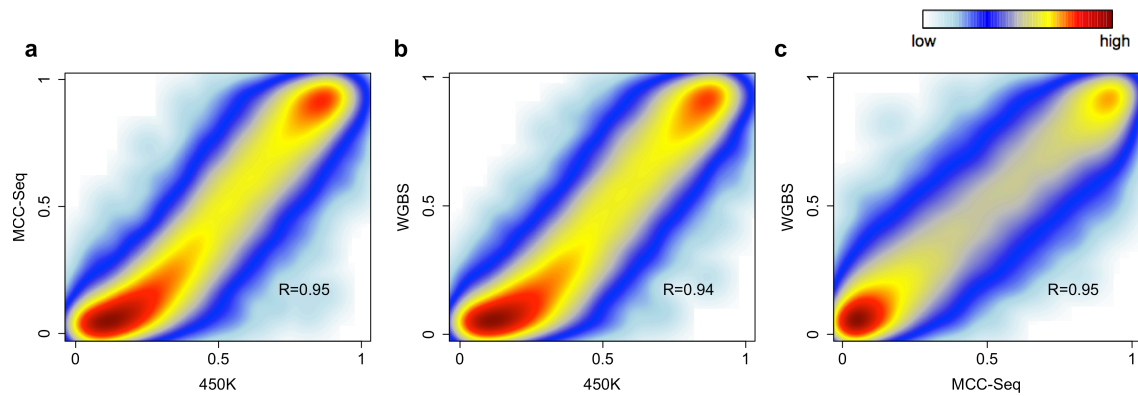
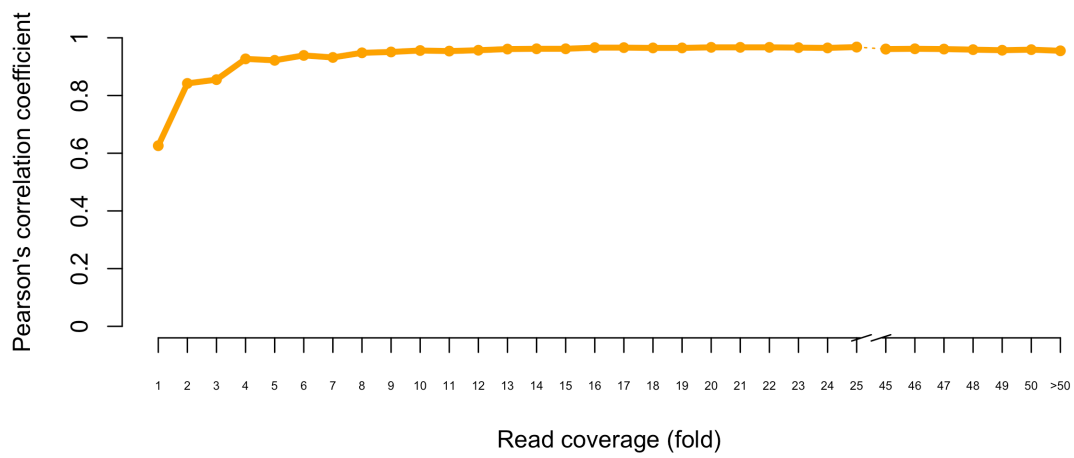


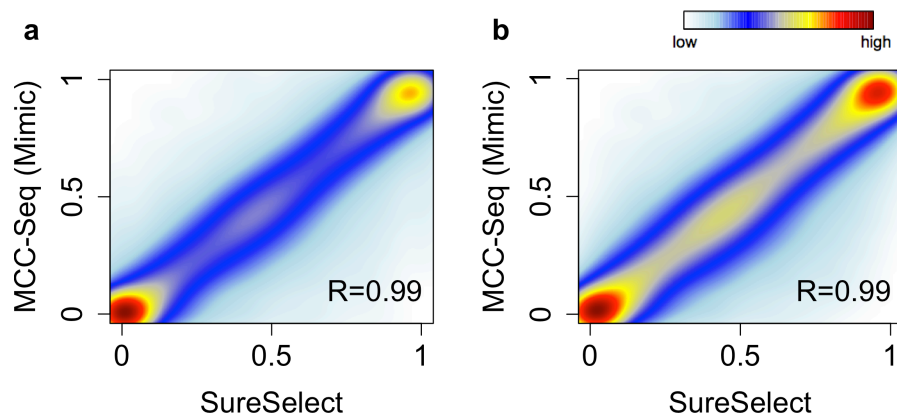
## Supplementary Figures



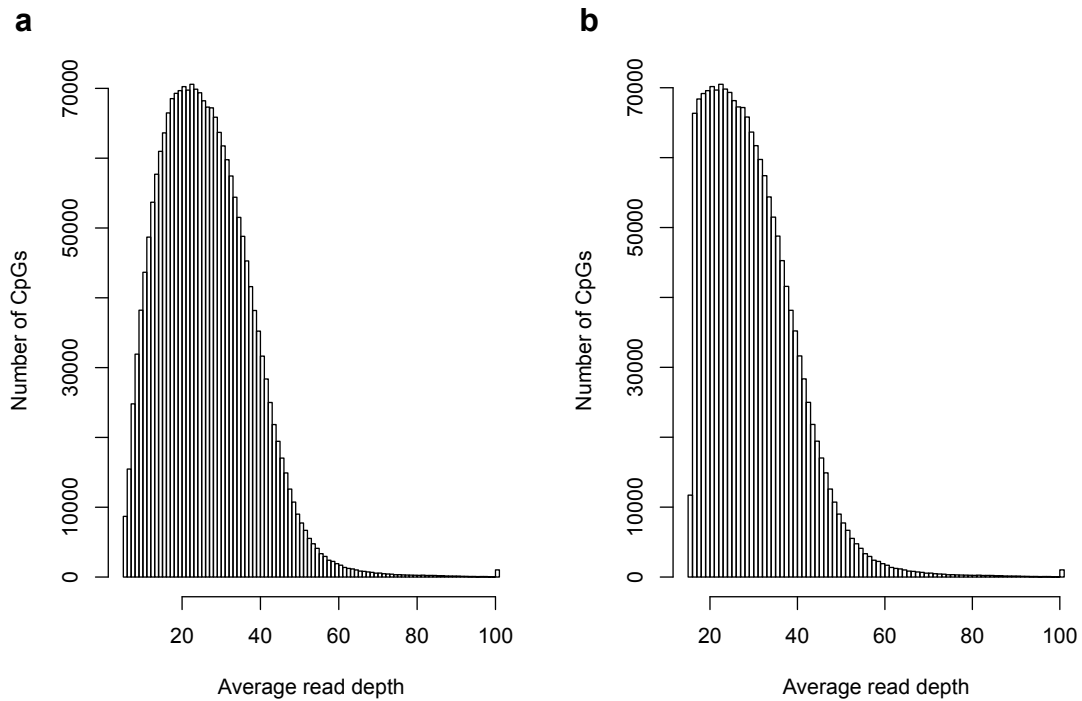
**Supplementary Figure 1. Extended comparison of MCC-Seq methylation calls with WGBS and the Illumina 450K array excluding completely hypo and hypermethylated CpGs** (a) Correlation between MCC-Seq<sub>4-plex</sub> and Illumina 450K array methylation calls for the same VAT DNA sample (R=0.95), (b) comparison between WGBS and Illumina 450K array results (R=0.94) and (c) comparison between WGBS and MCC-Seq<sub>4-plex</sub> results (R=0.95). Only CpGs with data available from all three techniques were included as well as excluding completely hypo and hypermethylated CpGs (N=150,898 CpGs); we required sequence coverage  $\geq 5X$  for MCC-Seq and WGBS; R is the Pearson correlation coefficient.



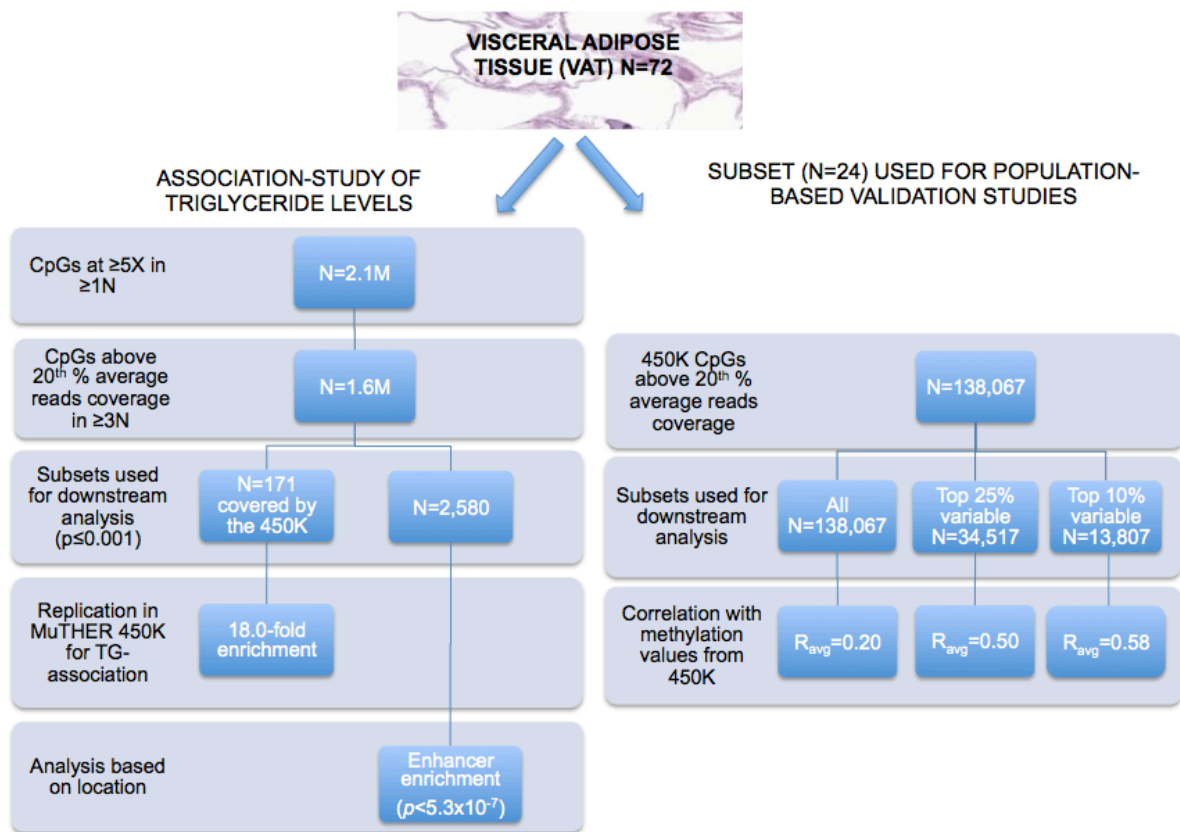
**Supplementary Figure 2. Correlation between Illumina 450K array and MCC-Seq methylation calls at different read coverage** Pearson correlation between Illumina 450K array and MCC-Seq methylation calls for the same VAT DNA sample is shown at different read depth (fold, x-axis).



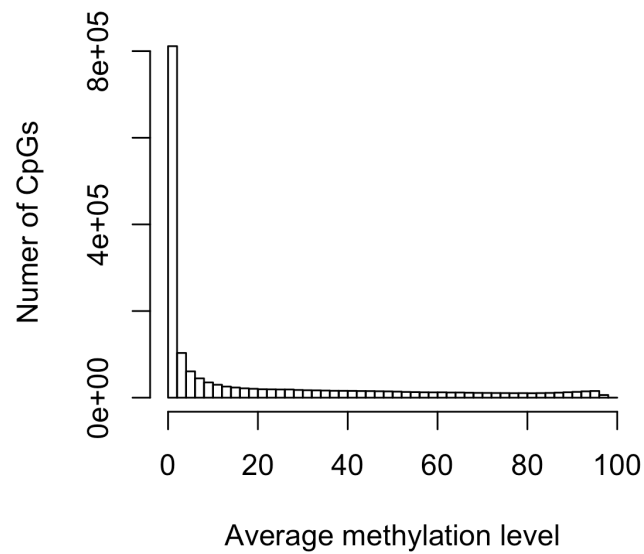
**Supplementary Figure 3. Comparison of MCC-Seq methylation calls with Agilent SureSelect** Correlations between MCC-Seq<sub>Mimic</sub> and Agilent SureSelect methylation calls for the LCL GM12878 cell line (a) using all overlapping CpGs (N=2,551,186; R=0.99) and (b) excluding hypo (0%) and hypermethylated CpGs (N=1,734,371; R=0.99). Only CpGs with data available from both techniques were included; we required sequence coverage  $\geq 5X$  for MCC-Seq and WGBS accordingly; R is the Pearson correlation coefficient; density scales are independent in the two panels.



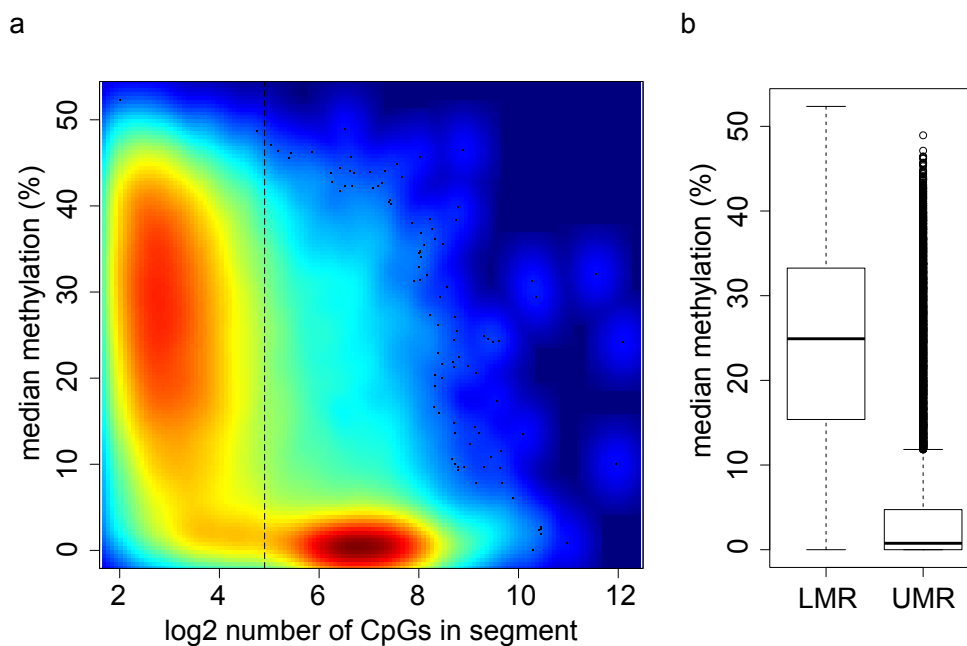
**Supplementary Figure 4. Distributions of sequence coverage at included CpG sites**  
Distributions of the average read depth across the 72 individuals of included CpG sites when considering (a) all CpG sites with  $\geq 5X$  and  $\leq 100X$  sequence depth and (b) sites meeting these criteria and with average sequence depth above the 20<sup>th</sup> percentile.



**Supplementary Figure 5. Outline of the trait association and population-based validation studies** In the left panel, an outline of the subsets used for different analysis in the association-study of methylation to triglyceride levels as well as main results are presented. In the right panel, the outline of the population-based validation studies comparing methylation values obtained with the 450K array and MCC-Seq in 24 individuals is shown.

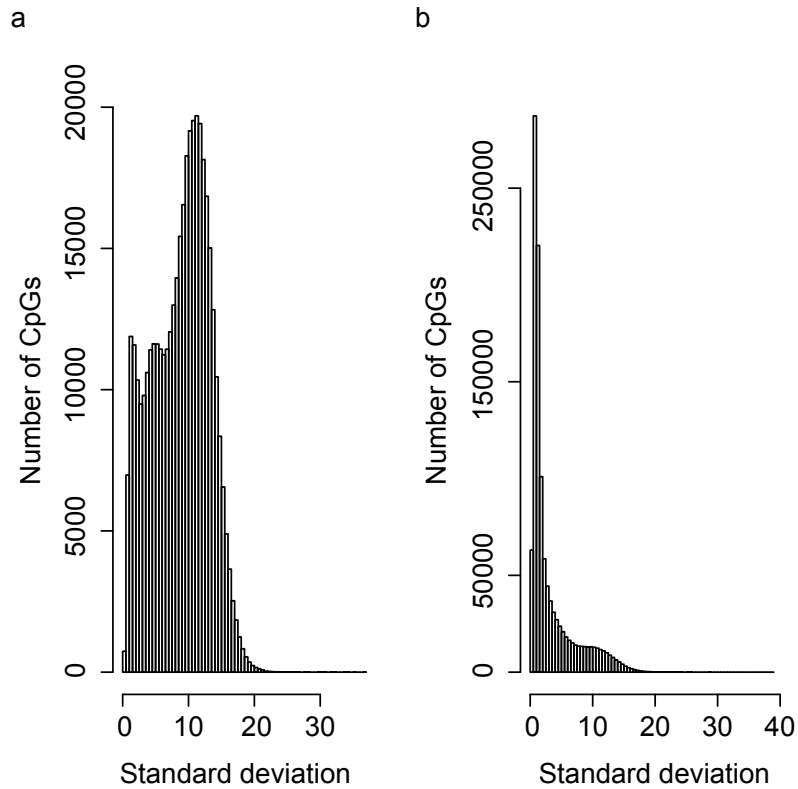


**Supplementary Figure 6. Average methylation pattern of CpGs captured with MCC-Seq Met V1 design** The figure shows the average methylation values (% , x-axis) for 72 visceral adipose tissue samples at on-target CpG sites above the 20<sup>th</sup> percentile average reads coverage (N=1,710,209) from Met V1 capture experiments.



**Supplementary Figure 7. Characterization of adipose hypomethylated footprints**

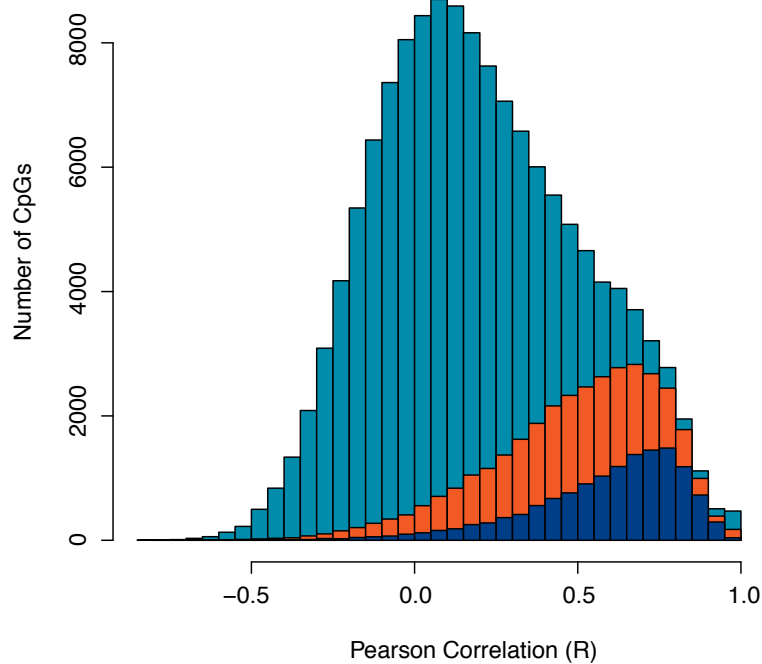
Hypomethylated footprints were generated from WGBS on AT using the R/Bioconductor package MethylSeekR. **(a)** Unmethylated regions (UMRs, right of dotted line) and low-methylated regions (LMRs, left of dotted line) were differentiated by a 30 CpG content threshold. **(b)** LMRs (N=45,065) and UMRs (N=20,195) show different median methylation patterns with LMRs having a broader range of methylation and UMRs being less variable in their methylation status and associated to low-methylated promoter regions. Boxplot whiskers represent 1.5\*IQR (inter quartile range).



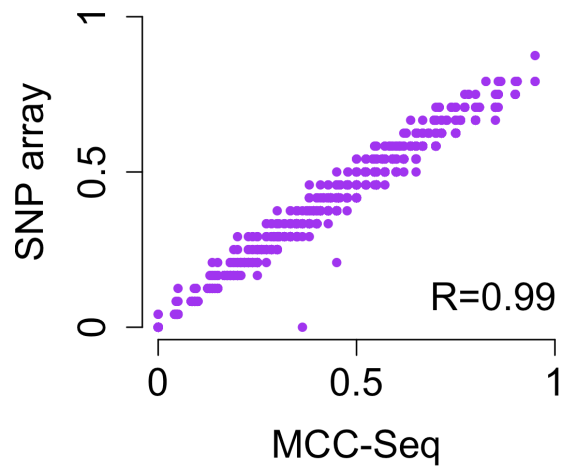
**Supplementary Figure 8. Variability of enhancer and promoter associated CpG sites**

Interrogated CpG sites were mapped to putative enhancers (H3K4me1 or LMR) or promoters (H3K4me3 and UMR). Assessing the standard deviation of the methylation status across individuals (a) CpGs mapping to putative enhancers were found to be more variable (median SD=9.4) than (b) those mapping to putative promoters (median SD=1.5).

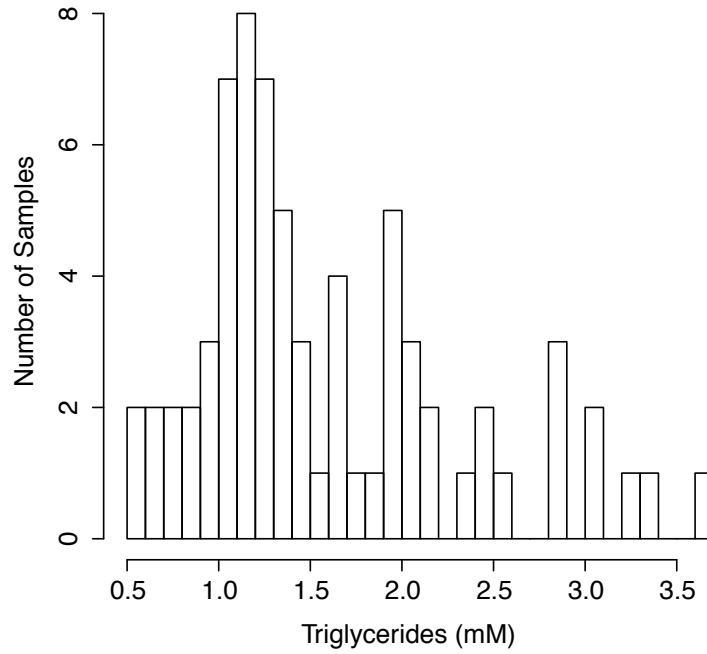




**Supplementary Figure 9. CpG-by-CpG correlation between Illumina 450K array and MCC-Seq methylation calls in 24 samples** The figure shows the distribution of spearman rank correlations for CpGs from the Illumina 450K array that had average reads coverage above the 20<sup>th</sup> percentile in the MCC-Seq method (teal; N=138,067 CpGs; mean R =0.20). The correlations were generally greater when we included only the top 25% variable CpGs (orange; N=34,517 CpGs; mean R=0.50) or the top 10% variable CpGs (blue; N=13,807 CpGs; mean R=0.58) from the MCC-Seq methylation calls.



**Supplementary Figure 10. Comparison of the observed heterozygosity from MCC-Seq and HumanOmni BeadChip array genotyping calls** Comparison between the observed heterozygosity based on the HumanOmni BeadChip array (SNP ratio, y-axis) and MCC-Seq<sub>plex</sub> (SNP ratio, x-axis) genotyping calls across  $\geq 20$  individuals (N=3,093 SNPs; R=0.99); we required sequence coverage  $\geq 5X$  for MCC-Seq; R is the Pearson correlation coefficient.



**Supplementary Figure 11. Distribution of triglycerides levels in the discovery cohort**  
Distributions of the triglyceride levels (mM, x-axis) across the discovery cohort (N=70, two outliers excluded).

Supplementary Table 1. Sequence statistics of the Met V1 pooled samples

	Plexing level	Sample ID	raw reads	Total aligned reads (%)	Total on-target reads (%)	Total on-target aligned reads (%)	On-target aligned reads	Average coverage on-target CpG sites	Total captured on-target CpG sites (%)	Total CpG sites with >=5 reads (%)	Total CpG sites with >=10 reads (%)
Individual	1	1	257,885,682	60	67	40	95,832,520	82	99	94	94
	2	1	151,069,388	60	71	43	58,871,218	53	99	91	90
	2	2	144,059,126	62	69	43	57,386,604	50	99	90	89
	4	3	65,799,220	70	79	55	35,355,720	33	99	82	80
	4	4	62,929,502	72	80	57	34,630,762	33	99	82	79
	4	5	57,375,348	72	79	57	31,638,544	30	99	80	77
	4	6	51,172,122	73	78	57	23,258,060	27	99	80	76
	6	1	65,153,260	74	56	41	28,090,152	24	99	80	76
	6	2	51,084,062	76	56	43	21,392,302	21	99	77	71
	6	7	59,331,638	73	55	40	23,132,120	21	99	77	71
	6	8	72,222,874	74	55	41	29,161,380	27	99	82	78
	6	9	65,481,242	73	54	39	25,251,428	24	99	80	75
	6	10	71,864,932	72	54	39	27,650,522	25	99	80	76
	10	11	36,474,056	75	59	44	15,739,422	14	98	65	54
	10	12	32,548,084	76	59	45	14,374,870	13	98	63	51
	10	13	32,058,534	76	59	45	14,139,984	13	98	62	49
	10	14	29,780,130	75	60	45	13,047,378	12	98	60	46
	10	15	38,922,258	74	58	43	16,445,576	15	98	67	56
	10	16	34,623,254	74	57	42	14,442,798	13	98	62	50
	10	17	40,850,638	75	51	38	15,457,642	14	98	66	55
10	18	28,196,414	74	58	43	11,877,752	10	98	55	38	
10	19	31,954,644	76	60	46	14,209,644	13	98	63	50	
10	20	32,427,388	75	59	44	14,171,532	13	98	62	49	
Average	1	NA	257,885,682	60	67	40	95,832,520	82	99	94	94
	2	NA	147,564,257	61	70	43	58,128,911	51	99	90	90
	4	NA	59,319,048	72	79	57	31,194,648	31	99	81	78
	6	NA	64,189,668	74	55	41	25,446,317	24	99	79	75
	10	NA	33,783,540	75	58	44	14,390,660	13	98	63	50

Supplementary Table 2. Sequence statistics of the Met V2 pooled samples

	Plexing level	Sample ID	raw reads	Total aligned reads (%)	Total on-target reads (%)	Total on-target aligned reads (%)	On-target aligned reads	Average coverage on-target CpG sites	Total captured on-target CpG sites (%)	Total CpG sites with >=5 reads (%)	Total CpG sites with >=10 reads (%)
Individual	6	21	52,965,668	71	62	44	23,013,966	13	98	62	50
	6	22	52,005,852	72	62	44	22,681,612	13	98	62	50
	6	23	76,717,544	73	62	45	34,152,304	20	98	73	67
	6	24	59,006,738	73	61	45	26,096,106	15	98	65	54
	6	25	53,915,196	71	62	44	23,447,444	14	98	63	51
	6	26	60,990,908	72	63	45	27,038,892	16	98	67	58
Average	6	NA	59,268,984	72	62	45	26,071,387	15	98	65	55

**Supplementary Table 3. Comparison of MCC-Seq methylation calls with Illumina 450K array and WGBS data at various read depths**

Technique comparisons	Pearson Correlation			
	>=5X (N=150,898 CpGs)	>=10X (N=144,868 CpGs)	>=20X (N=90,547 CpGs)	>=30X (N=17,852 CpGs)
450K-MCC-Seq	0.964	0.964	0.965	0.962
450K-WGBS	0.962	0.962	0.961	0.959
MCC-Seq-WGBS	0.974	0.974	0.974	0.972

Supplementary Table 4. Comparison of MCC-Seq methylation calls with Illumina 450K array and WGBS data at various read depths excluding completely hypo and hypermethylated CpGs

Technique comparisons	Pearson Correlation			
	>=5X (N=45,097 CpGs)	>=10X (N=44,414 CpGs)	>=20X (N=30,934 CpGs)	>=30X (N=7,182 CpGs)
450K-MCC-Seq	0.946	0.947	0.952	0.953
450K-WGBS	0.942	0.943	0.946	0.947
MCC-Seq-WGBS	0.949	0.950	0.955	0.958





**Supplementary Table 6. ATAC-Seq PCR amplification primers**

ID	Adaptor Sequence	Primer Sequence
Ad1	n/a	AATGATACGGCGACCACCGAGATCTACACTCGTCGGCAGCGTCAGATGTG
Ad2.1	TAAGGCGA	CAAGCAGAAGACGGCATAACGAGATTCGCCTTAGTCTCGTGGGCTCGGAGATGT
Ad2.2	CGTACTAG	CAAGCAGAAGACGGCATAACGAGATCTAGTACGGTCTCGTGGGCTCGGAGATGT
Ad2.3	AGGCAGAA	CAAGCAGAAGACGGCATAACGAGATTTCTGCCTGTCTCGTGGGCTCGGAGATGT
Ad2.4	TCCTGAGC	CAAGCAGAAGACGGCATAACGAGATGCTCAGGAGTCTCGTGGGCTCGGAGATGT
Ad2.5	GGACTCCT	CAAGCAGAAGACGGCATAACGAGATAGGAGTCCGTCTCGTGGGCTCGGAGATGT
Ad2.6	TAGGCATG	CAAGCAGAAGACGGCATAACGAGATCATGCCTAGTCTCGTGGGCTCGGAGATGT
Ad2.7	CTCTCTAC	CAAGCAGAAGACGGCATAACGAGATGTAGAGAGGTCTCGTGGGCTCGGAGATGT
Ad2.8	CAGAGAGG	CAAGCAGAAGACGGCATAACGAGATCCTCTCTGGTCTCGTGGGCTCGGAGATGT
Ad2.9	GCTACGCT	CAAGCAGAAGACGGCATAACGAGATAGCGTAGCGTCTCGTGGGCTCGGAGATGT
Ad2.10	CGAGGCTG	CAAGCAGAAGACGGCATAACGAGATCAGCCTCGGTCTCGTGGGCTCGGAGATGT
Ad2.11	AAGAGGCA	CAAGCAGAAGACGGCATAACGAGATTGCCTCTTGTCTCGTGGGCTCGGAGATGT
Ad2.12	GTAGAGGA	CAAGCAGAAGACGGCATAACGAGATTCCTCTACGTCTCGTGGGCTCGGAGATGT
Ad2.13	GTCGTGAT	CAAGCAGAAGACGGCATAACGAGATATCACGACGTCTCGTGGGCTCGGAGATGT
Ad2.14	ACCACTGT	CAAGCAGAAGACGGCATAACGAGATACAGTGGTGTCTCGTGGGCTCGGAGATGT
Ad2.15	TGGATCTG	CAAGCAGAAGACGGCATAACGAGATCAGATCCAGTCTCGTGGGCTCGGAGATGT
Ad2.16	CCGTTTGT	CAAGCAGAAGACGGCATAACGAGATACAAACGGGTCTCGTGGGCTCGGAGATGT
Ad2.17	TGCTGGGT	CAAGCAGAAGACGGCATAACGAGATACCCAGCAGTCTCGTGGGCTCGGAGATGT
Ad2.18	GAGGGGTT	CAAGCAGAAGACGGCATAACGAGATAACCCCTCGTCTCGTGGGCTCGGAGATGT
Ad2.19	AGGTTGGG	CAAGCAGAAGACGGCATAACGAGATCCCAACCTGTCTCGTGGGCTCGGAGATGT
Ad2.20	GTGTGGTG	CAAGCAGAAGACGGCATAACGAGATCACCACAGTCTCGTGGGCTCGGAGATGT
Ad2.21	TGGTITTC	CAAGCAGAAGACGGCATAACGAGATGAAACCCAGTCTCGTGGGCTCGGAGATGT
Ad2.22	TGGTCACA	CAAGCAGAAGACGGCATAACGAGATTGTGACCAGTCTCGTGGGCTCGGAGATGT
Ad2.23	TTGACCCT	CAAGCAGAAGACGGCATAACGAGATAGGGTCAAGTCTCGTGGGCTCGGAGATGT
Ad2.24	CCACTCCT	CAAGCAGAAGACGGCATAACGAGATAGGAGTGGGTCTCGTGGGCTCGGAGATGT

**Supplementary Table 7. ATAC-Seq Q-PCR primers**

<b>ID</b>	<b>Sequence</b>
Mit_+ve_F	CTAAATAGCCCACACGTTCCC
Mit_+ve_R	AGAGCTCCCGTGAGTGGTTA
GAPDH_+ve_F	CTGTCCCTTCAGTAGCTGCC
GAPDH_+ve_R	GAAGAGAGTGGGTTGGTGGG
GAPDH_-ve_F	TCTGGATGGCCTGAAGGAGA
GAPDH_-ve_R	GCCAGCAGCACTCATGTTTC
ACTB_+ve_F	GAGTCCTTAGGCCGCCAG
ACTB_+ve_R	TCCGACCAGTGTTTGCCTTT
ACTB_-ve_F	CATCTCGTGTCCAGTGCAGA
ACTB_-ve_R	CCATGCAATGTGGGAGTCCT