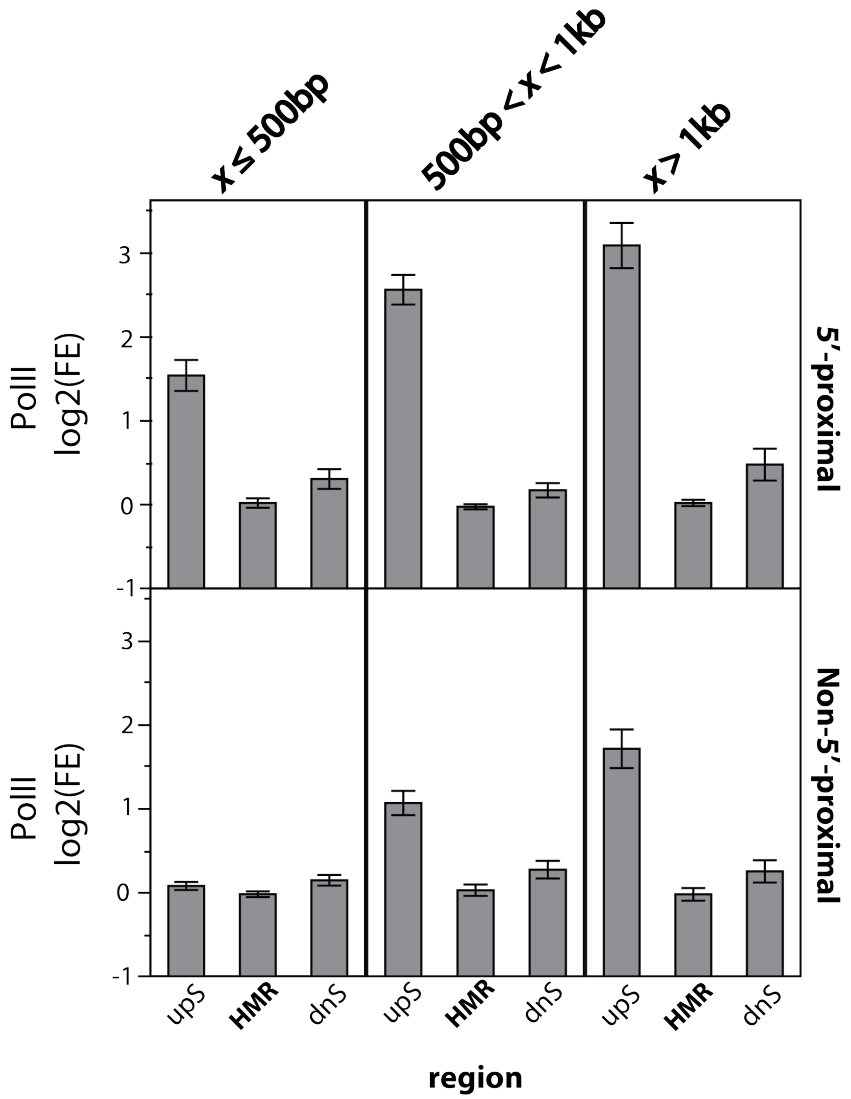

Figure S6: RNA polymerase II ChIP enrichment (log2 fold enrichment over input) within highly methylated regions (HMRs) as well as 1kb regions in the 5' and 3' directions (upS and dnS, respectively), split both by HMR proximity to a gene start, as well as grouped into 3 HMR length classes. All comparisons are significant below the $p<0.0001$ level.

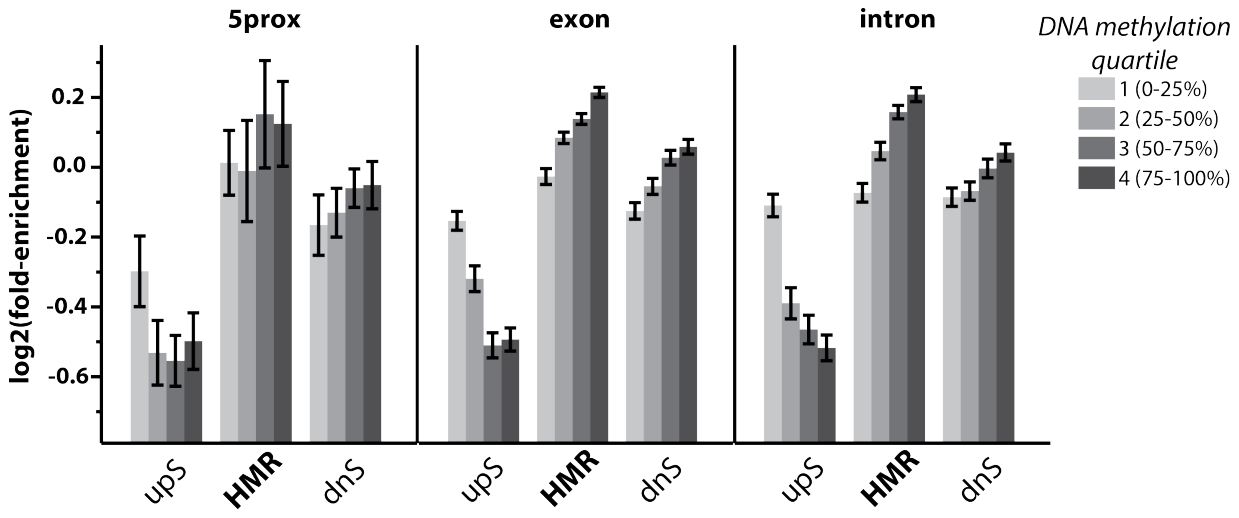

Figure S7: H3K4me1 is enriched within highly methylated regions (HMRs), independent of genic context. H3K4me1 values shown for HMRs, and 1kb bins up- and downstream (upS and dnS, respectively) of HMR for HMRs overlapping the region -2kb-0kb from gene starts (5prox), exons, and introns. HMR DNA methylation levels were split into 4 equally-sized ascending quartiles to illustrate opposite relationship between DNA methylation level and H3K4me1 enrichment between regions upstream of HMRs and HMRs themselves.