Supporting Information

Rauch *et al.* 10.1073/pnas.0812399106

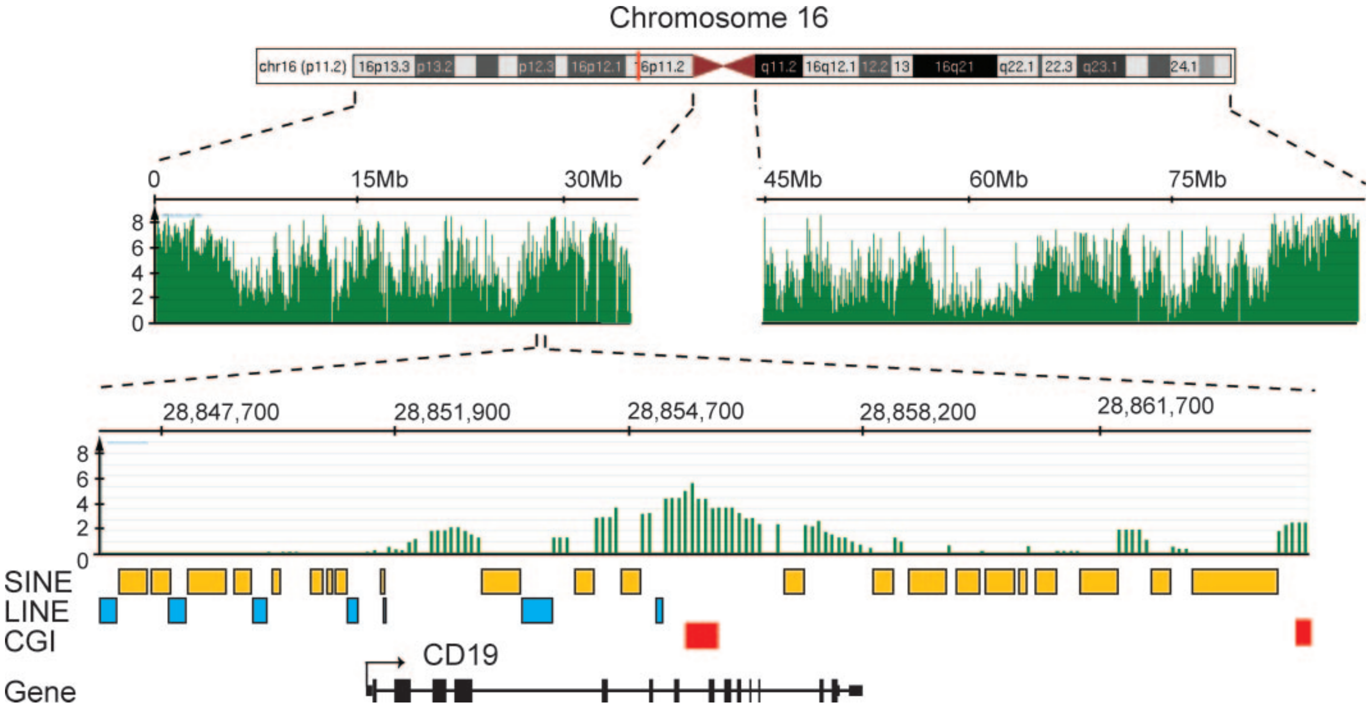


Fig. S1. Analysis of CpG methylation at the *CD19* locus on chromosome 16. The *CD19* gene on chromosome 16 was analyzed. The methylation profile is shown plotted along the chromosome as a −log10 *P* value score (green). The *P* value score was obtained by NimbleScan software and is derived from the Kolmogorov-Smirnov test comparing the log2 ratios (MIRA vs. input) within a 750-bp window centered at each probe and the rest of the data on the array. The *CD19* gene, its exons, and the direction of transcription are indicated. SINE and LINE sequences are shown as yellow and blue boxes, respectively. The red boxes show the two CpG islands (CGI) located in this region. The highest methylation signals are seen in the body of the gene and at its 3′ end, but methylation is absent at the promoter.

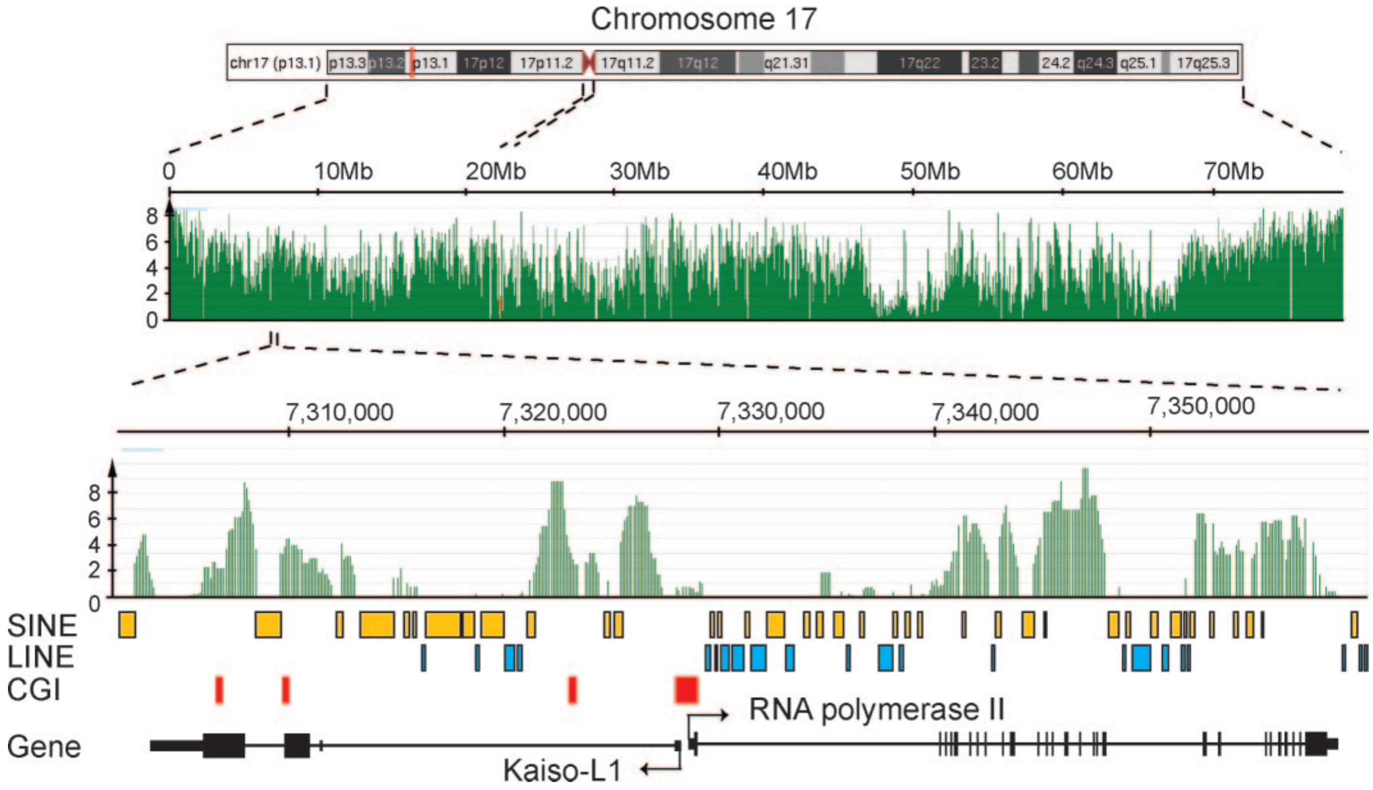


Fig. S2. Analysis of CpG methylation at the RNA polymerase II locus on chromosome 17. The RNA polymerase II and Kaiso-L1 genes on chromosome 17 were analyzed. The methylation signal is shown plotted along the chromosome as a −log10 *P* value score (green). The 2 genes, their exons, and the directions of transcription are indicated. SINE and LINE sequences are shown as yellow and blue boxes, respectively. The red boxes show CpG islands (CGI). The highest methylation signals are seen in the gene bodies, whereas methylation levels in the bidirectional promoter are low.

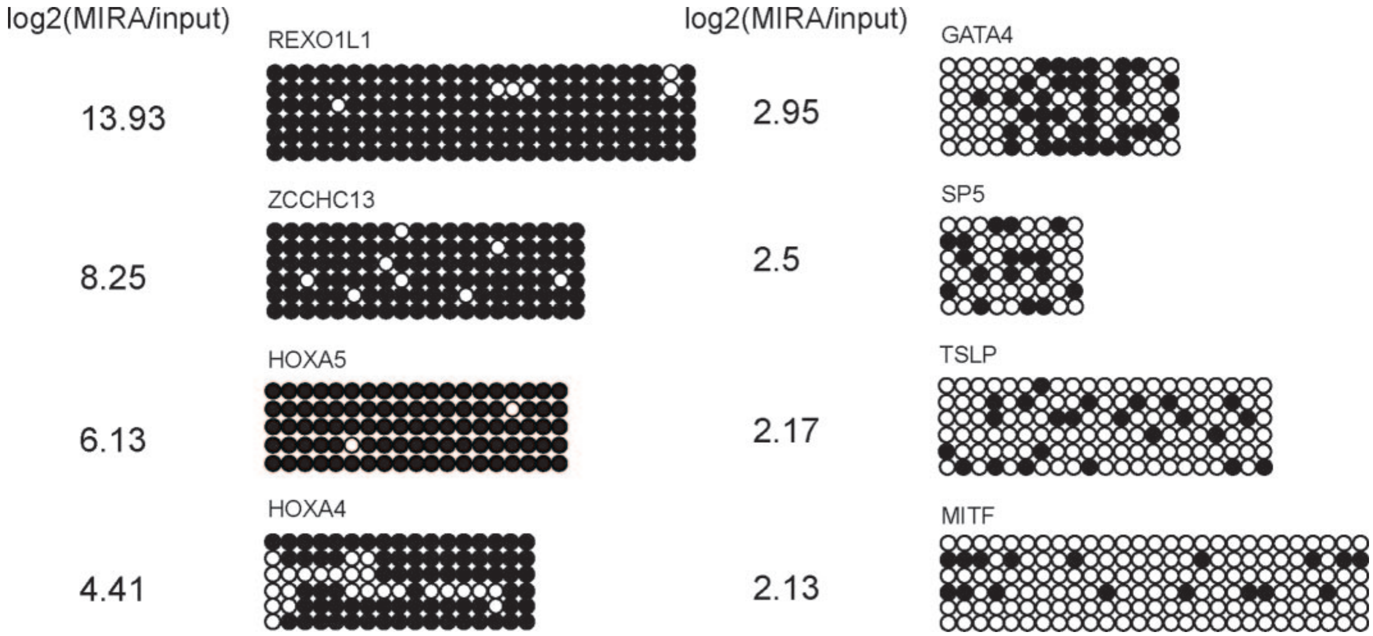


Fig. S3. Correlation of array data with bisulfite sequencing data. An enrichment factor representing the signal score of each identified MRI was calculated by averaging the log2 ratios of MIRA-enriched DNA vs. input DNA of probes within the MRI. Gene promoters overlapping with MRIs showing a range of enrichment factors were chosen at random. Bisulfite sequencing analysis was performed for the gene targets shown. Gene targets with the highest enrichment factors show the densest CpG methylation patterns.

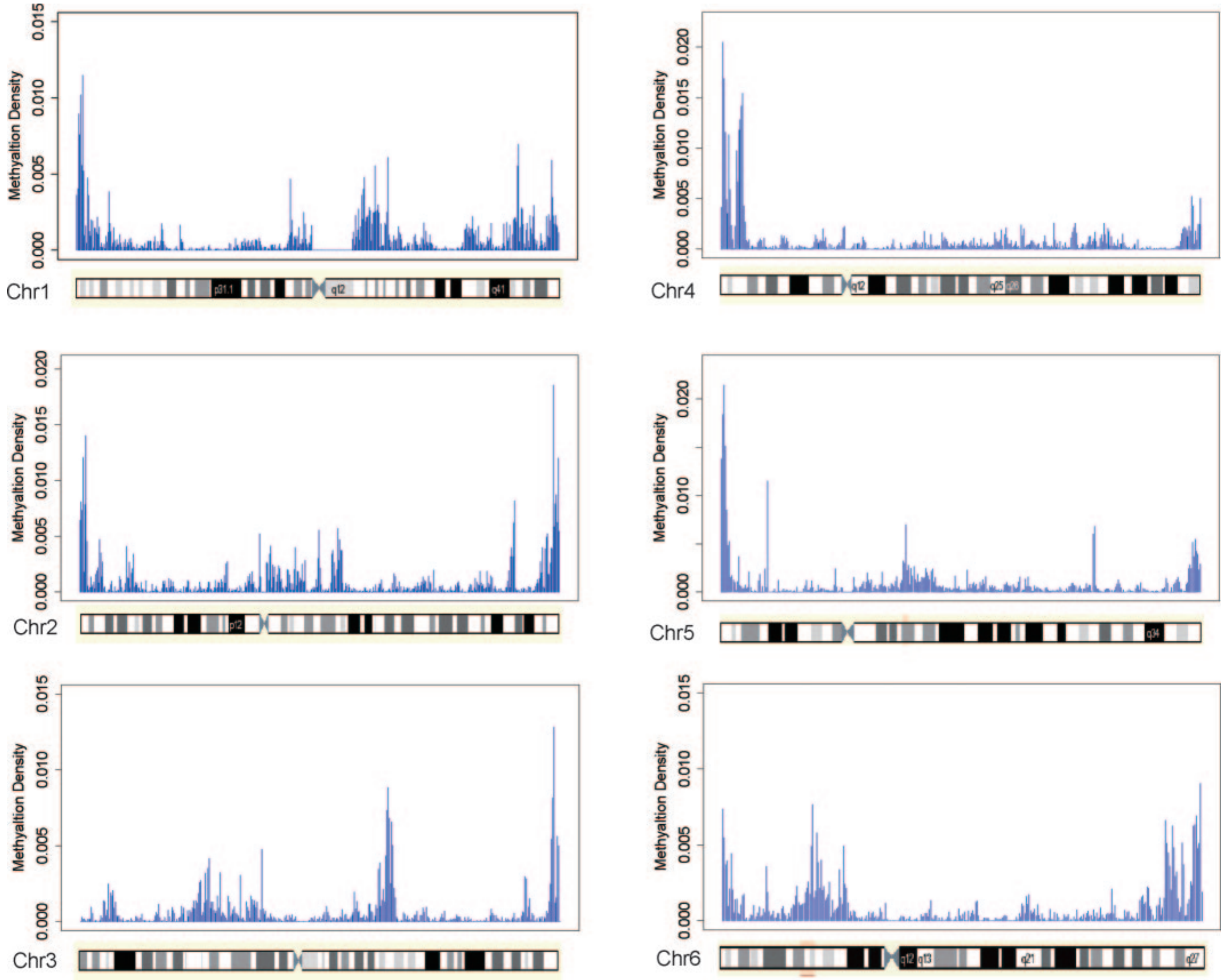


Fig. S4. Methylation patterns along human chromosomes 1–6. Chromosomal methylation density maps are shown for chromosomes 1–6. See main text for details. The chromosome banding pattern was derived from the UCSC Genome Browser.

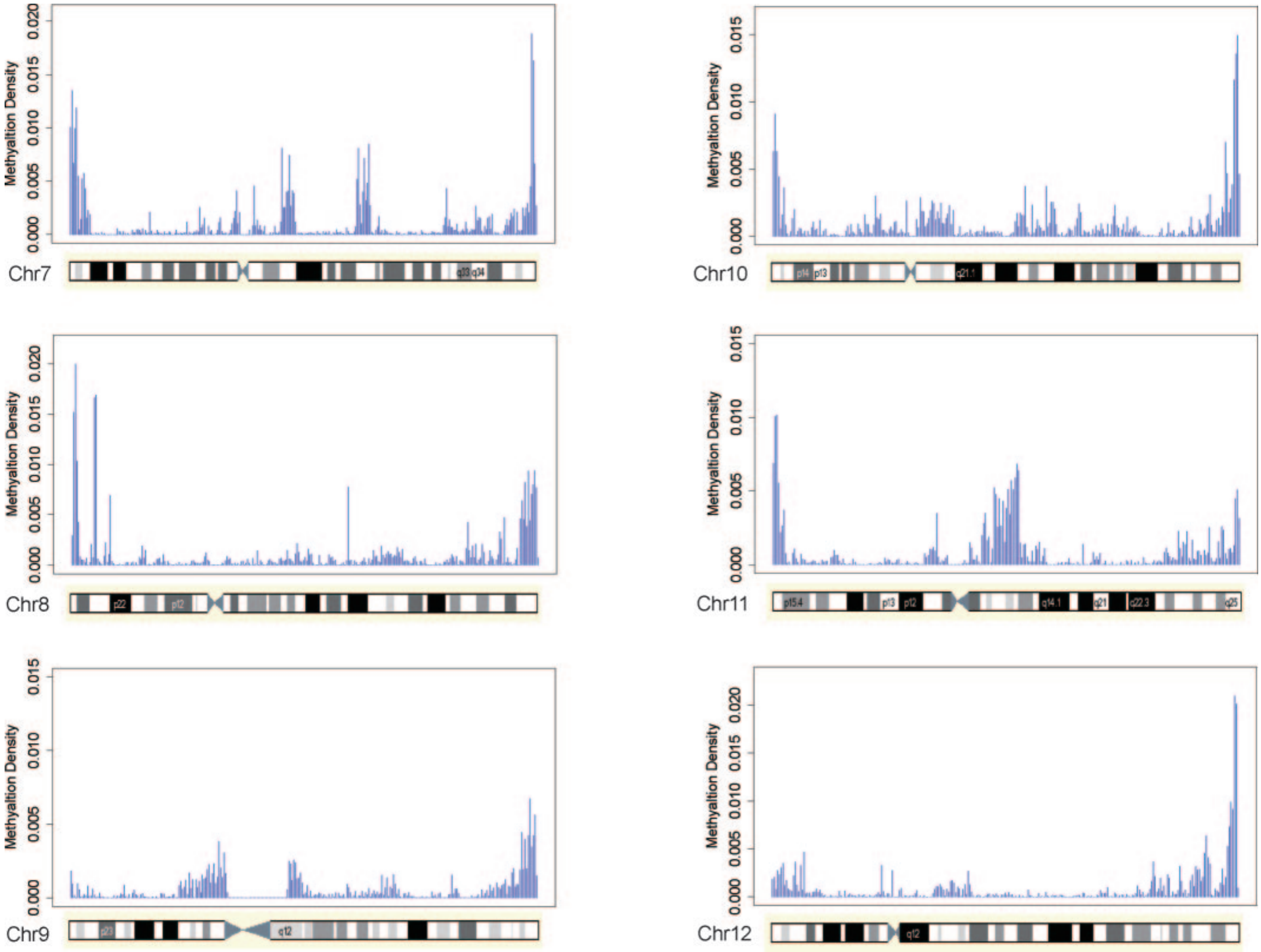


Fig. S5. Methylation patterns along human chromosomes 7–12. Chromosomal methylation density maps are shown for chromosomes 7–12.

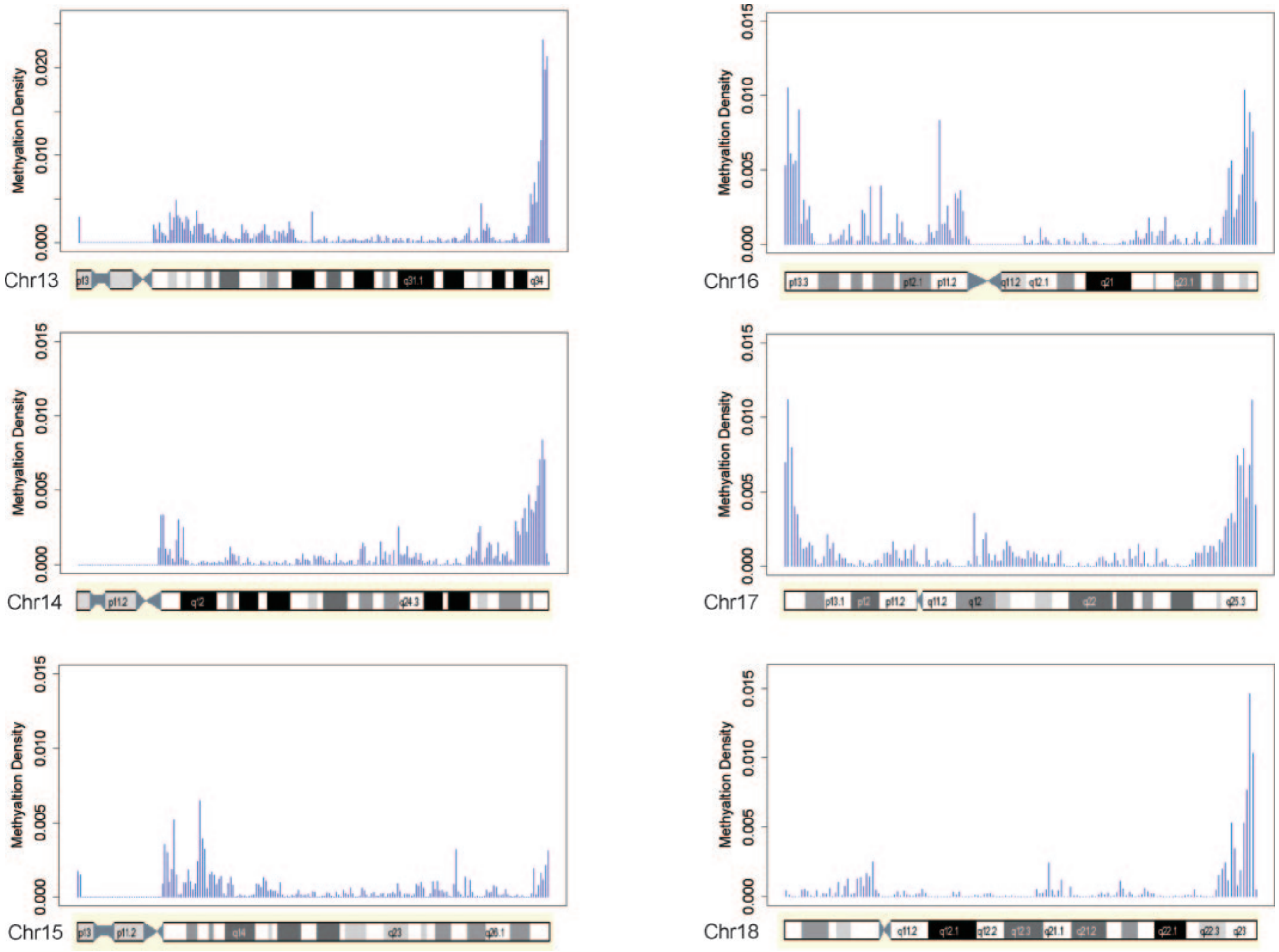


Fig. S6. Methylation patterns along human chromosomes 13–18. Chromosomal methylation density maps are shown for chromosomes 13–18.

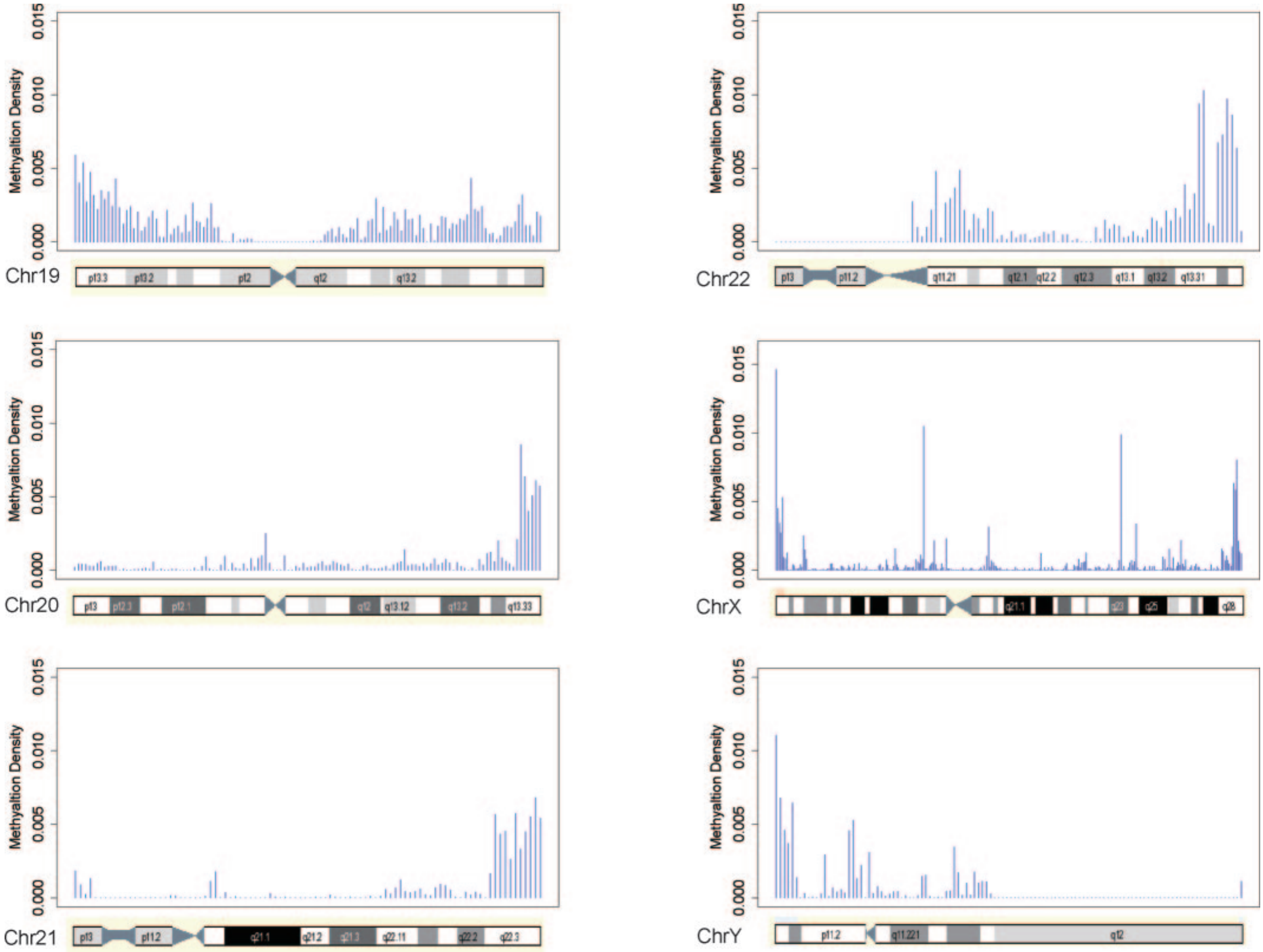
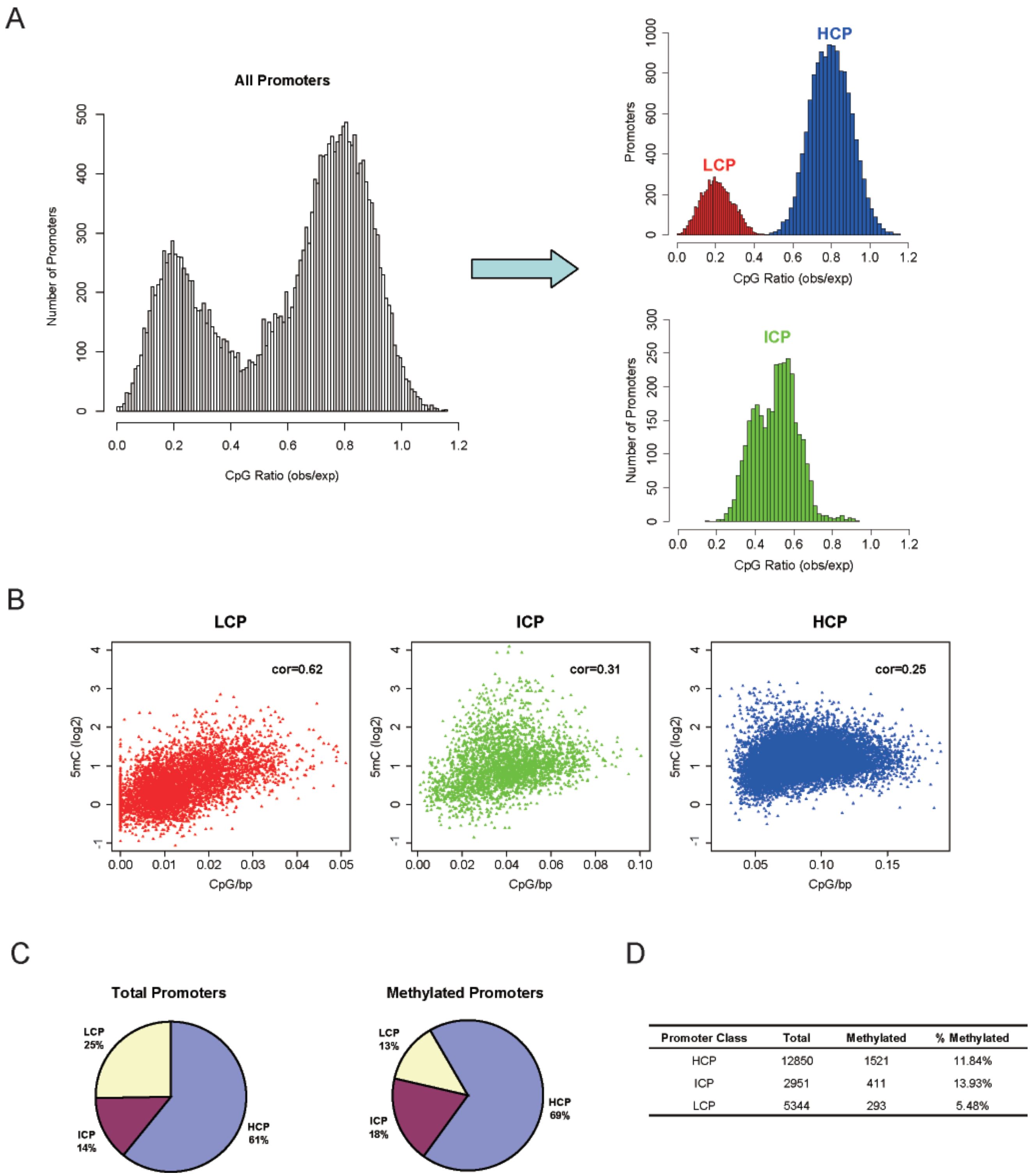


Fig. S7. Methylation patterns along human chromosomes 19–22, X, and Y. Chromosomal methylation density maps are shown for chromosomes 19–22, X, and Y. The short arm of chromosome 22 is not covered by probes, owing to a series of tandem repeat structures.



Promoter Class	Total	Methylated	% Methylated
HCP	12850	1521	11.84%
ICP	2951	411	13.93%
LCP	5344	293	5.48%

Fig. S8. Levels of DNA methylation in different promoter classes. (*A*) Promoters were classified according to CpG density as described by Weber *et al.* (2007) *Nature Genet* 39:457–466. Low CpG (LCP), intermediate CpG (ICP), and high CpG (HCP) promoters were defined. (*B*) The scatterplots show the DNA methylation levels (log2 of MIRA signals vs. input) for the 3 classes of promoters. Each spot represents 1 promoter. (*C* and *D*). The pie charts and table show the relative frequency of the promoter classes among all promoters and methylated promoters, the latter being defined as overlapping an MRI (see main text for definition). ICP and HCP promoters are overrepresented among the methylated promoters.

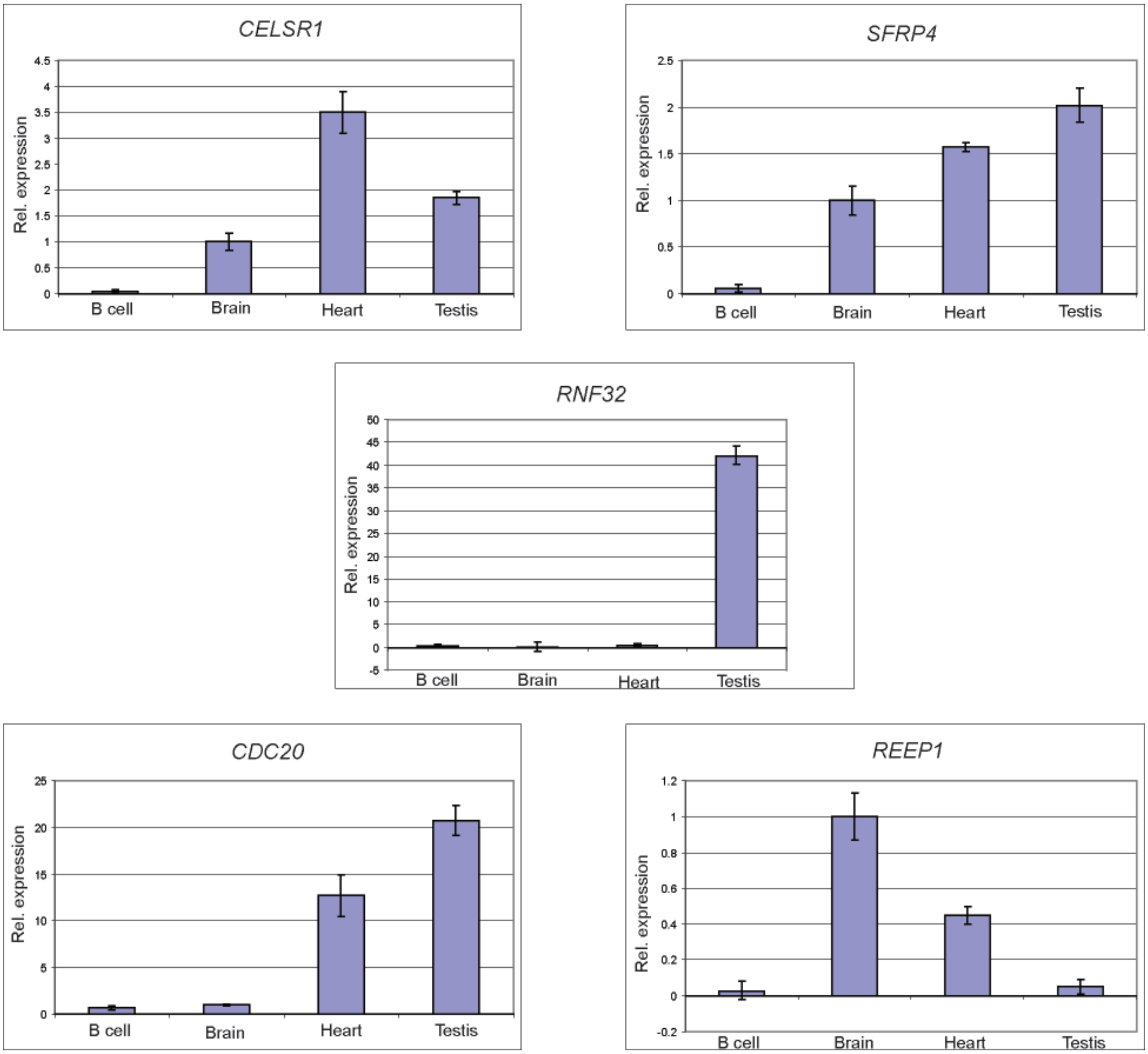


Fig. S9. RT-PCR analysis of promoter-methylated genes. Five genes methylated at their promoters in B cells were analyzed by real-time RT-PCR. These genes (*CELSR1*, *SFRP4*, *RNF32*, *CDC20*, and *REEP1*) are expressed at very low levels in B cells but are expressed substantially in brain, heart, or testis.

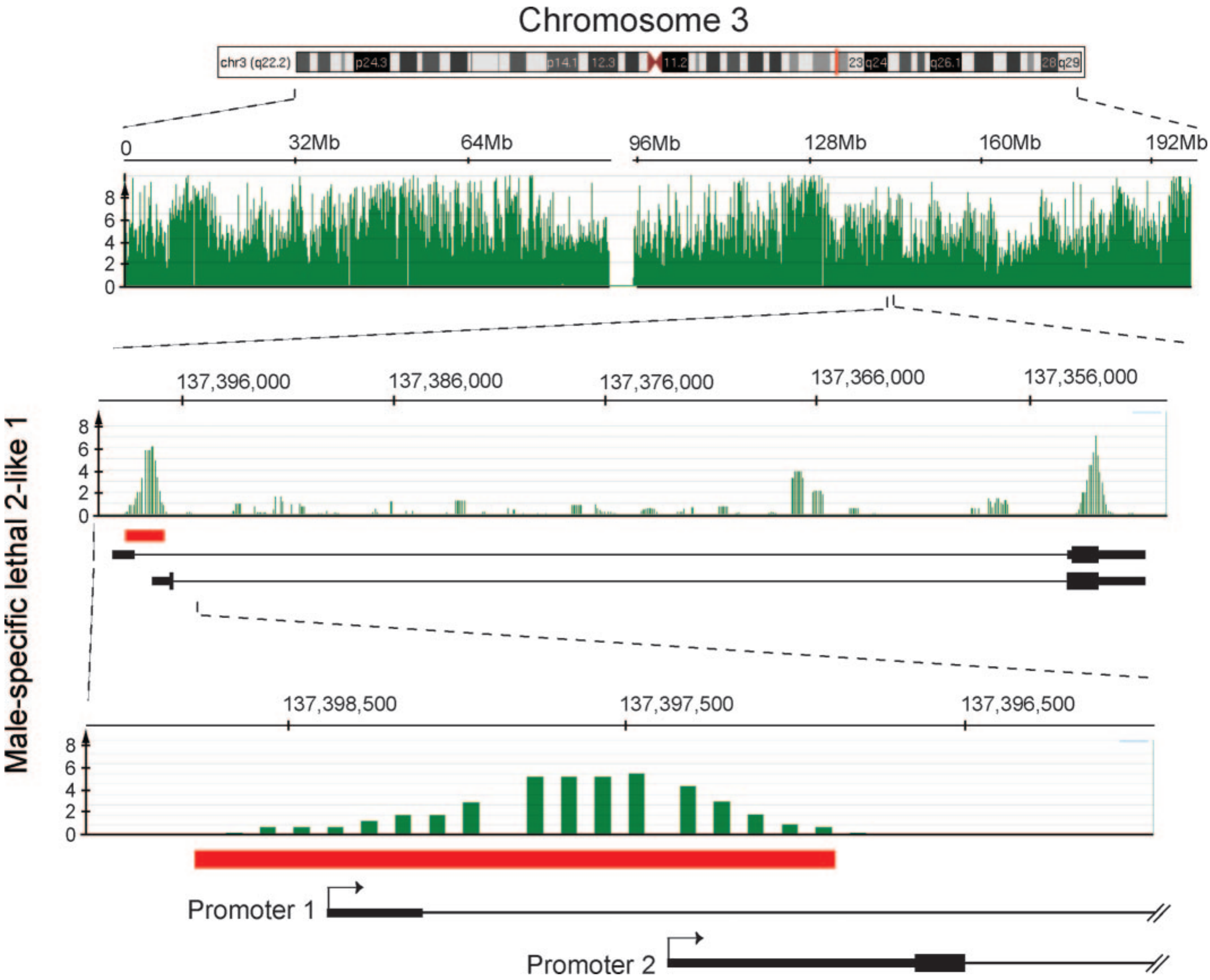


Fig. S10. Expression of the methylated male-specific lethal 2-like 1 gene is explained by alternative promoter usage. The methylation profile is in green, and the red box indicates a methylated CpG island associated with the 5′ end of this gene. The gene is expressed in B cells according to expression microarray databases. Promoter 2 is highly methylated. Using 5′-RACE, we identified an alternative transcript that emanates from an undermethylated region of the CpG island (promoter 1).

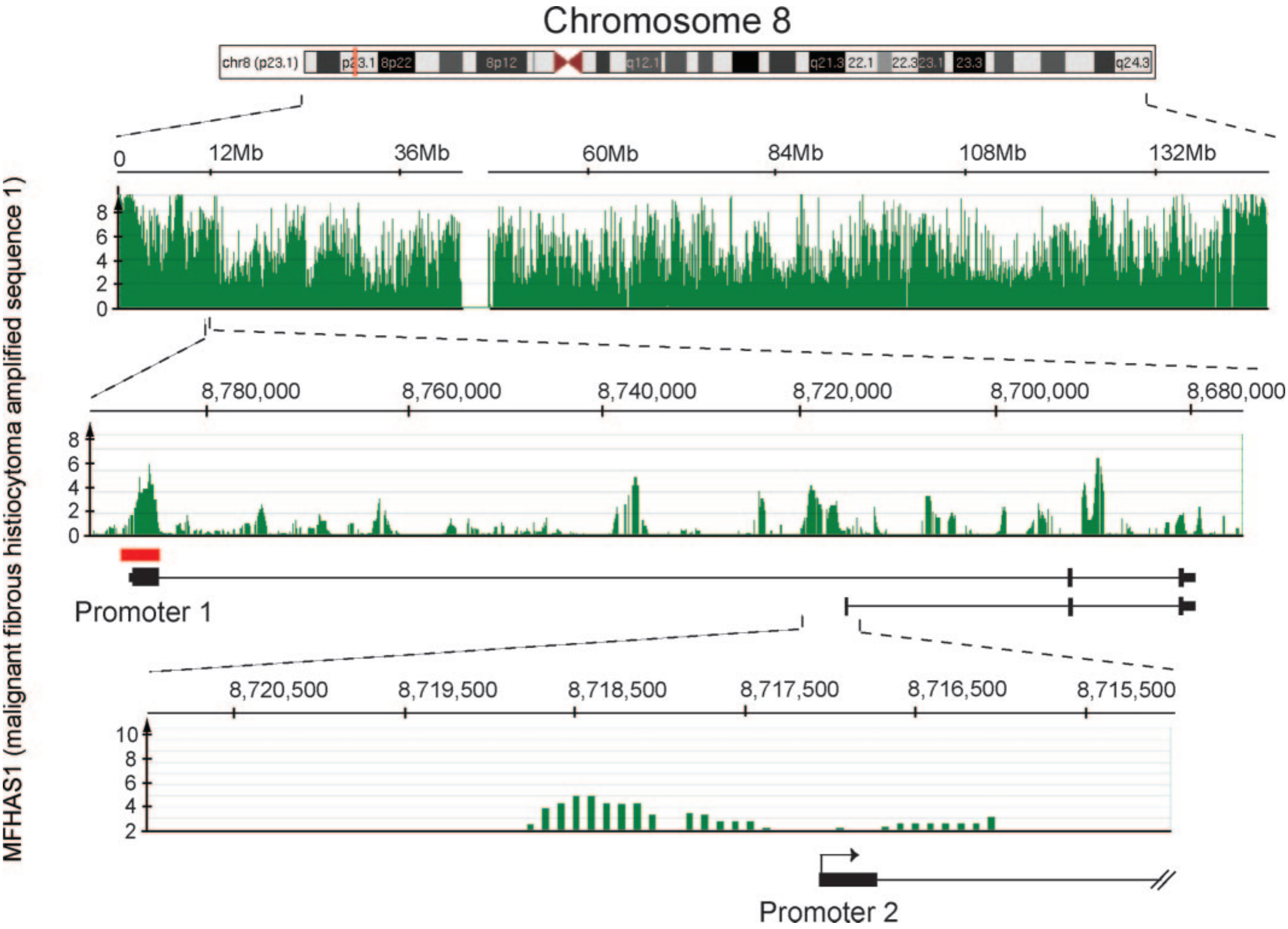


Fig. S11. Expression of the methylated *MFHAS1* gene is explained by alternative promoter usage. Data for the *MFHAS1* gene are shown. The methylation profile is in green, and the red box indicates a methylated CpG island associated with promoter 1. The gene is expressed in B cells according to expression microarray databases. Using 5′-RACE, we identified an alternative transcript that emanates from an unmethylated promoter (promoter 2).

Table S1. Peak summary

Chromosome	No. of MRIs*	Chromosome length	Probe coverage	% Coverage	Methylated[†]	% Methylated
chr1	3,219	247,249,719	114,350,500	46.25	1,929,456	1.69
chr2	3,950	242,951,149	126,623,400	52.12	2,381,662	1.88
chr3	2,240	199,501,827	100,243,800	50.25	1,317,996	1.31
chr4	2,829	191,273,063	94,850,500	49.59	1,769,971	1.87
chr5	2,746	180,857,866	91,535,000	50.61	1,710,863	1.87
chr6	2,595	170,899,992	87,608,200	51.26	1,558,995	1.78
chr7	2,684	158,821,424	79,698,100	50.18	1,672,219	2.10
chr8	2,145	146,274,826	73,138,600	50.00	1,507,987	2.06
chr9	1,348	140,273,252	61,475,900	43.83	763,946	1.24
chr10	2,118	135,374,737	69,473,900	51.32	1,314,458	1.89
chr11	1,951	134,452,384	66,295,300	49.31	1,117,955	1.69
chr12	1,983	132,349,534	64,957,800	49.08	1,196,696	1.84
chr13	1,906	114,142,980	51,347,600	44.99	1,230,765	2.40
chr14	1,110	106,368,585	45,427,900	42.71	672,916	1.48
chr15	854	100,338,915	42,605,200	42.46	503,248	1.18
chr16	1,573	88,827,254	39,836,400	44.85	957,833	2.40
chr17	1,436	78,774,742	40,469,400	51.37	810,637	2.00
chr18	706	76,117,153	40,769,300	53.56	456,164	1.12
chr19	1,544	63,811,651	23,691,600	37.13	804,685	3.40
chr20	707	62,435,964	30,194,500	48.36	391,222	1.30
chr21	538	46,944,323	18,295,000	38.97	320,232	1.75
chr22	1,083	49,691,432	17,943,600	36.11	679,994	3.79
chrX	1,094	154,913,754	61,022,300	39.39	732,435	1.20
chrY	485	57,772,954	9,618,100	16.65	379,048	3.94
Total	42,844	3,080,419,480	1,451,471,900	47.12	26,181,383	1.80

*For definition of MRI, see Table 1 and main text.

[†] The number is the sum of the length of all identified MRIs on each chromosome.

Table S2. Broad MRIs (>10 kb)

Chromosome	Location	Start	End	Length	Ratio	Features
chr4	4P16.1	8,935,462	8,979,842	44,381	6.54	USP17, segmental duplication
chr8	8P23.3	1,328,022	1,343,471	15,450	8.34	Many small CpG islands, simple tandem repeat, segmental duplication
chr4	4q36.2	188,117,137	188,131,786	14,650	6.36	Large CpG island, simple tandem repeat, also segmental duplication
chr7	7q22.1	100,420,733	100,434,982	14,250	4.14	Simple tandem repeat, also segmental duplication
chr15	15q26.3	100,110,103	100,123,352	13,250	6.11	Simple tandem repeat, also segmental duplication
chr14	14q32.33	104,766,142	104,778,791	12,650	16.00	Intron of BRF1, simple tandem repeat, segmental duplication
chr8	8p23.1	12,023,169	12,035,492	12,324	4.69	Segmental duplication
chr1	1q21.3	150,542,395	150,553,167	10,773	4.35	FLG exon, simple tandem repeat, segmental duplication
chr3	3q29	196,990,413	197,000,862	10,450	3.39	MUC4 exon, simple tandem repeat, segmental duplication
chrX	Xp22.31	9,332,329	9,342,619	10,291	5.17	Simple tandem repeat, segmental duplication