

## **Supplemental Material to:**

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**Integrated detection of both 5-mC and 5-hmC by high-throughput tag sequencing technology highlights methylation reprogramming of bivalent genes during cellular differentiation**

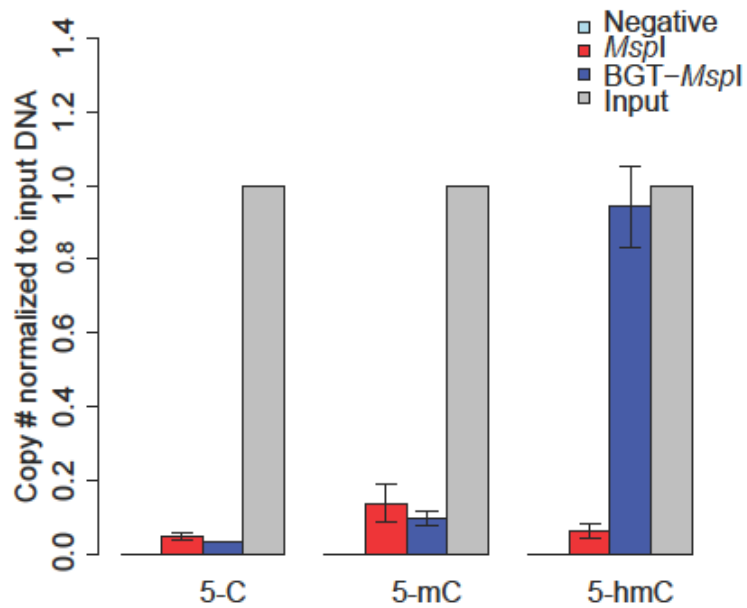
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**<http://www.landesbioscience.com/journals/epigenetics/article/24280/>**

## Supplementary Files

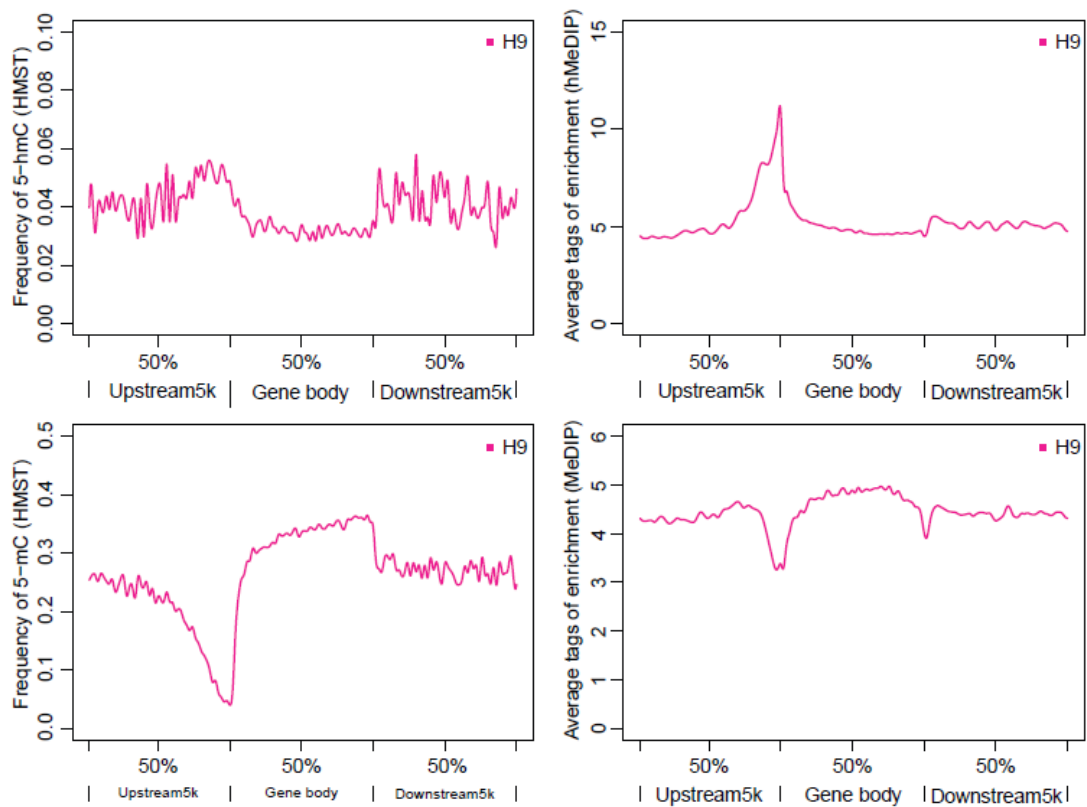
Figure S1



**Figure S1. Real-time QPCR analysis of *MspI* and *HpaII* sensitivity to glucosylation of 5-hmC.**

Double stranded oligonucleotides containing either a single 5-hmC, 5-mC or 5-C residue within the *MspI* recognition site on both strands are either first glucosylated and then digested by *MspI* (BGT-*MspI*) or directly digested by *MspI*. Two types of controls were set, in which no oligonucleotides are included in negative controls (Negative) while no *MspI* enzyme is included in "Input" controls. All data with values of mean  $\pm$ s.d. from three independent experiments are normalized to "Input" controls.

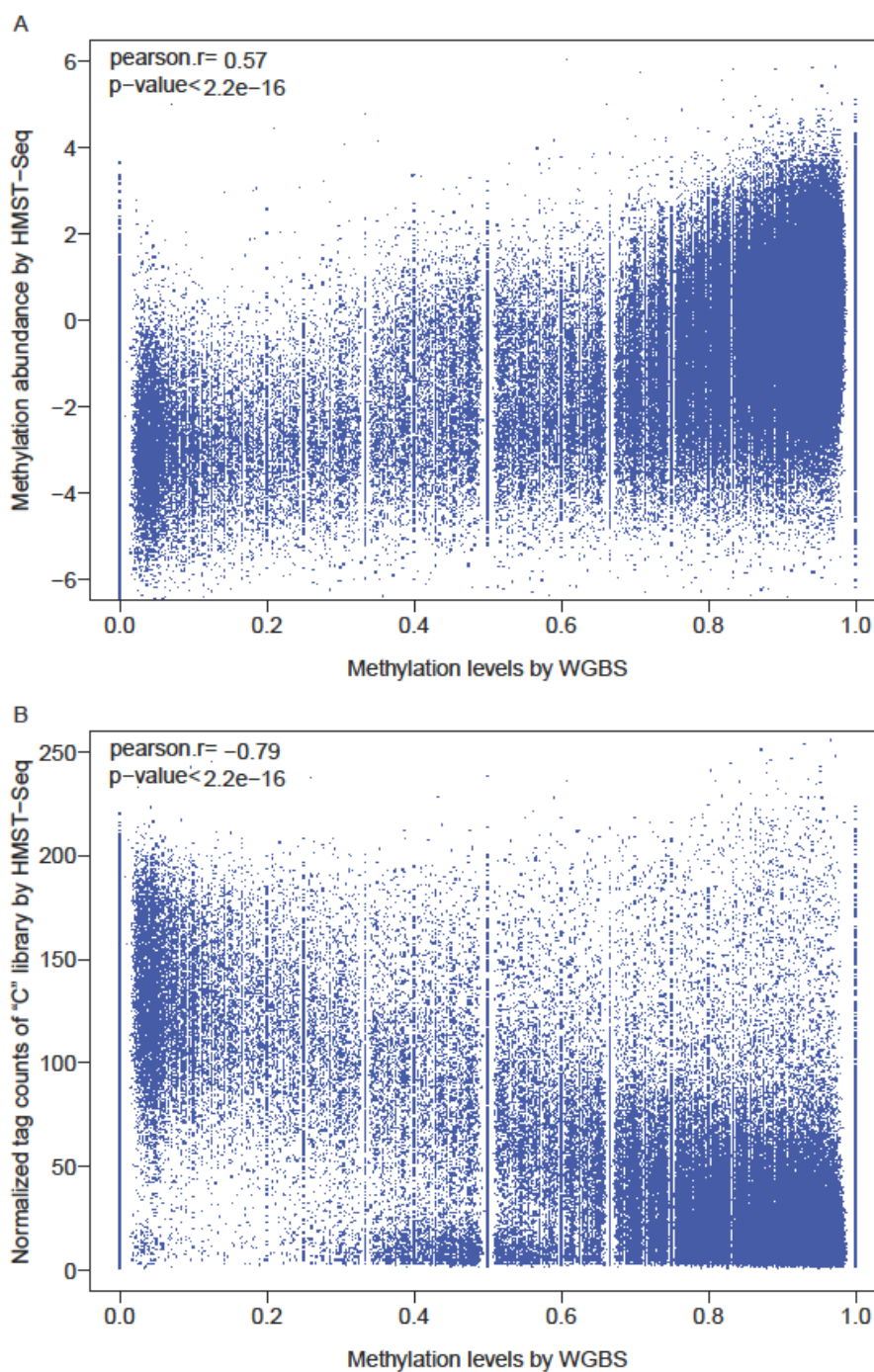
**Figure S2**



**Figure S2. Distribution of DNA hydroxymethylation or methylation in genic regions generated by HMST-seq or (h)MeDIP in H9 hESCs.**

Frequency of 5-hmC (top left) or 5-mC (bottom left), and average tags enriched by hMeDIP (top right) or MeDIP (bottom right) in genic regions are presented, where 5kb upstream region from transcription start site (TSS), gene body and 5kb downstream region from transcription end site (TES) were equally divided into 50 windows separately.

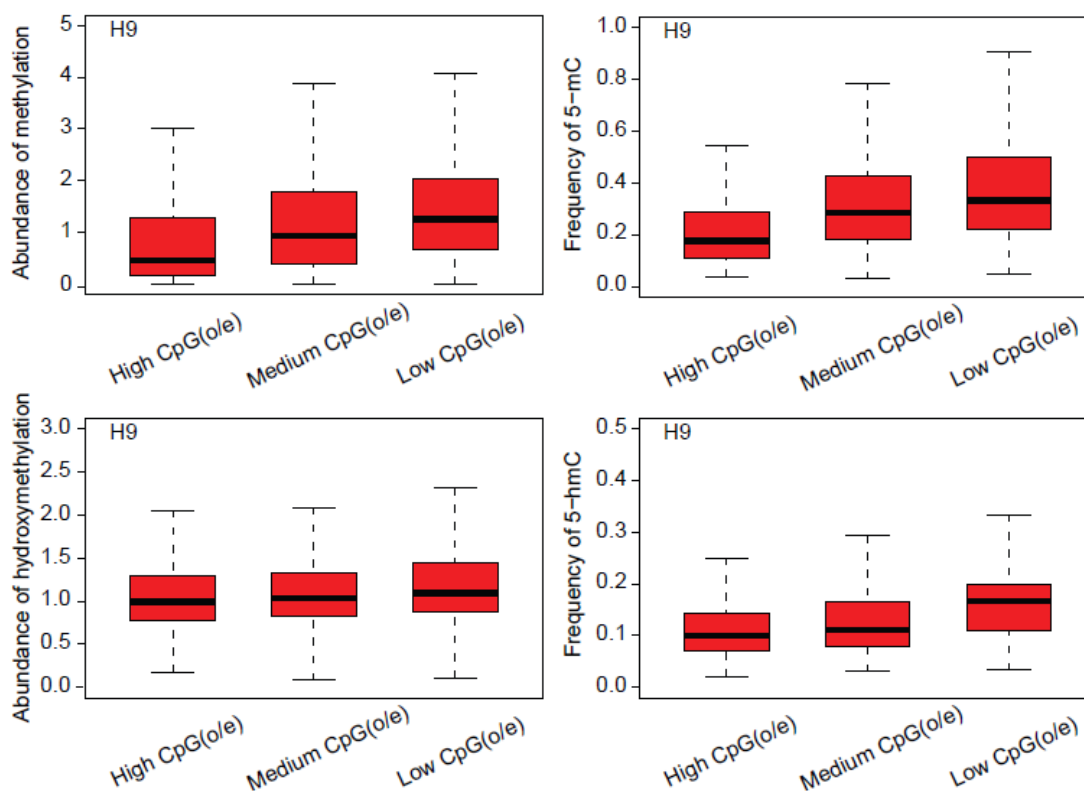
**Figure S3**



**Figure S3. Pair-wise comparison of methylation levels detected by WGBS and methylation levels deduced by HMST-Seq.**

(A). Scatter analysis for methylation levels detected by WGBS and methylation abundance deduced by HMST-Seq. The methylation abundance was determined as the ratio between tag counts of "C+mC" and "C" libraries. (B). Scatter analysis for methylation levels detected by WGBS and normalized tag counts of "C" library by HMST-Seq. Pearson correlation coefficients and *P*-values are provided.

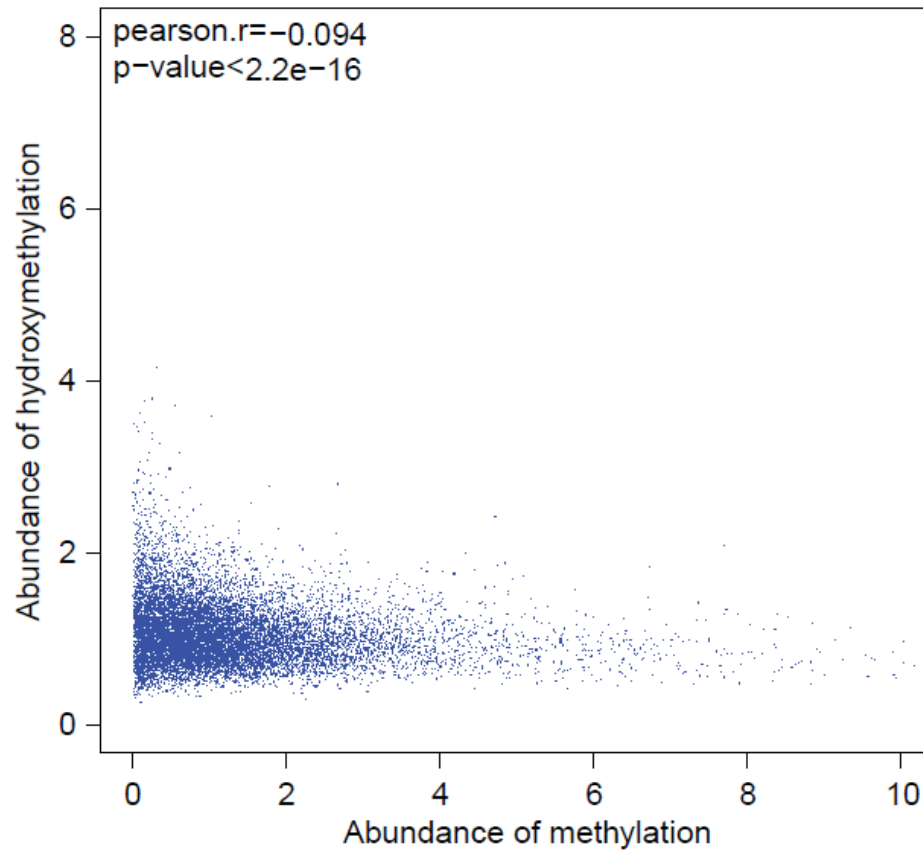
**Figure S4**



**Figure S4. Boxplots of methylation or hydroxymethylation abundances characterized by CpG density in H9 hESCs.**

Boxplots of methylation or hydroxymethylation abundances of 10kb windows across the genome that contain at least one 5-mC site (top) or 5hmC site (bottom) are showed. These windows are classified as high (Observed/expected ratio $\leq$  0.3), medium (Observed/expected ratio $>$  0.3 and Observed/expected ratio $\leq$  0.5) and low (Observed/expected ratio $>$  0.5) CpG density.

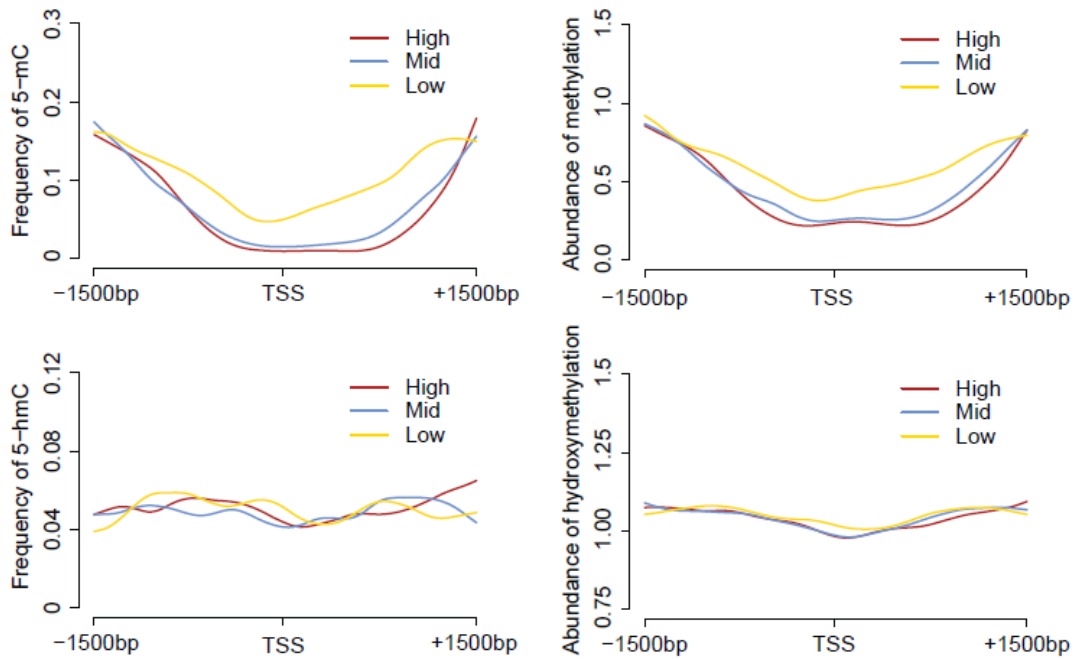
**Figure S5**



**Figure S5. Correlation analysis of abundances of methylation and hydroxymethylation at CCGG sites.**

Scatter analysis for the abundances of methylation and hydroxymethylation at CCGG sites in H9 ESCs is performed, in which Pearson correlation coefficients and *P*-value are provided.

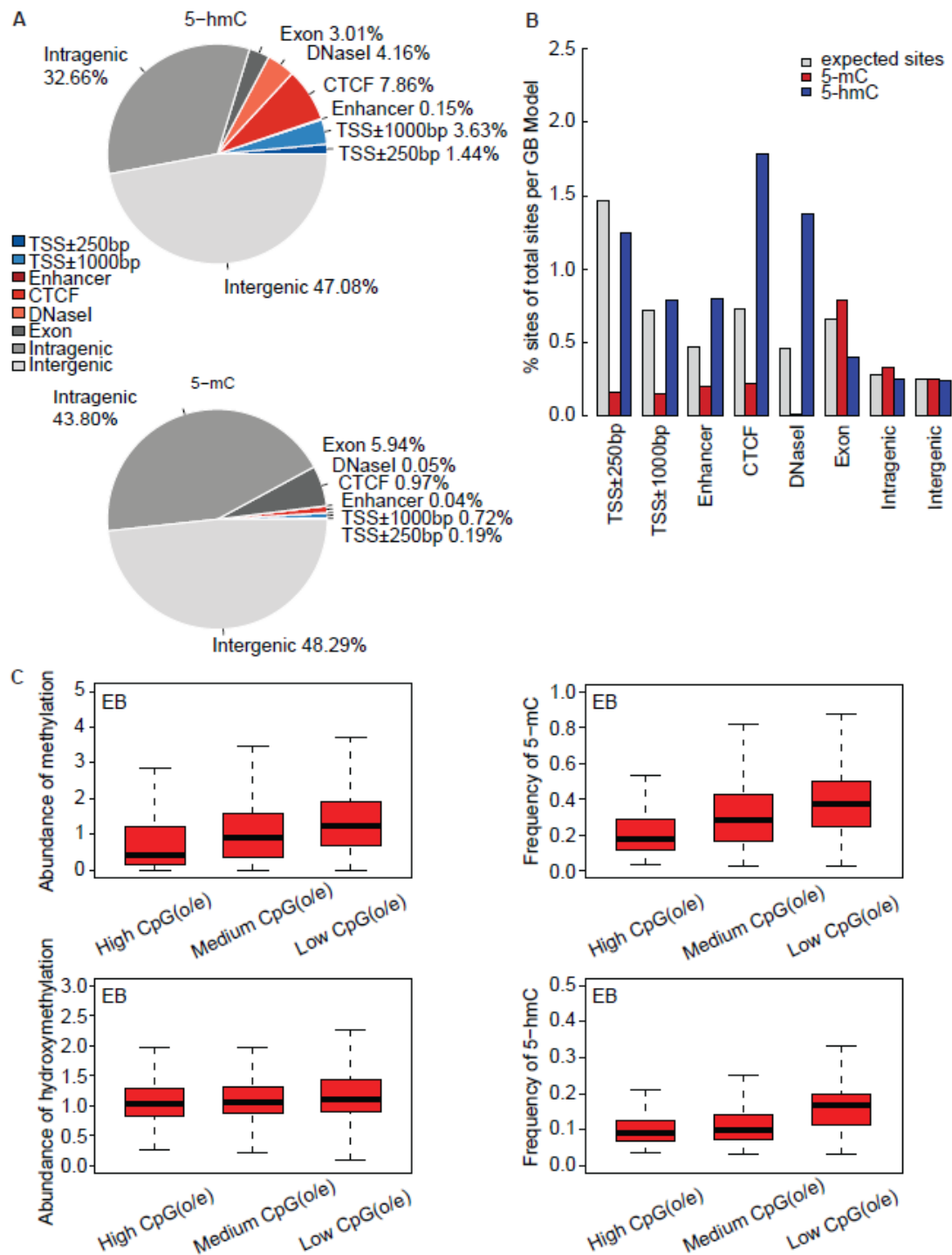
**Figure S6**



**Figure S6. Association between DNA methylation or hydroxymethylation and gene expression in H9 cells.**

Frequency of 5-mC or 5-hmC around TSS of lowly (yellow,  $TPM \leq 0.01$ ), moderately (blue,  $0.01 < TPM \leq 2.70$ ) or highly (red,  $TPM > 2.70$ ) expressed genes, which are equally classified by their expression levels. The  $\pm 1500$ bp around transcription start sites are equally divided into 100 windows.

**Figure S7**



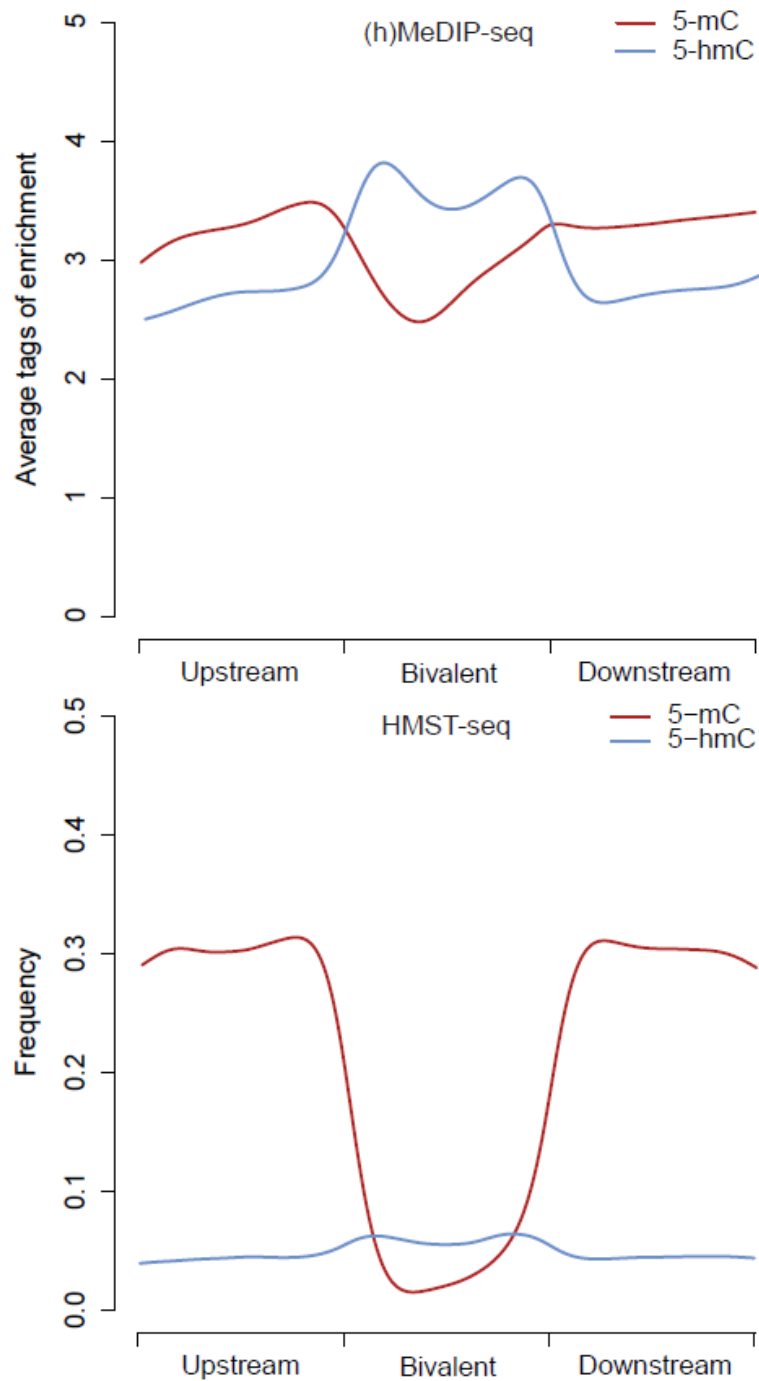
**Figure S7. Genomic distribution of 5-mC and 5hmC sites in EBs.**

(A). Overlap of 5-hmC and 5-mC with genomic regions. Genic features were extracted from the UCSC hg19 database. Regulatory elements (CTCF and DNase I) were download from previously published data generated by CHIP-Seq and DNase-Seq experiments and enhancer were download from VISTA Enhancer Browser database.



Each 5-hmC or 5-mC site is counted once: the cytosines located in previous regions are excluded counterclockwise from TSS $\pm$ 250bp. Blue: promoter-proximal elements; Red: distal-regulatory elements; Gray: genic regions and intergenic regions. (B). Relative enrichment of modified sites within several genomic elements. The observed percentage of 5-mC (red) or 5-hmC (blue) counts within these genomic elements out of all modified CCGG sites in human genome and the expected percentage of CCGG counts (gray) within these genomic elements out of all CCGG counts in human genome are presented. All values of counts were normalized to the length of each region (per Gb). (C). Boxplots of methylation or hydroxymethylation abundances of 10kb windows across the genome that contain at least one 5-mC site (top) or 5hmC site (bottom) are showed. These windows are classified as high (Observed/expected ratio $\leq$  0.3), medium (Observed/expected ratio $>$  0.3 and Observed/expected ratio $\leq$  0.5) and low (Observed/expected ratio $>$  0.5) CpG density.

**Figure S8**



**Figure S8. Distribution of methylation and hydroxymethylation around genomic regions with bivalent marks in H9 ESCs.**

Distribution of methylation (red) and hydroxymethylation (blue) generated by HMST-seq (bottom) or (h)MeDIP-seq (top) around bivalent regions that contain both H3K4me3 and H3K27me3 marks. The bivalent regions and their upstream and downstream regions are equally divided into 50 windows separately.

Supplementary Table S1. Control DNA sequence and associated primers information of Q-PCR assay for efficacy of glucosyla

control DNA 5'-CAGTGAAGTTGGCAGACTGAGCCAGGTCCCACAGATGCAGTGA**CCGG**AGTCATTGCCAAACTCTGCA  
GGAGAGCAAGGGCTGTCTATAGGTGGCAAGTCA-3'  
3'-GTCACTTCAACCGTCTGACTCGGTCCAGGGTGTCTACGTCACT**GGCCT**CAGTAACGGTTTGAGACGT  
CCTCTCGTTCCCGACAGATATCCACCGTTCAGT-5'  
FW primer 5'- CAGTGAAGTTGGCAGACTGAGC -3'  
REV primer 5'- CTGACTTGCCACCTATAGACAGC -3'

Supplementary Table S2. Copy numbers of control DNA with different modification after MspI digestion detected by Q-PCR assay.

	5C (Mean±SD)	5mC (Mean±SD)	5hmC (Mean±SD)
Negative	6.58E-05±9.35E-05	7.09E-05±1.02E-04	2.45E-05±3.50E-05
MspI	4.64E-02±8.93E-03	1.38E-01±5.27E-02	6.29E-02±1.78E-02
BGT-MspI	3.20E-02±5.49E-04	9.57E-02±1.85E-02	9.43E-01±1.09E-01
Input	1	1	1

Supplementary Table S3. Data summary and repeatability of replicates

Replicate	Sample	Library	Total reads	Clean reads	Clean rate	Accepted reads	Accepted rate	Average informative tags	Informative sites	Average depth	Replicates correlation (R <sup>2</sup> )	Replicates correlation (p-value)
1	H9	"C"	66,500,038	61,346,150	92.25%	40,235,240	65.59%	36,750,132	1,119,146	32.84	0.92	<2.2e-16
2	H9	"C"	81,725,204	76,757,324	93.92%	41,515,419	54.09%					
1	H9	"C+mC"	70,109,727	64,723,835	92.32%	35,280,109	54.51%	32,832,035	1,378,153	23.82	0.81	<2.2e-16
2	H9	"C+mC"	92,136,726	86,747,336	94.15%	43,316,899	49.93%					
1	H9	"C+mC+hmC"	67,791,420	62,338,830	91.96%	34,610,284	55.52%	29,712,569	1,368,519	21.71	0.71	<2.2e-16
2	H9	"C+mC+hmC"	91,776,060	86,459,211	94.21%	36,341,900	42.03%					
1	EB	"C"	66,030,587	61,327,289	92.88%	34,641,227	56.49%	34,355,941	1,157,123	29.69	0.93	<2.2e-16
2	EB	"C"	81,669,796	76,886,385	94.14%	42,068,546	54.72%					
1	EB	"C+mC"	91,215,821	87,130,009	95.52%	52,026,070	59.71%	42,098,840	1,376,473	30.58	0.96	<2.2e-16
2	EB	"C+mC"	92,995,981	87,646,708	94.25%	49,187,305	56.12%					
1	EB	"C+mC+hmC"	84,358,222	78,275,663	92.79%	47,450,979	60.62%	35,653,132	1,361,478	26.19	0.90	<2.2e-16
2	EB	"C+mC+hmC"	94,716,146	88,968,269	93.93%	38,299,920	43.05%					

Supplementary Table S4. UPLC–MS/MS analysis of global DNA methylation and hydroxymethylation

Sample	Calculated Concentration of mdC	Calculated Concentration of hmdC	Calculated Concentration of dG	Percentage of 5-mC (mdC/dG)	Adjusted percentage of 5-mCG* (mCG/CG)	Percentage of 5-mC by HMST-Seq	Percentage of 5-hmCG (hmdC/dG)	Adjusted percentage of 5-hmCG* (hmCG/CG)	Percentage of 5-hmC by HMST-Seq
EB	1.51E-07	4.77E-09	4.92E-06	3.07%	63.45%	31.37%	0.10%	2.00%	1.95%
H9	1.07E-07	5.67E-09	3.87E-06	2.77%	57.25%	28.47%	0.15%	3.03%	3.28%

\* As methylation and hydroxymethylation mainly occur at CG sites, correcting computed percentage of 5-mCG or 5-hmCG can be defined as: (mdC/dG)\*(number of G sites)/(number of CG sites) or (hmdC/dG)\*(number of G sites)/(number of CG sites)

Number of CG: 57400172

Number of G: 1186291952

Supplementary Table S5. Digital gene expression levels of DNMTs and TETs in H9 hESCs and EBs

Gene	H9 (TPM)	EB (TPM)
DNMT1	62.89	53.13
DNMT3A	81.73	89.66
DNMT3B	613.08	528.14
DNMT3L	0.85	0.86
TET1	56.41	58.67
TET2	9.81	9.10
TET3	12.64	14.14

Supplementary Table S6. Gene expression levels of DMRs- or DhMRs-associated genes

chr	DMR start	DMR end	# of CCGG	H9 abundance	EB abundance	p-value	Symbol	H9 TPM	EB TPM	Hyper-expressed sample	Bivalent gene
chr1	15,736,141	15,736,551	5	0.32	0.10	3.17E-02	EFHD2	26.40	31.67	NA	bivalent
chr1	36,022,701	36,023,567	6	0.41	0.17	4.33E-03	NCDN	22.22	20.05	NA	bivalent
chr1	36,022,701	36,023,567	6	0.41	0.17	4.33E-03	KIAA0319L	75.25	60.64	NA	bivalent
chr1	38,511,999	38,512,488	6	0.43	0.15	2.16E-03	POU3F1	6.60	8.3	NA	bivalent
chr1	40,781,764	40,783,132	6	0.47	0.16	4.11E-02	COL9A2	7.61	13.84	NA	bivalent
chr1	154,971,941	154,973,595	6	0.42	0.14	1.52E-02	ZBTB7B	16.19	22.26	NA	bivalent
chr1	182,991,805	182,993,133	5	0.47	0.20	1.59E-02	LAMC1	41.74	53.69	NA	NA
chr10	81,366,471	81,368,967	5	0.75	1.12	1.59E-02	SFTPA1	NA	NA	NA	NA
chr10	102,891,280	102,891,552	5	0.38	0.13	1.59E-02	TLX1	0.01	0.12	NA	NA
chr10	102,891,280	102,891,552	5	0.38	0.13	1.59E-02	TLX1NB	NA	NA	NA	NA
chr10	104,676,924	104,679,412	6	0.39	0.17	2.60E-02	CNNM2	3.22	3.51	NA	bivalent
chr10	123,357,621	123,358,240	5	0.34	0.13	3.17E-02	FGFR2	58.49	57.5	NA	bivalent
chr11	35,965,004	35,965,712	5	0.33	0.12	3.17E-02	LDLRAD3	14.16	24.48	NA	bivalent
chr12	121,479,798	121,482,801	5	0.73	1.08	3.17E-02	OASL	NA	NA	NA	NA
chr12	122,150,344	122,151,517	5	0.45	0.21	3.17E-02	TMEM120B	12.47	15.87	NA	bivalent
chr12	133,194,982	133,196,658	5	0.38	0.14	3.17E-02	P2RX2	3.27	3.14	NA	NA
chr13	41,238,886	41,241,600	6	0.34	0.09	1.52E-02	FOXO1	35.71	25.09	NA	NA
chr14	94,392,707	94,393,816	5	1.50	0.72	3.17E-02	C14orf86	NA	NA	NA	NA
chr14	103,563,704	103,567,024	9	0.80	0.36	5.64E-03	C14orf73	NA	NA	NA	NA
chr15	65,066,703	65,070,250	7	0.36	0.12	1.11E-02	RBPMS2	106.33	140.46	NA	bivalent
chr15	90,358,931	90,363,985	5	0.62	1.22	3.17E-02	ANPEP	0.56	0.49	NA	bivalent
chr15	91,471,413	91,474,066	6	2.45	1.39	4.11E-02	UNC45A	40.89	41.14	NA	bivalent
chr16	572,491	574,652	5	2.23	1.02	3.17E-02	SOLH	31.36	29.09	NA	bivalent
chr16	734,710	736,058	5	3.82	1.15	3.17E-02	JMJD8	25.55	23.55	NA	bivalent
chr16	1,146,161	1,147,799	7	1.80	0.81	2.62E-02	C1QTNF8	0.11	0.01	NA	NA
chr16	3,624,392	3,630,429	9	2.21	0.65	1.06E-02	NLRC3	0.34	0.74	NA	NA
chr16	4,737,300	4,740,171	8	5.50	1.63	6.99E-03	NUDT16L1	9.36	13.47	NA	bivalent
chr16	8,966,801	8,968,385	5	0.54	0.91	1.59E-02	CARHSP1	119.30	127.85	NA	bivalent
chr16	33,964,251	33,965,655	6	3.22	1.66	4.11E-02	MIR1826	NA	NA	NA	NA
chr16	46,781,587	46,785,471	5	2.34	1.14	3.17E-02	MYLK3	2.09	3.57	NA	NA
chr16	69,140,090	69,141,044	5	0.39	0.14	3.17E-02	HAS3	16.41	16.05	NA	bivalent
chr16	88,720,466	88,722,655	6	2.98	1.15	8.66E-03	CYBA	88.78	116.04	NA	bivalent
chr16	88,729,181	88,730,291	6	0.33	0.13	4.11E-02	MGC23284	1.52	1.41	NA	bivalent
chr16	88,729,181	88,730,291	6	0.33	0.13	4.11E-02	MVD	53.87	27.86	NA	bivalent
chr16	88,935,300	88,938,017	5	3.06	1.06	7.94E-03	PABPN1L	0.01	0.49	NA	NA
chr17	681,524	683,907	5	1.09	1.72	3.17E-02	RNMTL1	12.13	11.44	NA	bivalent
chr17	1,173,263	1,175,048	6	0.59	0.24	1.52E-02	BHLHA9	0.01	0.12	NA	bivalent
chr17	1,953,942	1,957,387	11	0.42	0.18	1.51E-02	MIR132	NA	NA	NA	bivalent
chr17	1,953,942	1,957,387	11	0.42	0.18	1.51E-02	MIR212	NA	NA	NA	NA
chr17	1,953,942	1,957,387	11	0.42	0.18	1.51E-02	HIC1	0.23	0.31	NA	NA
chr17	4,642,789	4,644,276	5	0.40	0.16	3.17E-02	ZMYND15	0.45	0.43	NA	NA
chr17	4,642,789	4,644,276	5	0.40	0.16	3.17E-02	CXCL16	0.96	1.17	NA	bivalent
chr17	6,538,465	6,540,792	5	2.37	1.07	1.59E-02	TXNDC17	211.98	131.91	NA	bivalent
chr17	7,321,160	7,322,532	5	0.55	0.28	1.59E-02	SPEM1	NA	NA	NA	NA
chr17	61,779,439	61,781,803	5	1.78	0.85	1.59E-02	LIMD2	40.56	31.24	NA	bivalent
chr17	76,185,107	76,189,400	5	5.37	2.01	3.17E-02	TK1	33.67	35.91	NA	bivalent
chr17	79,632,662	79,636,336	7	0.82	0.43	2.62E-02	C17orf90	2.09	4.8	EB	bivalent
chr19	460,220	462,557	6	0.35	0.13	4.33E-03	SHC2	31.14	52.46	NA	NA
chr19	1,445,789	1,446,505	5	0.49	0.13	3.17E-02	APC2	0.01	0.18	NA	bivalent



chr19	1,752,858	1,754,892	9	0.46	0.16	7.77E-03	ONECUT3	NA	NA	NA	NA
chr19	1,819,539	1,822,000	5	4.56	1.39	7.94E-03	MIR1909	NA	NA	NA	NA
chr19	2,426,943	2,427,940	6	0.36	0.12	4.11E-02	TIMM13	110.33	123.12	NA	bivalent
chr19	8,808,334	8,809,121	5	1.29	0.68	7.94E-03	ACTL9	NA	NA	NA	NA
chr19	9,200,143	9,203,433	5	0.66	1.21	3.17E-02	OR1M1	NA	NA	NA	NA
chr19	11,311,103	11,313,267	5	1.52	0.90	7.94E-03	KANK2	16.41	15.99	NA	bivalent
chr19	12,899,651	12,901,132	5	0.41	0.12	3.17E-02	JUNB	7.28	9.84	NA	bivalent
chr19	50,920,365	50,921,802	7	1.09	0.37	4.08E-03	SPIB	3.84	3.81	NA	bivalent
chr19	51,300,818	51,303,717	6	4.41	1.61	1.52E-02	SNORD88B	NA	NA	NA	bivalent
chr19	56,137,245	56,141,036	5	0.97	1.73	3.17E-02	ZNF784	3.50	3.07	NA	bivalent
chr2	130,691,033	130,692,012	5	0.36	0.15	3.17E-02	LOC389033	0.17	0.61	NA	bivalent
chr2	198,314,641	198,316,307	5	0.42	0.82	3.17E-02	COQ10B	1.35	2.77	EB	bivalent
chr2	241,505,326	241,506,132	5	0.38	0.17	1.59E-02	RNPEPL1	5.81	6.76	NA	NA
chr2	241,563,008	241,564,722	5	1.06	0.56	3.17E-02	GPR35	0.68	0.49	NA	NA
chr2	242,089,281	242,090,099	5	0.33	0.10	1.59E-02	PPP1R7	26.85	45.45	NA	bivalent
chr2	242,089,281	242,090,099	5	0.33	0.10	1.59E-02	PASK	30.18	21.65	NA	bivalent
chr2	242,500,579	242,502,855	5	2.66	1.37	3.17E-02	BOKAS	NA	NA	NA	NA
chr20	20,347,751	20,350,563	5	0.41	0.13	3.17E-02	INSM1	0.28	1.66	EB	bivalent
chr20	21,377,635	21,378,549	6	0.46	0.15	2.16E-03	NKX2-4	NA	NA	NA	NA
chr20	48,770,151	48,770,857	5	0.35	0.15	1.59E-02	EM189-UBE2	NA	NA	NA	bivalent
chr20	48,770,151	48,770,857	5	0.35	0.15	1.59E-02	TMEM189	30.52	37.57	NA	NA
chr20	61,150,952	61,152,381	5	2.34	1.00	7.94E-03	MIR1-1	NA	NA	NA	bivalent
chr20	61,150,952	61,152,381	5	2.34	1.00	7.94E-03	C20orf200	3.61	5.23	NA	bivalent
chr20	61,159,408	61,162,340	7	2.07	0.85	2.33E-03	MIR133A2	NA	NA	NA	NA
chr20	62,663,524	62,665,155	5	2.99	1.93	1.59E-02	NCRNA00176	0.23	0.49	NA	NA
chr22	19,134,809	19,138,426	5	0.45	0.24	1.59E-02	DGCR14	7.95	9.72	NA	bivalent
chr22	25,421,713	25,424,299	5	0.70	0.39	3.17E-02	KIAA1671	35.42	33.88	NA	NA
chr22	39,155,631	39,156,825	5	1.70	0.96	3.17E-02	SUN2	4.91	5.47	NA	bivalent
chr22	46,398,043	46,402,130	6	2.13	1.22	4.11E-02	LOC730668	1.58	1.35	NA	NA
chr22	50,054,187	50,057,517	5	2.78	1.11	3.17E-02	C22orf34	0.01	0.18	NA	NA
chr22	50,350,856	50,353,222	5	1.43	0.69	1.59E-02	PIM3	18.90	22.88	NA	NA
chr22	50,528,388	50,529,266	6	2.11	0.86	2.60E-02	MOV10L1	NA	NA	NA	NA
chr22	50,528,388	50,529,266	6	2.11	0.86	2.60E-02	MLC1	NA	NA	NA	NA
chr22	50,623,336	50,624,295	5	0.74	0.24	3.17E-02	TRABD	23.30	16.11	NA	bivalent
chr3	52,090,259	52,092,251	5	0.43	0.10	3.17E-02	DUSP7	4.96	8.18	NA	bivalent
chr3	52,524,840	52,526,356	5	3.84	1.50	3.17E-02	STAB1	37.91	25.15	NA	NA
chr3	127,308,178	127,310,922	5	0.47	0.22	3.17E-02	TPRA1	21.77	16.79	NA	bivalent
chr3	127,390,433	127,392,355	6	0.52	0.22	4.11E-02	ABTB1	5.53	5.9	NA	bivalent
chr3	128,223,721	128,226,395	5	2.27	0.94	7.94E-03	LOC90246	0.01	0.37	NA	NA
chr3	160,170,249	160,171,014	5	2.91	1.23	3.17E-02	TRIM59	52.12	54.18	NA	bivalent
chr3	197,807,144	197,808,733	6	0.41	0.19	8.66E-03	LOC348840	2.03	2.34	NA	bivalent
chr4	5,991,307	5,996,369	8	2.24	1.02	6.99E-03	C4orf50	NA	NA	NA	NA
chr4	8,194,514	8,197,813	6	0.66	0.37	1.52E-02	SH3TC1	0.11	0.49	NA	NA
chr6	160,768,459	160,769,651	5	0.45	0.17	3.17E-02	SLC22A3	2.37	1.6	NA	bivalent
chr7	1,121,712	1,123,937	6	2.47	1.42	4.11E-02	GPER	0.01	0.18	NA	bivalent
chr7	1,598,998	1,599,869	6	1.59	0.70	1.52E-02	TMEM184A	4.51	5.04	NA	NA
chr7	5,111,792	5,112,381	5	0.40	0.16	3.17E-02	LOC389458	NA	NA	NA	NA
chr7	23,053,043	23,053,937	5	0.40	0.18	3.17E-02	FAM126A	21.49	26.87	NA	bivalent
chr7	44,184,941	44,186,290	5	0.48	0.11	3.17E-02	MYL7	11.68	7.5	NA	bivalent
chr7	100,491,753	100,495,072	6	0.40	0.15	1.52E-02	ACHE	5.36	8.55	NA	bivalent
chr7	129,410,506	129,412,947	5	0.79	0.34	1.59E-02	MIR182	NA	NA	NA	bivalent

chr8	145,753,758	145,754,635	5	0.39	0.13	7.94E-03	LRRC24	4.63	4.86	NA	bivalent
chr8	145,753,758	145,754,635	5	0.39	0.13	7.94E-03	MGC70857	NA	NA	NA	bivalent
chr9	4,792,422	4,793,992	5	0.43	0.18	1.59E-02	RCL1	26.12	26.51	NA	bivalent
chr9	82,185,134	82,188,283	5	0.46	0.17	3.17E-02	TLE4	18.61	22.08	NA	bivalent
chr9	101,983,963	101,984,769	5	0.38	0.18	7.94E-03	SEC61B	145.92	145.5	NA	bivalent
chr9	101,983,963	101,984,769	5	0.38	0.18	7.94E-03	ALG2	24.14	36.84	NA	bivalent
chr9	127,112,547	127,115,312	5	2.36	1.16	3.17E-02	OC10012903	12.47	10.45	NA	NA
chr9	136,448,864	136,450,776	5	0.92	0.51	3.17E-02	FAM163B	0.01	0.25	NA	NA
chr9	139,637,827	139,640,409	6	3.31	0.98	8.66E-03	LCN10	0.62	0.37	NA	NA
chr9	139,837,245	139,841,195	8	2.49	0.89	3.79E-02	C8G	0.11	0.43	NA	NA
chr9	139,837,245	139,841,195	8	2.49	0.89	3.79E-02	FBXW5	34.58	26.94	NA	NA
chr9	140,116,040	140,118,140	5	0.44	0.18	3.17E-02	C9orf169	1.24	1.66	NA	NA
chr9	140,116,040	140,118,140	5	0.44	0.18	3.17E-02	RNF208	6.26	7.69	NA	NA
chr9	140,116,040	140,118,140	5	0.44	0.18	3.17E-02	LOC643596	0.45	0.55	NA	NA
chr9	140,167,077	140,168,481	5	3.70	1.75	3.17E-02	C9orf167	4.46	4.24	NA	bivalent
chr9	140,196,040	140,196,866	5	0.37	0.15	1.59E-02	NRARP	20.08	29.76	NA	NA

Supplementary Table S7. The top four Gene Ontology enriched terms for DhMR-associated genes.

Category	Term	Count	%	Fold Enrichment	p-value	Gene symbols
GO MF	GO:0030528~	82	10.83	1.53	8.23E-05	TAF1C, HMX2, NR6A1, CNOT3, PAX3, GATA1, BRPF1, HOXC9, PAX7, MED26, SND1, NR2F6, SPIB, OLIG2, USP16, CIITA, EGR3, SNAPC4, MTA1, ARNTL, HMGA1, JMY, ZNF500, ZNF236, PFDN6, HES4, BHLHA9, MED8, HIPK2, DPRX, MED18, SIX1, CAND2, VGLL3, THOC1, CNBP, ONECUT1, GLIS2, TRRAP, CALR, SOX8, ZNF169, T, ZNF323, HOXA3, HOXA4, HAND1, C11ORF9, LHX1, TSC22D4, POU2F3, HOXA10, POU3F3, MLXIP, FOXD2, NFATC3, ETV5, SIM1, ZBTB48, FOXL2, UTF1, CEBPE, TAF8, TP53BP1, SAP18, LMX1A, NEUROG3, FOXP3, STAT3, USF2, CDKN1C, MED31, HOXB3, HOXB4, HOXB8, PHB2, EBF2, MLX, FOXE1, TCEB1, KDM4A, KLF4
GO MF	GO:0043565~	40	5.28	1.86	2.39E-04	HMX2, ONECUT1, NR6A1, PAX3, TERF2IP, GATA1, T, HOXC9, HOXA3, HOXA4, HAND1, LHX1, POU2F3, PAX7, HOXA10, NR2F6, POU3F3, SPIB, OLIG2, FOXD2, ETV5, TERT, CIITA, FOXL2, CEBPE, TP53BP1, MTA1, MCM2, LMX1A, FOXP3, HMGA1, STAT3, USF2, HOXB3, HOXB4, HOXB8, SIX1, DPRX, FOXE1, KLF4 HMX2, CNOT3, CBX8, RCBTB1, GATA1, BRPF1, HOXC9, MED26, SND1, OLIG2, CRY1, FIZ1, CIITA, RBL1, ARNTL, ZNF500, ZNF236, HES4, BHLHA9, MED18, DPRX, RDBP, VGLL3, CNBP, ONECUT1, GPBP1L1, SETD1B, TRRAP, ZNF514, DIDO1, SOX8, T, ZNF323, HOXA3, HOXA4, LHX1, HOXA10, ZBTB48, UTF1, ZNF625, TP53BP1, TAF8, SAP18, NEUROG3, FOXP3, ZBTB44, USF2, HOXB3, NOTCH3, HOXB4, HOXB8, MLX, EBF2, KLF4, ZNF410, TAF1C, ZFP41, NR6A1, PAX3, ZNF205, ZNF678, ANG, PAX7, RTF1, NR2F6, SPIB, USP16, EGR3, KHDRBS3, SNAPC4, CDK9, MCM2, HMGA1, CCNL2, MED8, HIPK2, CAND2, ZNF467, GLIS2, ZNF169, HAND1, TSC22D4, POU2F3, MLXIP, FOXD2, NFATC3, SIM1, FOXL2, CEBPE, ZNF667, LMX1A, STAT3, ZNF664, MED31, PHB2, FOXE1, KDM4C, TCEB1, KDM4A, RBM16
GO BP	GO:0006350~	100	13.21	1.36	8.86E-04	HMX2, GDF6, GMCL1L, PASK, CNOT3, CBX8, RCBTB1, GATA1, BRPF1, HOXC9, MED26, SND1, OLIG2, CRY1, FIZ1, CIITA, RBL1, MTA1, ARNTL, ZNF500, PTHLH, ZNF236, HES4, BHLHA9, DPRX, SIX1, MED18, RDBP, VGLL3, CNBP, ONECUT1, GPBP1L1, SETD1B, TRRAP, ZNF514, CALR, SOX8, T, IRAK3, ZNF323, HOXA3, HOXA4, LHX1, HOXA10, ZBTB48, UTF1, ZNF625, TAF8, TP53BP1, RFX7, SAP18, NEUROG3, FOXP3, ZBTB44, SFMBT1, USF2, HOXB3, NOTCH3, CDKN1C, HOXB4, CDKN1B, HOXB8, MLX, EBF2, KLF4, ZNF410, ZFP41, NR6A1, PAX3, TERF2IP, ZNF205, ZNF678, PAX7, RTF1, NR2F6, RHOA, SPIB, USP16, EGR3, KHDRBS3, SNAPC4, TLE6, CDK9, MCM2, HMGA1, SIGIRR, JMY, CCNL2, MED8, HIPK2, CAND2, ZNF467, GLIS2, TNFRSF4, ZNF169, HAND1, C11ORF9, TSC22D4, POU2F3, POU3F3, MLXIP, FOXD2, NFATC3, ETV5, SIM1, FOXL2, CEBPE, ZNF667, LMX1A, STAT3, ZNF664, MED31, RPS6KA4, PHB2, FOXE1, KDM4C, TCEB1, KDM4A, RBM15
GO BP	GO:0045449~	119	15.72	1.31	1.10E-03	ALOX15, TBXAS1, PTGIS, PTGDS, PDPN, RNPEPL1, PLA2G4C, DEGS1
GO BP	GO:0033559~	8	1.06	4.48	1.87E-03	HMX2, FOXL2, USH1G, TH, HOXB3, HOXB4, SPRY2, HOXA3, HOXC9, HOXA4, HOXB8, SIX1, FOXE1
GO BP	GO:0048562~	13	1.72	2.79	2.42E-03	CDKN1C, CCND1, CASP3, CDKN1B, CABLES2
GO MF	GO:0016538~	5	0.66	8.28	2.56E-03	RET, FOXL2, HMX2, NF2, USH1G, TH, PAX3, GREM1, HOXB3, T, HOXB4, SPRY2, HOXA3, HOXC9, HOXA4, HAND1, LHX1, HOXB8, SIX1, FOXE1, HOXA10, KLF4
GO BP	GO:0048598~	22	2.91	2.05	2.65E-03	HMX2, FOXL2, USH1G, TH, HOXB3, T, HOXB4, SPRY2, HOXA3, HOXC9, HAND1, HOXA4, HOXB8, SIX1, FOXE1
GO BP	GO:0048568~	15	1.98	2.49	2.88E-03	NDUFS6, CASP3, HTRA2, GPR37, SLC6A3, NDUFA6, SDHC, TH, NDUFA7, SLC18A2, UBE2J2, NDUFA1
KEGG pathway	hsa05012:Par	12	1.59	2.68	4.70E-03	HMX2, NR6A1, PAX3, GATA1, HOXC9, PAX7, NR2F6, SPIB, OLIG2, EGR3, SNAPC4, MTA1, ARNTL, HMGA1, ZNF500, ZNF236, SIX1, DPRX, CNBP, ONECUT1, SOX8, ZNF169, T, ZNF323, HOXA3, HOXA4, C11ORF9, HAND1, TSC22D4, LHX1, POU2F3, HOXA10, POU3F3, FOXD2, ETV5, NFATC3, SIM1, ZBTB48, FOXL2, CEBPE, NEUROG3, LMX1A, FOXP3, USF2, STAT3, HOXB3, HOXB4, HOXB8, MLX, FOXE1, KLF4
GO MF	GO:0003700~	51	6.74	1.47	5.09E-03	ALOX15, TBXAS1, PTGIS, PTGDS, PDPN, RNPEPL1, PLA2G4C
GO BP	GO:0006690~	7	0.92	4.25	5.64E-03	PTHLH, HOXB3, HOXB4, BMP1, HOXC9, HOXA3, HOXA4, HOXB8, SIX1, ANKRD11, IDUA
GO BP	GO:0048705~	11	1.45	2.80	5.83E-03	ALOX15, TBXAS1, PTGIS, PTGDS, RNPEPL1, DEGS1
GO BP	GO:0006636~	6	0.79	5.04	6.16E-03	GNAT1, NF2, OPR1, ANAPC1, PXX, TNNI3, FOXP3, TNFRSF4, SIGIRR, CDKN1C, PTHLH, IRAK3, SPRY2, CASP3, CYP27B1, CDKN1B, PSMB6, DUSP16, PSMC1, ZFYVE28, DUSP9, OPRD1
GO BP	GO:0044092~	22	2.91	1.88	6.99E-03	HOXB3, HOXA3, SIX1, FOXE1
GO BP	GO:0030878~	4	0.53	9.51	7.38E-03	HMX2, XRCC3, GATA1, BRPF1, HOXC9, MED26, BAHCC1, OLIG2, CRY1, CIITA, MTA1, TOPBP1, ARNTL, TOX4, ZNF500, ZNF236, HES4, BHLHA9, DPRX, SIX1, CNBP, HMGB3, ONECUT1, GPBP1L1, ZNF514, CALR, SOX8, T, ZNF323, PEG10, HOXA3, HOXA4, LHX1, HOXA10, ZBTB48, ZNF625, TAF8, TP53BP1, RFX7, NEUROG3, FOXP3, ZBTB44, USF2, HOXB3, HOXB4, HOXB8, POLD1, MLX, EBF2, PCNA, KLF4, BTBD11, ZNF410, TAF1C, ZFP41, NR6A1, PAX3, TERF2IP, ZNF205, ZNF678, ANG, PAX7, RTF1, NR2F6, SPIB, TERT, ZFP36, EGR3, SNAPC4, CDK9, MCM2, HMGA1, XPC, CDK2AP1, THOC1, H1FNT, C17ORF70, ZNF467, WRNIP1, GLIS2, ZNF169, C11ORF9, HAND1, TSC22D4, POU2F3, POU3F3, MLXIP, FOXD2, NFATC3, ETV5, FEN1, SIM1, BAHD1, FOXL2, CEBPE, HIST1H2BG, ZNF667, LMX1A, STAT3, ZNF664, SON, ZBED4, FOXE1, REPIN1
GO MF	GO:0003677~	104	13.74	1.26	8.07E-03	NDUFS6, CYBA, NDUFA6, NDUFA7, MIOX, GMPR, NDUFA1, CYB561, DHDH
GO MF	GO:0016651~	9	1.19	3.05	9.03E-03	