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Supplemental Material to:

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Conserved DNA methylation patterns in healthy blood cells and extensive changes in leukemia measured by a new quantitative technique

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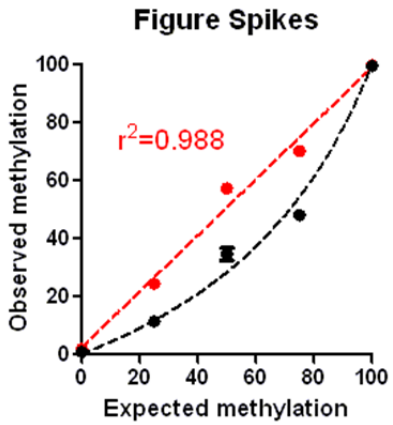
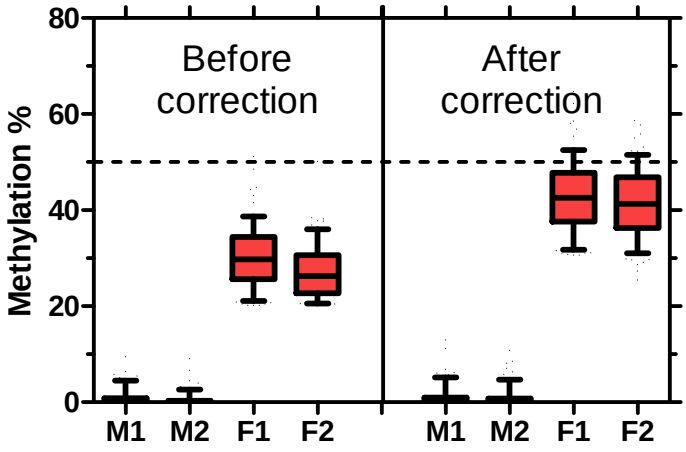
Supplementary Data



Supplementary Figure 1. Spiked-in methylation standards.

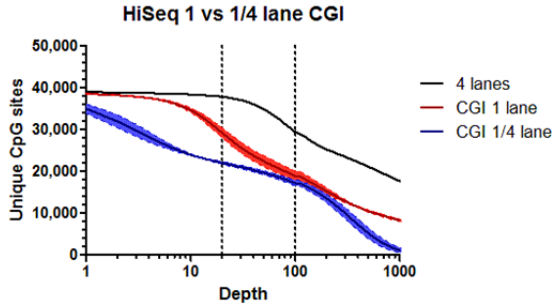
Black, values of DNA methylation measured by DREAM (observed methylation) plotted against expected methylation values based on the fraction of *M.Sss*I methylated DNA. Red, methylation values after correction computed as 100%*(c*sm/(c*sm+u)), where m/u is the expected ratio of methylated and unmethylated reads, while sm and u are observed numbers of methylated and unmethylated reads. Correction factor c was calculated as an antilog of the average difference between log ratios of ln(m/u) and ln(sm/u). Broken red line shows linear regression of corrected values. Broken black curve shows interpolation of uncorrected methylation values. Linear regression of corrected values showed an excellent fit (slope 0.967 and r^2=0.988).



Supplementary Figure 2. Uncorrected and corrected methylation values on the X chromosome.

Uncorrected and spike-corrected methylation values at 159 CpG sites in CpG islands that were subject to differential methylation on the inactivated X chromosome in white blood cells from two healthy males (M1, M2) and two healthy females (F1, F2). Methylation in males is below 1% while corrected methylation values in females are close to expected 50%.

A



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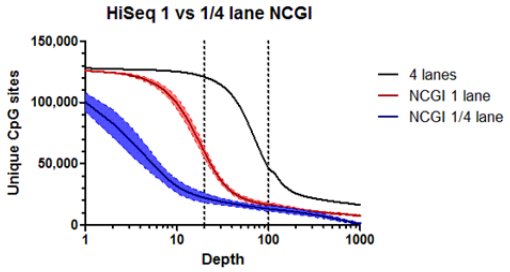
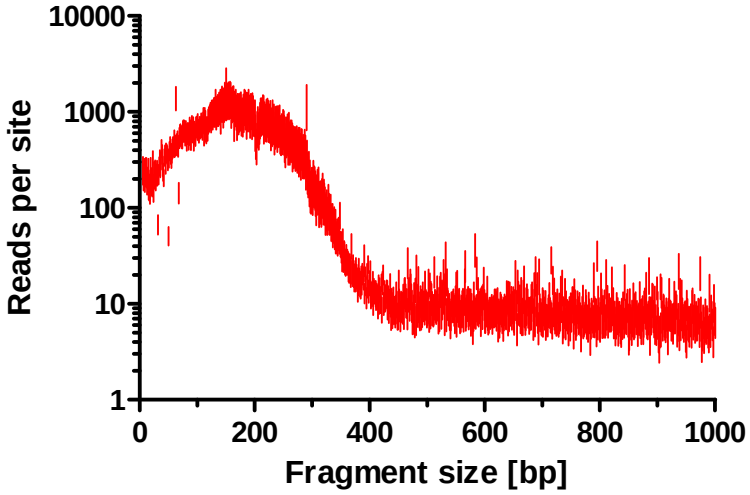


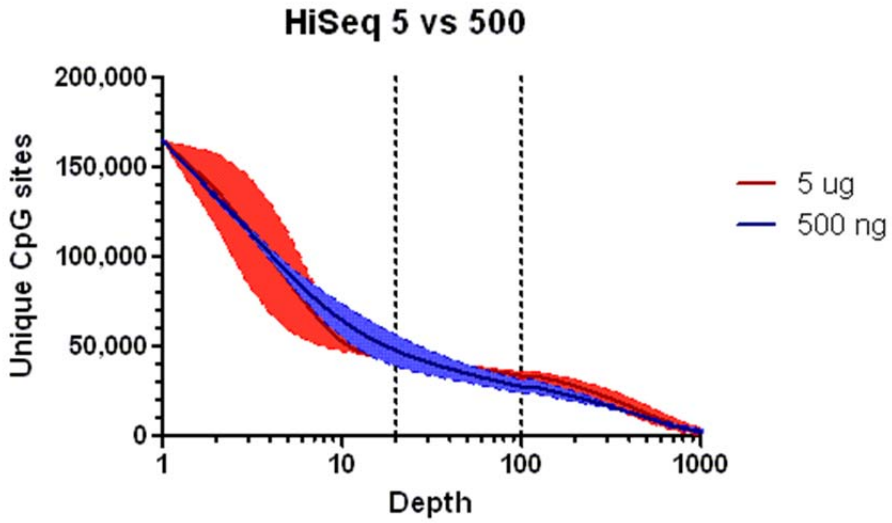
Supplementary Figure 3. Minimum sequencing depth and the numbers of unique CpG sites captured by DREAM. (A) CCCGGG sites in CpG islands. (B) CCCGGG sites outside of CpG islands. Black, 4 HiSeq lanes; red, single HiSeq lane, 4 samples; blue, ¼ of HiSeq lane, 4 samples. Solid lines show means; colored areas between broken lines show mean ± SEM.



Supplementary Figure 4. Sequencing depth based on the mutual distance of restriction sites.

The X axis shows distances of *Sma*I/*Xma*I sites to the nearest neighboring site. The graph shows average numbers of reads ± SEM (y axis) based on the distance between neighboring *Sma*I/*Xma*I sites (x axis). The sites with the nearest neighbor within 400 bases have 20-100-fold higher sequencing coverage than the remaining sites.

A



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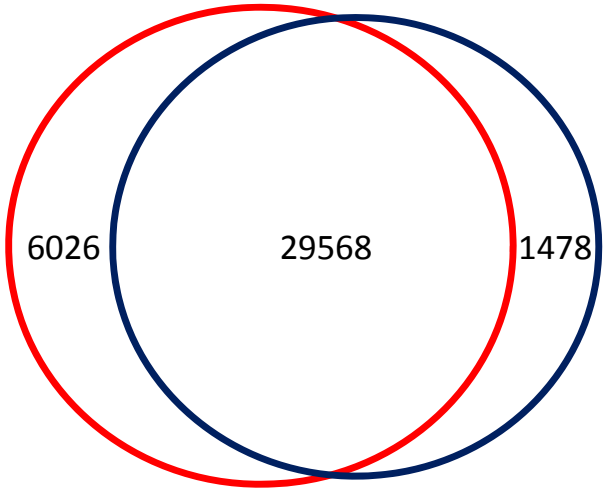
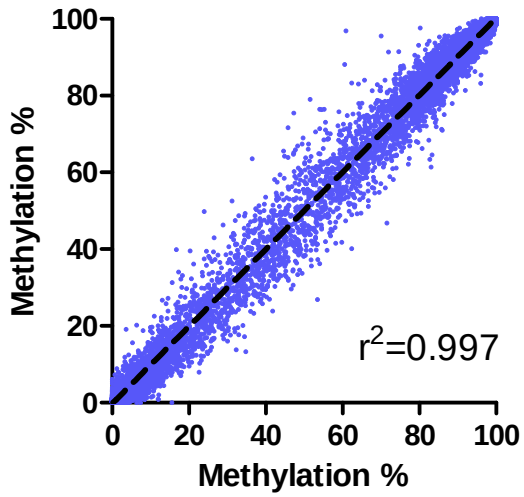
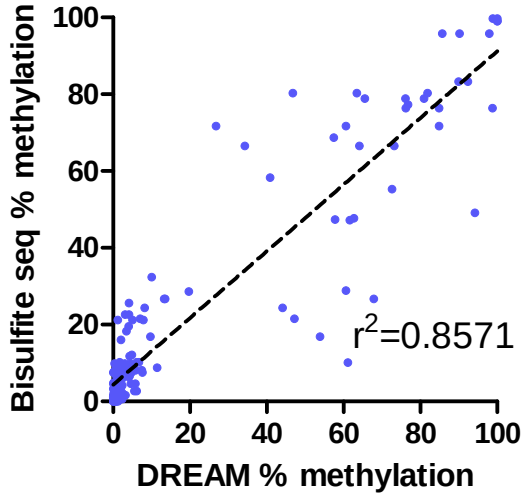


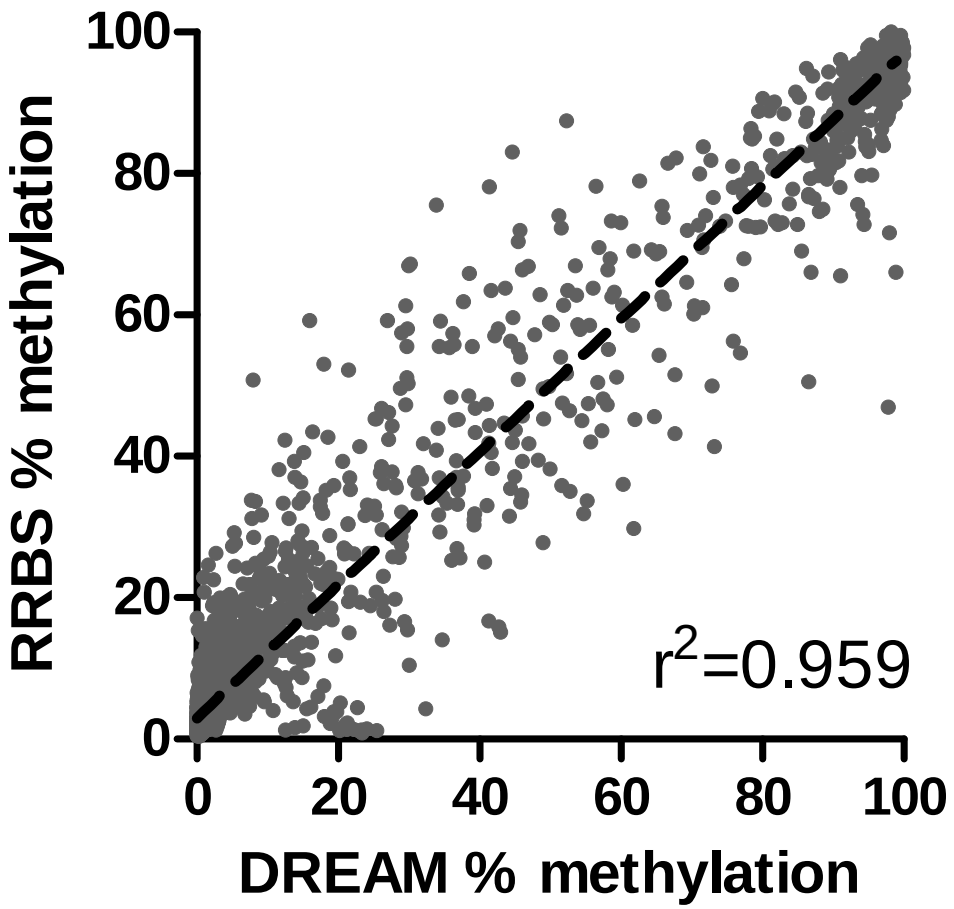
Supplementary Figure 5. (A) Sequencing depth for libraries made from 5 µg vs 500 ng healthy human white blood cell gDNA . Numbers of unique CCCGGG sites captured by DREAM. Red, 5 µg gDNA ¼ HiSeq lane, 2 samples; blue, 500 ng gDNA ¼ of HiSeq lane, 2 samples. Solid lines show means; colored areas between broken lines show mean ± SEM. (B) The coverage of CCCGGG sites was comparable with a 80% overlap of sites covered by 100+ reads in both libraries. Red, 5 µg gDNA; blue 500 ng gDNA.



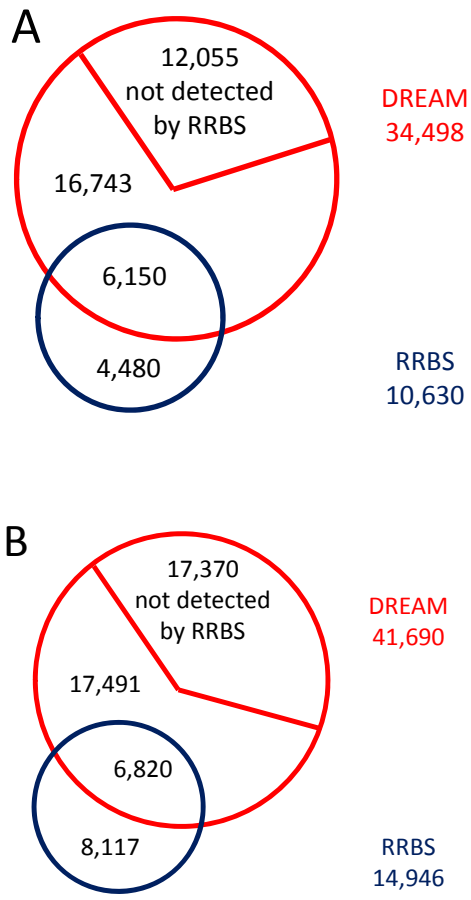
Supplementary Figure 6. Correlation between technical replicates. DNA methylation values at 28,522 CCCGGG sites covered by 100+ reads. Libraries from the same sample of healthy WBC DNA were prepared and sequenced on separate occasions.



Supplementary Figure 7. Validation of DREAM results. Bisulfite sequencing vs DREAM. Methylation at CCCGGG sites and neighboring CpG sites. Linear regression r^2=0.857, P<0.0001.

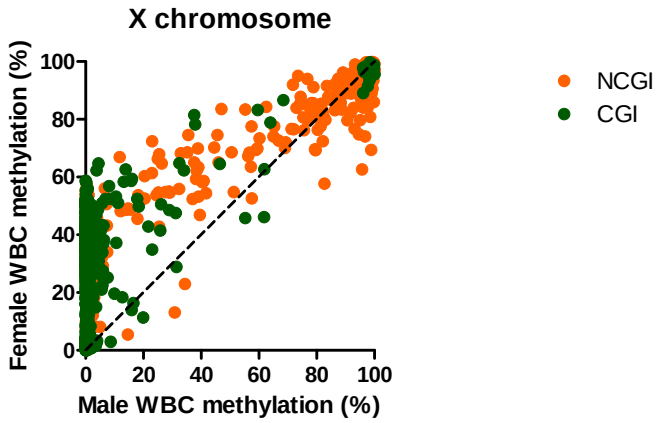


Supplementary Figure 8. Validation of DREAM results. Reduced representation bisulfite sequencing, normal leukocytes vs DREAM. Minimum coverage 50+ reads. Linear regression r^2=0.959, P<0.0001.



Supplementary Figure 9. Overlap between the CCCGGG sites covered by RRBS and DREAM by 50+ reads was relatively small, 16% for normal WBCs (A) and 14% for K562 cells (B). We detected 12,055 and 17,370 sites in normal WBCs and K562 cells, respectively, that had zero coverage by RRBS. Approximately two thirds of these sites were not in CpG islands.

A



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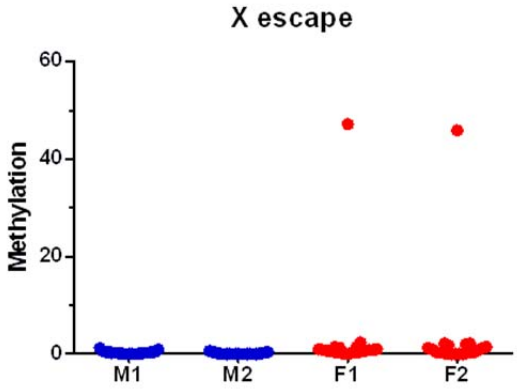
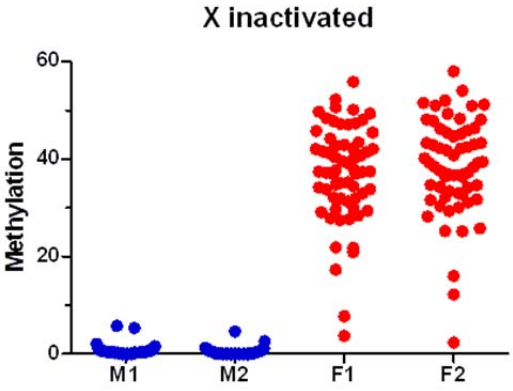
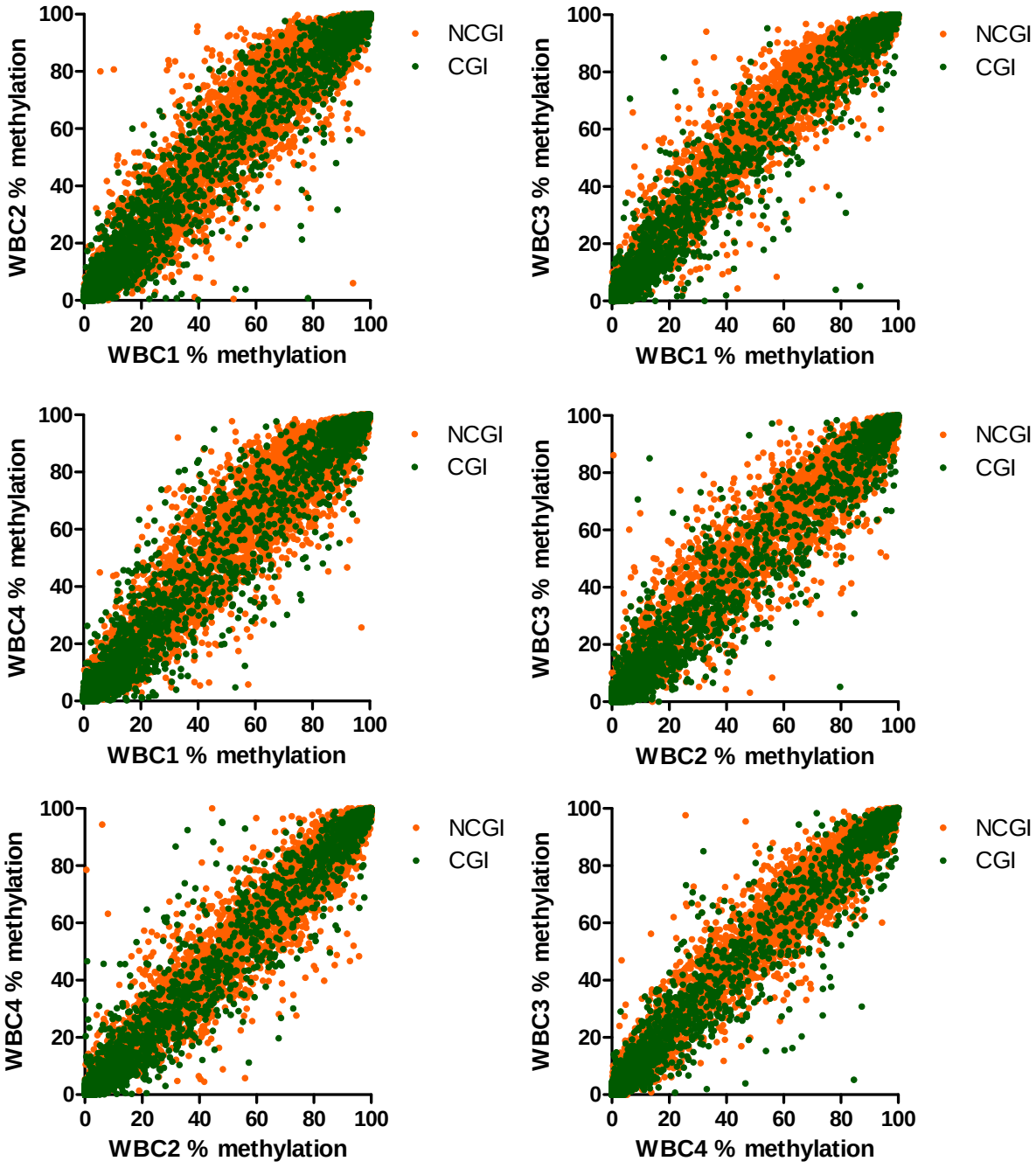


C



Supplementary Figure 10. Methylation on the X chromosome in female and male DNA. (A) Scatter plot showing methylation at 614 CCCGGG sites. Green, sites in CpG islands. Orange, sites outside CpG islands. (B) Sites in CpG islands within 1 kb TSS from TSS of genes reported as expressed on inactive X chromosome. (C) Sites in CpG islands within 1 kb TSS from TSS of genes reported as silenced on inactive X chromosome (Carrel and Willard 2005).



Supplementary Figure 11. Small differences in DNA methylation between pairs of WBC samples from healthy adults. Green, sites in CpG islands. Orange, sites outside of CpG islands.

Supplementary Table 1: Summary of sequencing data

Sample*	Number of lanes[#]		Reads in repeats	Uniquely mapped reads	Unique CpG sites with minimum coverage	
	GAII	HiSeq			20 reads	100 reads
NWBC1	6	0	13,614,227	28,033,541	46,967	40,396
NWBC2	0	3.25	38,884,926	72,110,046	123,505	47,965
NWBC3	2	0	7,216,231	13,368,413	40,487	32,165
NWBC4	2	0.5	31,303,581	51,251,441	64,881	42,202
CB1	0	2	64,859,753	120,476,642	133,273	48,486
CB2	0	2	63,084,746	113,023,565	145,810	50,921
AML	2	0	3,343,599	9,178,473	39,514	28,721
K562	2	0	6,386,209	10,075,503	49,630	31,121
HEL	2	0	5,129,563	7,169,746	53,202	25,316

*NWBC1-NWBC4, normal white blood cells; CB1, CB2, cord blood cells; AML, bone marrow from a patient with acute myeloid leukemia; K562, HEL, leukemia cell lines.

[#] GAII, Illumina Genome Analyzer II; HiSeq, Illumina HiSeq 2000.

Supplementary Table 2. Analysis of DNA inserts in DREAM libraries by cloning and Sanger sequencing.

DNA sample	WBC1	WBC3	HEL	K562
Informative ends*	91%	65%	67%	56%
SmaI sites both ends	85%	42%	60%	50%
SmaI sites single end	11%	46%	13%	13%
No SmaI site	4%	13%	27%	38%

Informative ends of fragment map to SmaI/XmaI site and start with GGG or CCGGG.

Supplementary Table 3. Primers for bisulfite PCR

Gene	PCR step	Primer name	Sequence
ACOT12	single	ACOT12-222F	ggttttaggagaagtttgggaag
		ACOT12-222R	aatcaaacatccaccaatcca
ADAM8	single	ADAM8-F387	ttttggaattatttatTAGGTAGT
		ADAM8-R387	ccttcccctccaaaacctac
CRIP1	1st	CRIP1-F385	gttttggggagggtaggatt
		CRIP1-R385	aaacccaaaacttaaaccctaaaa
	2nd	CRIP1-F385	gttttggggagggtaggatt
		CRIP1-R353	tctaaacccaaaacccaaaa
DPYS	1st	DPYS-F482	gggtatTTTGGTGTTTTGGTG
		DPYS-R482	aaactccaacccaaccttcc
	2nd	DPYS-F439	TTTATGAAGGGGAATTGTATGTG
		DPYS-R482	aaactccaacccaaccttcc
FAM65B	single	FAM65B-F351	gggtaaatgaagatttttgagattgtt
		FAM65B-R351	cctcaacttccaccaccctaa
IKBKB	1st	IKBKB-548F1	ggtttgtttatttttttttagttgtttagagattat
		IKBKB-373R	caaattcccctcaaaacca
	2nd	IKBKB-373F2	aGAGTAGGAAGTGTTTGAGGAAG
		IKBKB-373R	caaattcccctcaaaacca
PRRRG4	1st	PRRG4-153F	GTAGGGTTTGGATGGTTTT
		PRRG4-153R	accaaatatccccaaataac
	2nd	PRRG4-F126	TAAATTTGGGAAGGGTTTGG
		PRRG4-R126	caaatatccccaaataacc
RIPK4	single	RIPK4-F322	gggagagagaggtaggattt
		RIPK4-R322	CACCTAAACACCTAAACAACC
SLC35F3	single	SLC35F3-F136	gggatattgggtagtttgaggt
		SLC35F3-R136	tccccaataccctatcctaaa
TMEM132D	single	TMEM132D-F231	ggagtgatagttgtgagtttt
		TMEM132D-R231	aaaaacctcccctaaacctaaa
TTC22	1st	TTC22-F373	ttttgtgatttgggtaagtttt
		TTC22-R373	CCCTCTACTCCCCTAACCTCA
	2nd	TTC22-F245	tttttggtatatttggttgtgtaatg
		TTC22-R373	CCCTCTACTCCCCTAACCTCA
TUSC3	1st	TUSC3-F499	tggttttagattgaggttttaggg
		TUSC3-R499	tccattctacCTCCTTTTTCTTC
	2nd	TUSC3-F499	tggttttagattgaggttttaggg
		TUSC3-R347	ACAAAACAATATCTCCTCCAC

Supplementary Table 4. Methylation standards

Methylation 0%
>LA248
TCGAAAAAGAGCAGCACAGTGATGCCCGGGGAGGATACGTTTCACTATGAGAGCCTGCGTGGACGTTATGTGAGCGT
GATGGCCGGACCGGTTTTACAAATCAGTAAGCAGGTCAGTGCGTACGCCATGGCCGGAGTGGCTCACAGTCGGTGGT
CCGGCAGTACAATGGATTACCGTAAGACGGAAATCACTCCCGGGTATATGAAAGAGACGACCACTGCCAGGGACGAA
AGTGCAATGCGGCATAC

Methylation 25%
>L306
CCCTTTGATTGGATGGTTTGTAGGTGAGTTCCCGGGCGTTAATCAAAGAGGCGAACTGTGTGTGAGAGGTCCTATGA
TTATGTCCGGTTATGTAAACAATCCGGAAGCGACCAACGCCTTGATTGACAAGGATGGATGGCTACATTCTGGAGAC
ATAGCTTACTGGGACGAAGACGAACACTTCTTCATCGTTGACCGCCTGAAGTCTCTGATTAAGTACAAAGGCTATCA
GGTGGCTCCCGCTGAATTGGAATCCATCTTGCTCCAACACCCGGGAGAGGTTGAGTGATGGAGGTGAAGGGCGAA

Methylation 50%
>G414
TGTGGAATTGTGAGCGGATAACAATTTCACACAGGAAACAGCTATGACCATGATTACGCCAAGCTCAGAATTAACCC
TCACTAAAGGGACTAGTCCTGCAGGTTTAAACGAATTCGCCCTTCCCGGGTGGTGCCCATCCTGGTCGAGCTGGACG
GCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAG
TTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGACCTACGGCGTGCAGTGCTT
CAGCCGCTACCCGGGAAGGGCGAATTCGCGGCCGCTAAATTCAATTCGCCCTATAGTGAGTCGTATTACAATTCACT
GGCCGTCGTTTTACAACGTCGTGACTGGG

Methylation 75%
>T353
AGGACTTTCCCCGGCAACTCGCCCTCATCAAGGAGCTGGTGGACCTCCTGGGGCTGGCGCGCCTCGAGGTCCCGGGC
TACGAGGCGGACGACGTCCTGGCCAGCCTGGCCAAGAAGGCGGAAAAGGAGGGCTACGAGGTCCGCATCCTCACCGC
CGACAAAGACCTTTACCAGCTCCTTTCCGACCGCATCCACGTCCTCCACCCCGAGGGGTACCTCATCACCCCGGCCT
GGCTTTGGGAAAAGTACGGCCTGAGGCCCGACCAGTGGGCCGACTACCGGGCCCTGACCGGGGACGAGTCCGACAAC
CTTCCCGGGGTCAAGGGCATCGGGGAGAAGACGGCGAGGAAGCTT

Methylation 100%
>T324
GCGGGACATCCACACGGAGACCGCCAGCTGGATGTTCGGCGTCCCCCGGGAGGCCGTGGACCCCCTGATGCGCCGGG
CGGCCAAGACCATCAACTTCGGGGTCCTCTACGGCATGTCGGCCCACCGCCTCTCCCAGGAGCTAGCCATCCCTTAC
GAGGAGGCCCAGGCCTTCATTGAGCGCTACTTTCAGAGCTTCCCCAAGGTGCGGGCCTGGATTGAGAAGACCCTGGA
GGAGGGCAGGAGGCGGGGGTACGTGGAGACCCTCTTCGGCCGCCGCCGCTACGTGCCAGACCTAGAGGCCCGGGTGA
AGAGCGTGCGGGAGGC