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Supplemental Information

Genome-wide, Single-Cell DNA Methylomics Reveals Increased Non-CpG Methylation during Human Oocyte Maturation

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Figure S1 (i). Representative photographs of oocytes collected at different maturation stages; (ii). Representative library fragment size distribution on the Bioanalyzer platform. Related to Table 1.

i) A



i) B



i) C



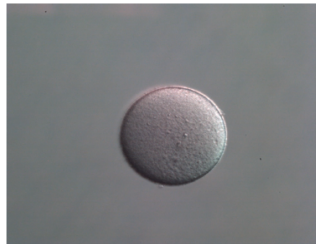
i) D



i) E



i) F



ii)

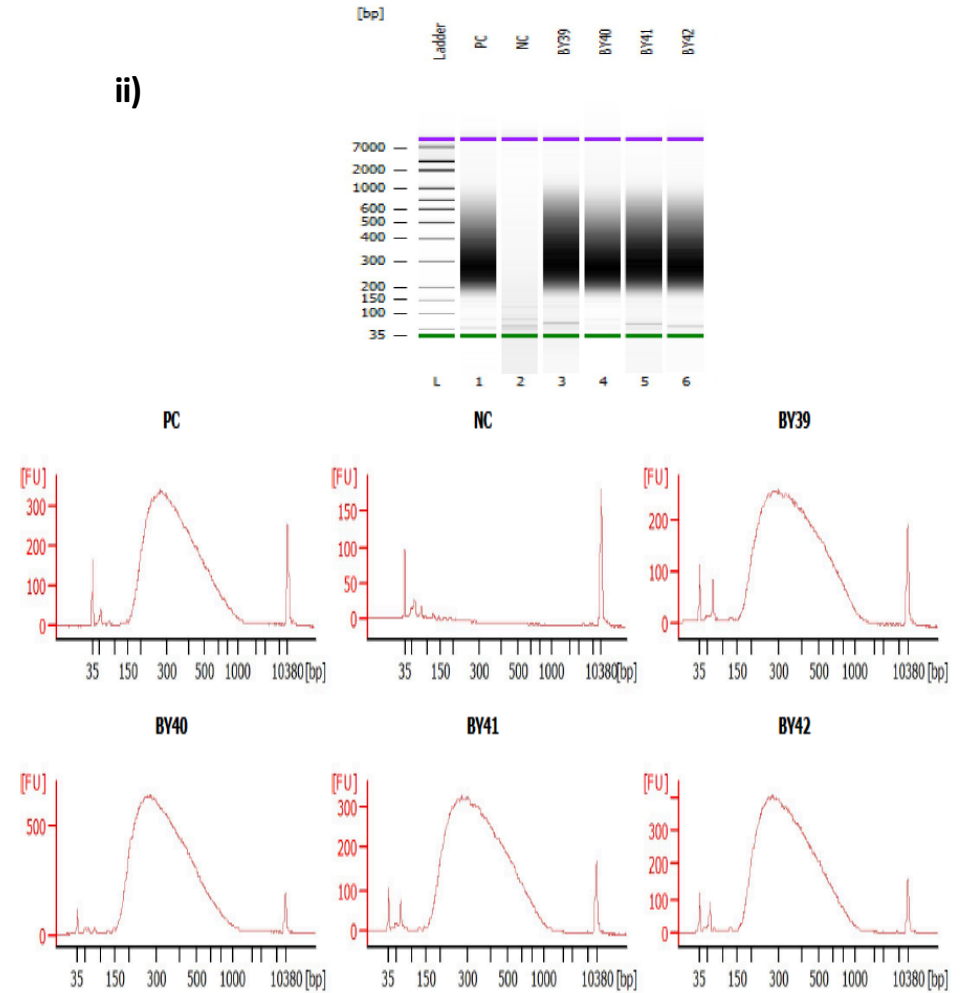


Table S1. Comparison with published SC-WGBS methods. Related to Table 1.

	Yu et al	Farlik et al	Smallwood et al
One-tube cell lysis and bisulfite conversion	Yes	Yes	Yes
Simultaneous bisulfite conversion and fragmentation	Yes	Yes	Yes
Post-bisulfite adaptor tagging	Yes	Yes	Yes
Commercially available library preparation	pico Methyseq kit (Zymo Research)	EpiGnome Methyl-Seq kit (Epicenter)	No
Compatible with Illumina primers	Yes	Yes	No
Compatible with paired-end sequencing	Yes	Yes	Yes
Species/Cell type	human oocyte	human, mouse cell lines	mouse oocyte, ESC
Sequencing read length	150bp, single-end	100bp, paired-end	100bp, paired-end
Bisulfite conversion rate	≥98.8%	>99%	≥97.7%
Median total sequencing reads per single cell	23,738,203	4,002,609	15,707,312
Median unique sequencing reads per single cell	997,952	617,336	2,746,323
Mean mapping efficiency	29.4%	NP*	20.1%
Median CpG site coverage per single cell	1,211,444 **	1,334,685	3,372,259
Bias towards CpG island	0.35	1.4	2.6
Median non-CpG coverage	18,848,813	NP*	71,512,185

*NP: Not presented in the paper

** for diploid cells (GV, MI oocytes); 972,851 if include haploid MII oocytes

Table S2. HiSeq raw data overview. Related to Table 1.

Sample_ID	Pt_ID	Oocyte stage	No. quality reads	% mapped reads	% Duplicated reads	No. CpG	No. CHG or CHH	BS conversion rate
BY06	B	GV	23,215,291	20.6%	77.6%	1,018,198	20,581,390	98.9%
BY07	B	GV	13,126,625	25.4%	77.6%	760,889	14,873,484	99.1%
BY11	I	GV	105,931,981	40.0%	95.7%	715,876	13,840,703	99.2%
BY16	F	GV	25,196,170	29.8%	81.6%	1,406,393	26,873,721	99.1%
BY17	F	GV	40,063,804	49.6%	92.3%	1,197,153	22,791,936	99.1%
BY19	G	GV	34,359,315	11.9%	77.8%	987,963	18,547,068	98.8%
BY20	A	GV	25,901,314	46.2%	82.8%	1,932,869	38,097,390	99.4%
BY21	A	GV	28,891,197	44.6%	93.5%	561,901	11,076,421	99.3%
BY39	K	GV	18,790,726	22.5%	81.9%	1,225,734	21,800,916	99.2%
BY42	L	GV	27,339,925	18.3%	86.1%	1,314,527	23,373,907	99.2%
BY02	D	MI	55,372,126	39.1%	83.6%	3,281,304	62,699,783	99.1%
BY05	B	MI	24,261,115	22.7%	78.4%	1,250,165	23,872,234	99.1%
BY08	C	MI	19,446,155	22.7%	70.7%	1,313,443	26,296,644	99.1%
BY09	H	MI	97,947,116	44.1%	95.2%	773,021	15,430,945	99.0%
BY13	A	MI	20,838,975	47.9%	84.9%	1,347,536	26,506,071	99.0%
BY14	A	MI	18,544,265	45.8%	86.9%	1,139,071	19,900,514	99.1%
BY18	A	MI	34,274,533	10.6%	78.4%	786,087	15,294,217	98.8%
BY40	K	MI	24,276,996	13.3%	69.1%	1,621,459	29,287,409	99.2%
BY41	L	MI	34,803,285	21.5%	86.7%	1,585,397	28,577,652	99.3%
BY57	L	MI	19,283,410	30.2%	79.5%	1,084,221	20,559,123	99.2%
BY03	D	MII	11,977,640	33.0%	81.7%	782,631	14,432,582	99.0%
BY23	J	MII	20,541,564	20.4%	83.9%	571,683	12,039,595	99.0%
BY27	J	MII	18,802,263	38.8%	84.4%	957,739	19,150,558	99.3%
BY29	J	MII	15,762,979	33.9%	83.8%	811,224	16,161,880	99.3%
BY52	M	MII	25,245,473	35.7%	92.1%	467,504	8,957,291	99.2%
BY55	M	MII	17,886,342	14.7%	70.6%	440,056	7,824,409	99.2%
BY56	M	MII	21,068,306	32.0%	82.6%	607,245	12,512,158	99.2%
BY50	N	MII	21,349,864	27.6%	87.4%	954,668	17,042,046	99.3%
BY51	N	MII	25,199,652	21.2%	91.9%	297,668	5,725,853	99.3%
BY54	N	MII	19,100,521	18.6%	81.1%	472,642	8,481,367	99.2%

Table S3. Correlation between the methylation levels of 68 oocyte-specific imprinted regions in each of our oocytes and those of different cell types published in Okae *et al.*, 2014. Pearson correlation coefficients are listed. Related to Table 1.

Correlation with	Oocyte	Sperm	Blastocyst	H9.ES	H1.ES	HUES6.ES	Blood
BY06	0.90	-0.54	0.69	-0.36	-0.36	-0.13	-0.22
BY07	0.98	-0.54	0.81	-0.30	-0.23	-0.22	-0.16
BY11	0.76	-0.48	0.66	-0.39	-0.39	-0.59	-0.52
BY16	0.79	-0.42	0.84	-0.31	-0.23	-0.09	-0.26
BY17	0.96	-0.58	0.89	0.01	0.13	0.06	0.30
BY19	0.96	-0.41	0.73	-0.30	-0.26	-0.26	-0.23
BY20	0.24	0.03	0.19	0.44	0.48	0.64	0.61
BY21	0.71	-0.29	0.68	-0.29	-0.35	-0.22	-0.20
BY39	0.98	-0.37	0.92	-0.18	-0.16	-0.02	-0.06
BY42	0.88	-0.18	0.70	0.20	0.11	0.29	0.17
BY02	0.38	-0.02	0.35	0.41	0.42	0.41	0.49
BY05	0.90	-0.25	0.79	-0.21	-0.41	-0.28	-0.07
BY08	0.92	-0.35	0.88	0.04	-0.10	0.17	0.29
BY09	0.87	-0.33	0.83	-0.53	-0.58	-0.36	-0.52
BY13	0.97	-0.13	0.81	-0.12	-0.21	-0.17	-0.15
BY14	0.88	-0.60	0.72	-0.11	-0.05	0.07	-0.25
BY18	0.82	-0.70	0.53	-0.12	-0.04	0.12	-0.04
BY40	0.89	-0.49	0.78	-0.21	-0.19	-0.19	-0.22
BY41	0.94	-0.28	0.93	-0.03	-0.12	-0.04	-0.05
BY57	0.97	-0.62	0.80	-0.35	-0.23	-0.08	-0.19
BY03	0.71	-0.64	0.27	-0.29	-0.21	-0.03	-0.08
BY23	0.91	-0.42	0.95	0.08	0.11	0.09	0.10
BY27	0.39	-0.18	0.38	0.41	0.41	0.46	0.51
BY29	0.88	-0.12	0.58	-0.38	-0.44	-0.46	-0.38
BY50	0.75	-0.13	0.70	0.27	0.47	0.36	0.36
BY51	0.99	-0.50	0.53	-0.55	-0.80	-1.00	0.17
BY52	0.56	-0.61	0.21	-0.36	-0.41	-0.35	-0.43
BY54	0.97	-0.69	0.73	-0.42	-0.33	-0.15	-0.08
BY55	0.99	-0.47	0.76	0.37	0.28	0.72	0.63
BY56	0.83	-0.43	0.93	-0.51	-0.53	-0.33	-0.42

Table S4. Correlation between the average CpG methylation level of each single oocyte with that of the merged sample of each maturation stage. Pearson correlation coefficients are listed.

Insert on the right: one example plot of a single oocyte sample (BY16) vs the pooled GV oocytes. Pearson Correlation coefficient = 0.93. Related to Table 1.

Sample ID	Coefficient
BY06	0.91
BY07	0.91
BY11	0.91
BY16	0.93
BY17	0.93
BY19	0.93
BY20	0.82
BY21	0.91
BY39	0.92
BY42	0.93
BY02	0.79
BY05	0.91
BY08	0.91
BY09	0.91
BY13	0.92
BY14	0.92
BY18	0.91
BY40	0.91
BY41	0.93
BY57	0.92
BY03	0.92
BY23	0.91
BY27	0.86
BY29	0.91
BY50	0.85
BY51	0.91
BY52	0.87
BY54	0.92
BY55	0.91
BY56	0.90

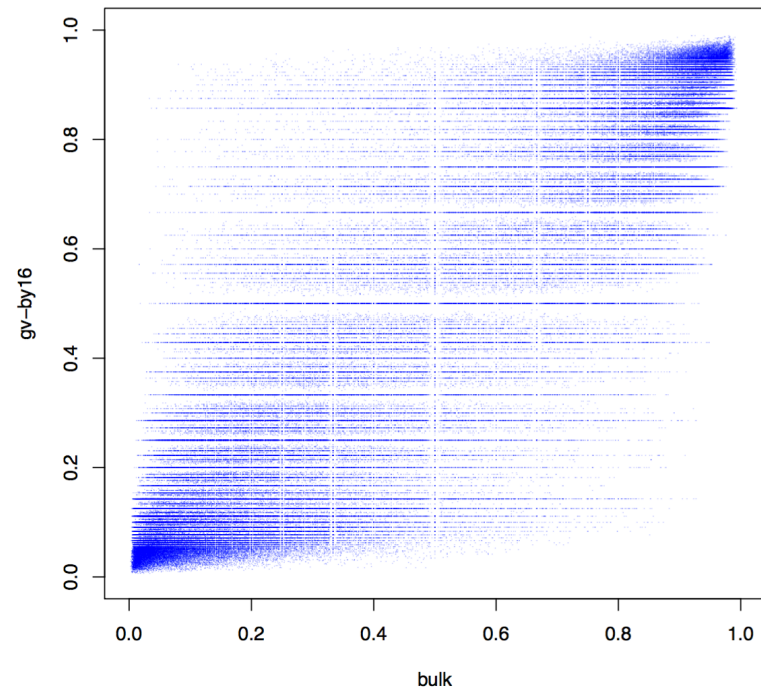
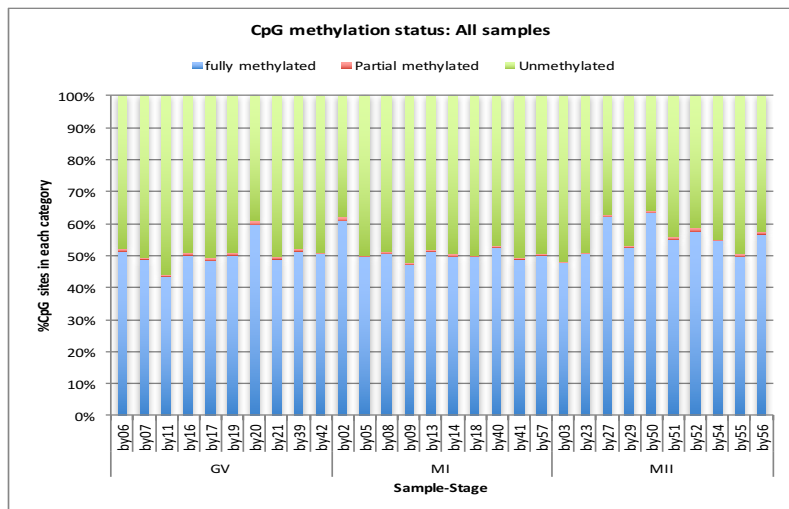
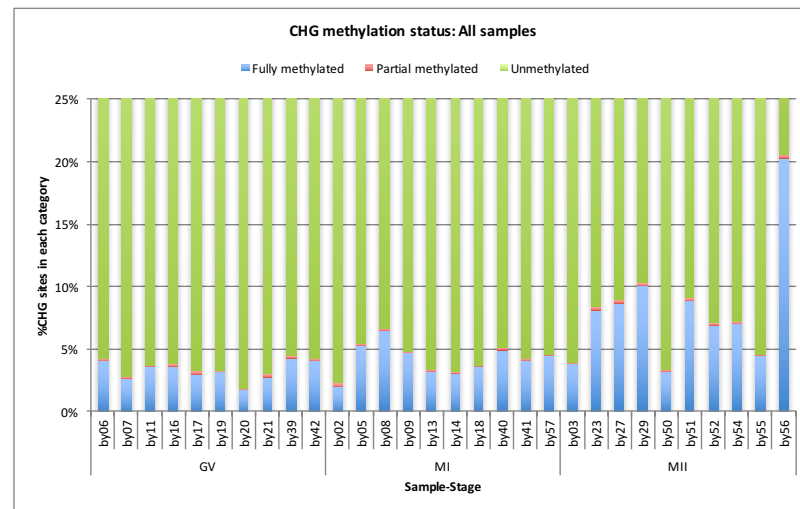


Figure S2. Methylation levels of individual samples. Related to Figure 1.

A



B



C

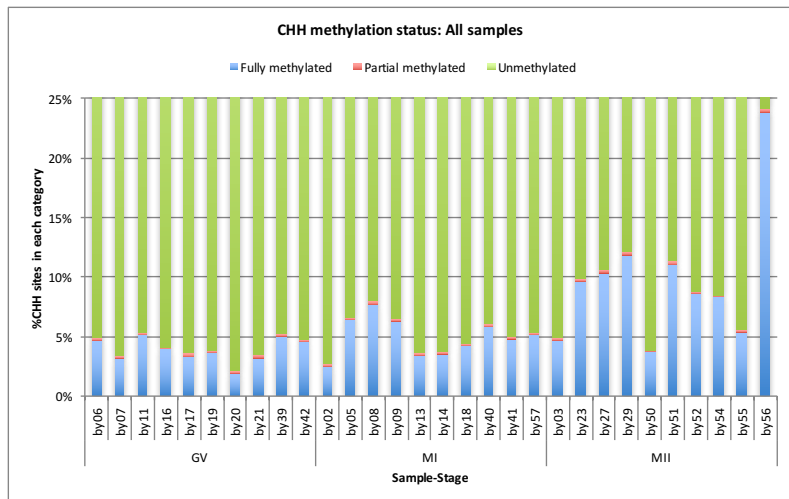
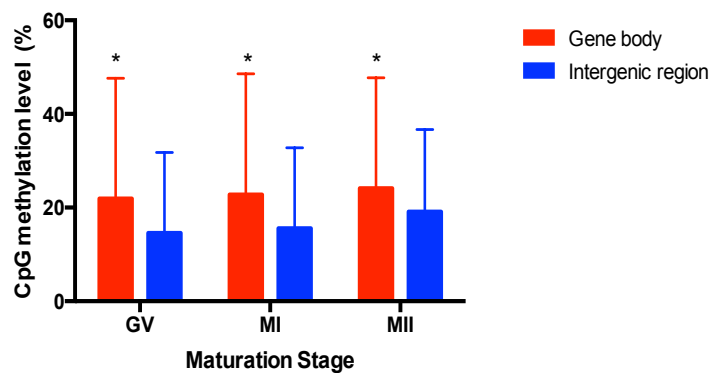


Figure S3. Gene body methylation in relation to intergenic region methylation and gene expression level. Related to Figure 2. n=10 oocytes per maturation stage.

A



* Compared to intergenic region, p-value < 0.05, two-sided paired t-test

B

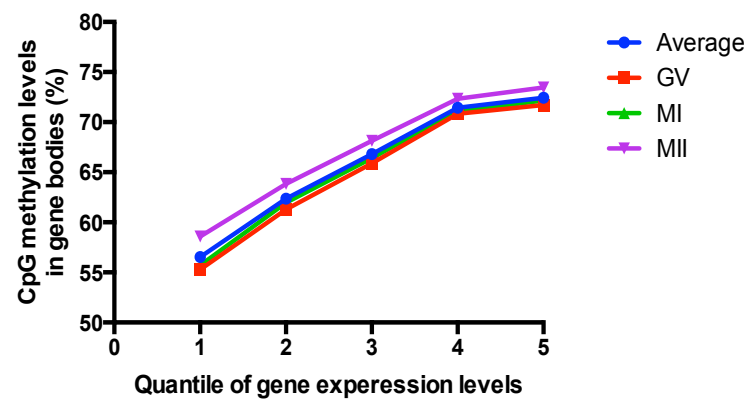


Table S5. Pearson correlation coefficients in Figure 3b,c. Related to Figure 3.

	GV_CpG	GV_CHG	GV_CHH	MI_CpG	MI_CHG	MI_CHH	MII_CpG	MII_CHG	MII_CHH
GV_CpG	-	0.44	0.48	0.87	0.32	0.33	0.80	0.14	0.14
GV_CHG		-	0.68	0.42	0.28	0.26	0.39	0.14	0.13
GV_CHH			-	0.45	0.27	0.30	0.42	0.13	0.15
MI_CpG				-	0.35	0.36	0.80	0.14	0.14
MI_CHG					-	0.75	0.30	0.14	0.13
MI_CHH						-	0.31	0.13	0.15
MII_CpG							-	0.22	0.21
MII_CHG								-	0.85
MII_CHH									-

	GV_MI_CpG	GV_MI_CHG	GV_MI_CHH	MI_MII_CpG	MI_MII_CHG	MI_MII_CHH
GV_MI_CpG	-	0.08	0.09	-0.16	-0.02	-0.02
GV_MI_CHG		-	0.59	-0.01	-0.11	-0.07
GV_MI_CHH			-	-0.01	-0.08	-0.10
MI_MII_CpG				-	0.16	0.16
MI_MII_CHG					-	0.79
MI_MII_CHH						-

P-values $<10^{-10}$ in all cases

Figure S4. Venn diagram and genomic distribution of Differentially Methylated Regions (DMRs). Related to Figure 3. n=10 oocytes per maturation stage.

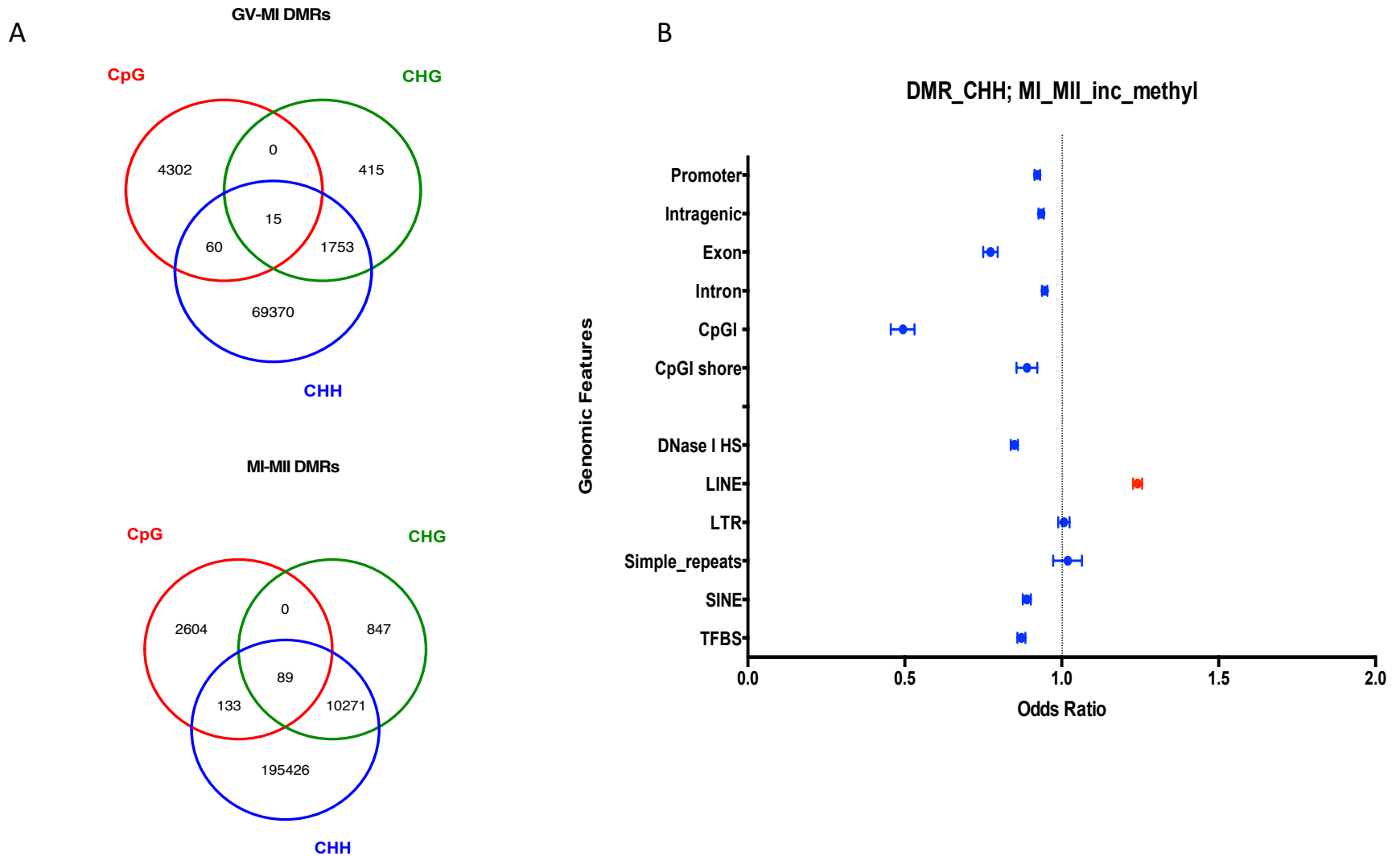


Table S6. Significantly enriched pathways in GO analysis. Related to Figure 3.

GO Term	Fold Enrichment	P-Value	FDR	Stage	Methylation Change
GO:0044459~plasma membrane part	2.00	5.24E-10	6.90E-07	GV-MI	increase
GO:0005886~plasma membrane	1.61	1.45E-08	1.91E-05	GV-MI	increase
GO:0005887~integral to plasma membrane	2.03	1.43E-05	1.88E-02	GV-MI	increase
GO:0031226~intrinsic to plasma membrane	1.98	2.43E-05	3.19E-02	GV-MI	increase
GO:0030054~cell junction	2.43	2.72E-04	3.57E-01	GV-MI	increase
GO:0006811~ion transport	2.33	2.23E-05	3.75E-02	GV-MI	increase
GO:0031012~extracellular matrix	4.36	8.57E-05	1.07E-01	MI-MII	decrease
GO:0005578~proteinaceous extracellular matrix	4.31	2.15E-04	2.67E-01	MI-MII	decrease