Supplementary Figures and Tables

Whole-genome fingerprint of the DNA methylome during human B-cell differentiation

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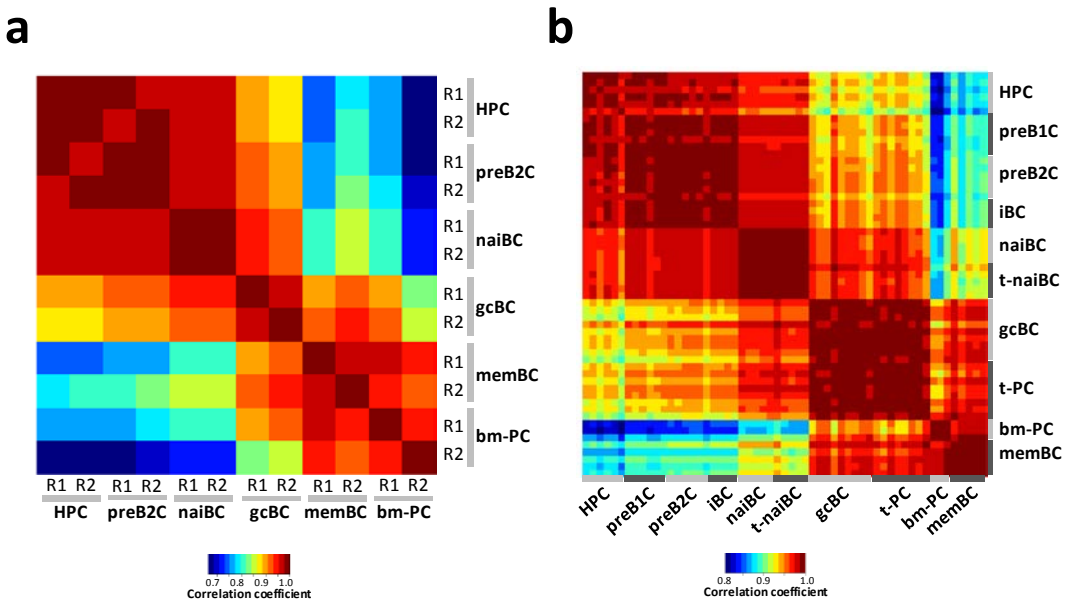
Supplementary figures



Supplementary Fig. 1

Reproducibility of DNA methylation data generated by WGBS and 450k microarrays. Heatmap showing the correlation matrices (Pearson correlation coefficient) of pair wise comparisons using 16.1 million CpGs with methylation estimates across all samples by WGBS (a) and 475,030 sites by 450k arrays (b). HPC: hematopoietic progenitor cell. preB1C: pre-B-I cell. preB2C: pre-B-II cell. iBC: immature B cell. naiBCs: naive B cell from peripheral blood. t-naiBCs: naive B cell from tonsil. gcBC: germinal center B cell. t-PC: plasma cell from tonsil. memBC: memory B cell from peripheral blood. bm-PCs: plasma cell from bone marrow. R1: first set of replicates. R2: second set of replicates.

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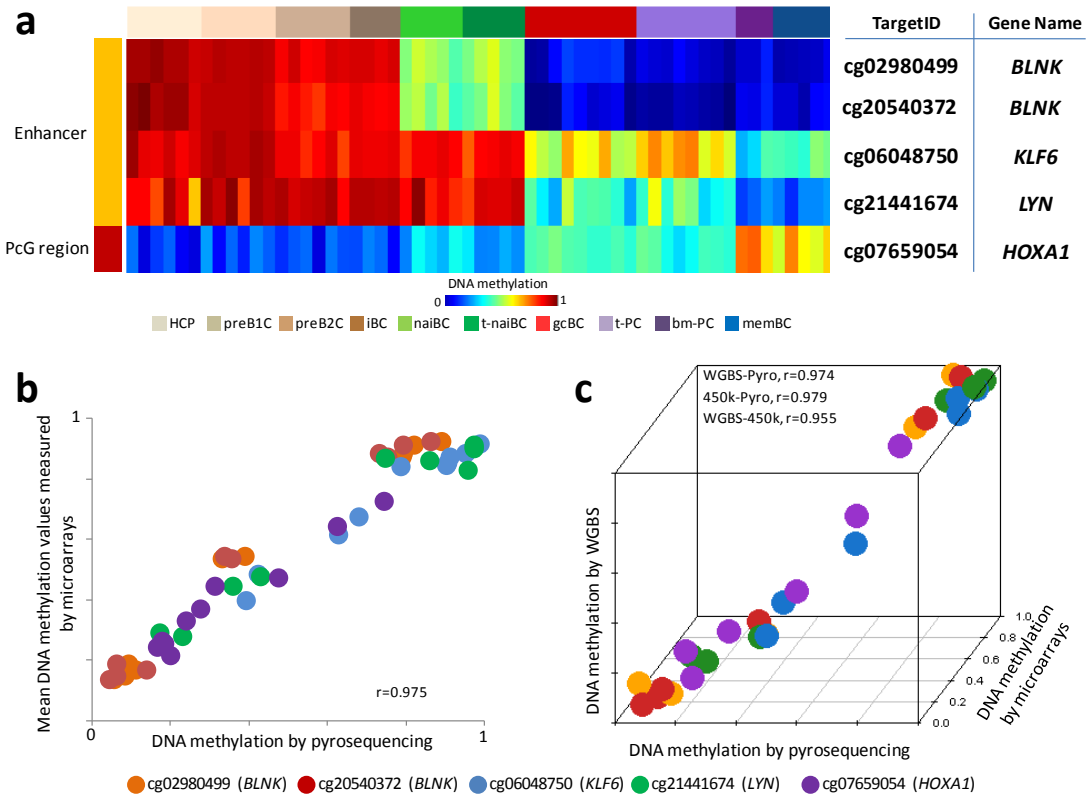

Supplementary Fig. 2

Validation of WGBS and microarray DNA methylation data by bisulfite pyrosequencing. (a) Five CpGs with distinct methylation patterns throughout B-cell differentiation, as measured by microarrays, were selected for validation by bisulfite pyrosequencing. (b) Scatter plot showing the correlation between DNA methylation values generated by microarrays and bisulfite pyrosequencing. c) 3D scatter plot showing the correlation among WGBS, microarrays and bisulfite pyrosequencing. These analyses show that all three techniques generate highly reproducible DNA methylation estimates, being all correlation coefficients of pair-wise comparisons above 0.95. All three techniques were done with independent sorted cell subpopulations and therefore, in addition to the technical reproducibility, this analysis also underscores the high biological reproducibility. HPC: hematopoietic progenitor cell. preB1C: pre-B-I cell. preB2C: pre-B-II cell. iBC: immature B cell. naiBCs: naive B cell from peripheral blood. t-naiBCs: naive B cell from tonsil. gcBC: germinal center B cell. t-PC: plasma cell from tonsil. memBC: memory B cell from peripheral blood. bm-PCs: plasma cell from bone marrow

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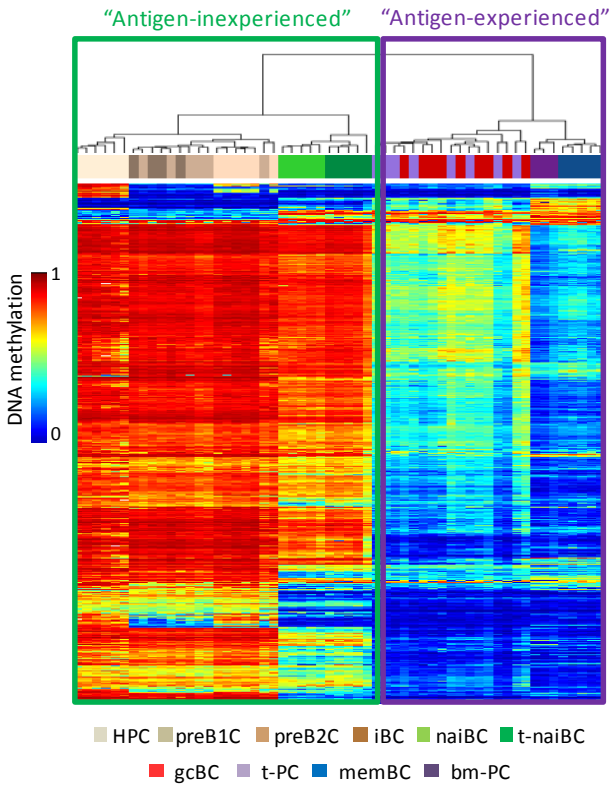

Supplementary Fig. 3

Unsupervised clustering of DNA methylation data of all B-cell subpopulations. Hierarchical clustering of the 81,468 CpGs with the most variable methylation levels (SD > 0.1) among all the samples. Two major clusters can be identified: "antigen-inexperienced" B-cell subpopulations (i.e. HPCs, preBCs and naiBCs) marked with green box and "antigen-experienced" B-cell subpopulations (i.e. gcBCs, memBCs and PCs) marked with violet box. HPC: hematopoietic progenitor cell. preB1C: pre-B-I cell. preB2C: pre-B-II cell. iBC: immature B cell. naiBCs: naive B cell from peripheral blood. t-naiBCs: naive B cell from tonsil. gcBC: germinal center B cell. t-PC: plasma cell from tonsil. memBC: memory B cell from peripheral blood. bm-PCs: plasma cell from bone marrow.

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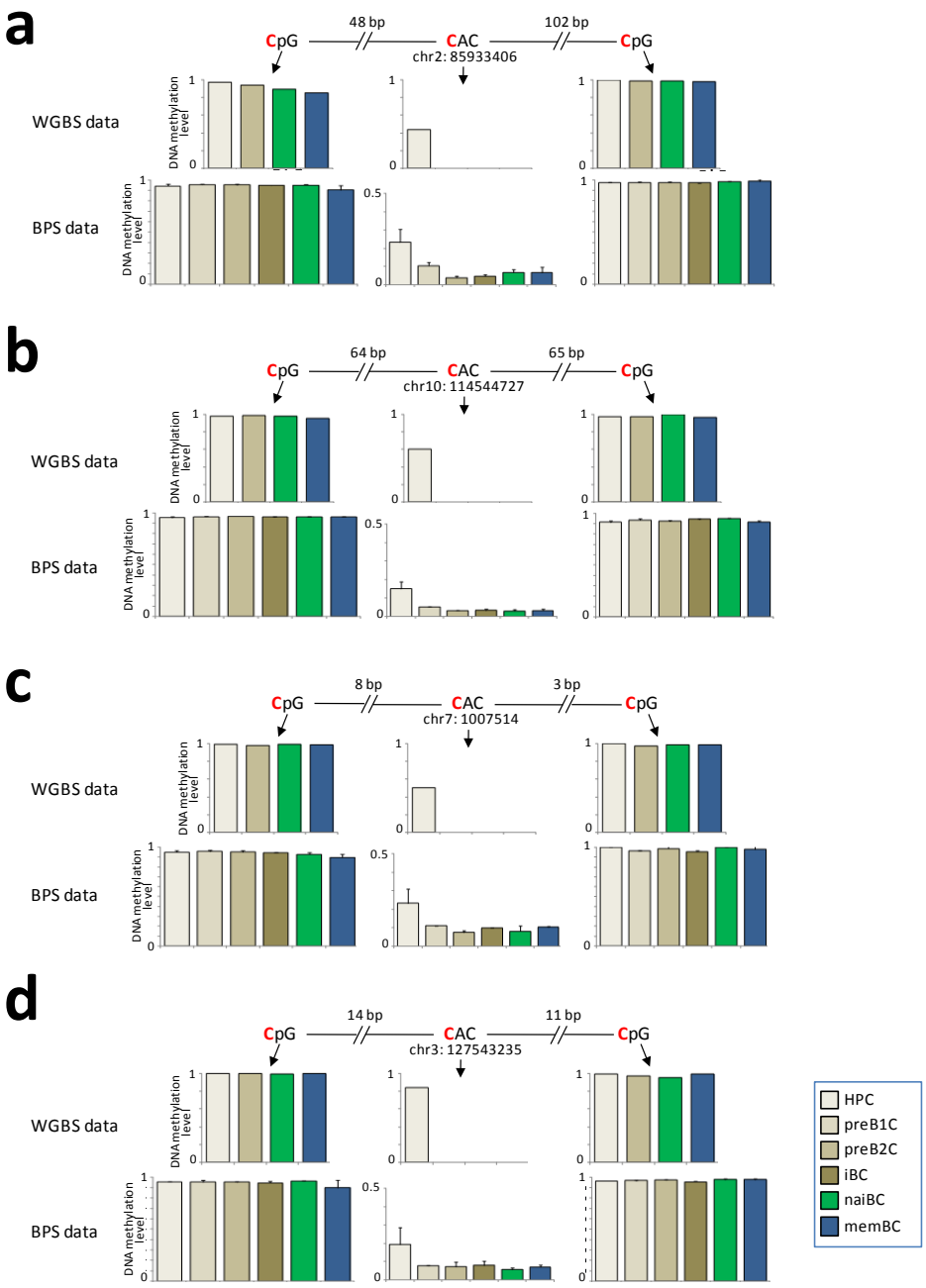

Supplementary Fig. 4

Validation by bisulfite pyrosequencing (BPS) of the presence of non-CpG methylation in HPCs and demethylation upon B- cell commitment independent of changes in flanking CpG sites. The four different regions shown in panels a to d confirm that non-CpG methylation sharply decreases upon B-cell commitment in the absence of simultaneous demethylation of flanking CpGs (independently of the distance between non-CpG and CpGs sites). In the subsequent maturation stages non-CpG methylation is at the detection threshold of BPS, and therefore can be considered negligible. Above BPS data representation, we also show data obtained by WGBS. The two techniques were performed in two independent biological replicates, confirming that non-CpG methylation of the studied sites in HPCs is conserved. HPC: hematopoietic progenitor cell. preB1C: pre-B-I cell. preB2C: pre-B-II cell. iBC: immature B cell. naiBCs: naive B cell from peripheral blood. memBC: memory B cell from peripheral blood.

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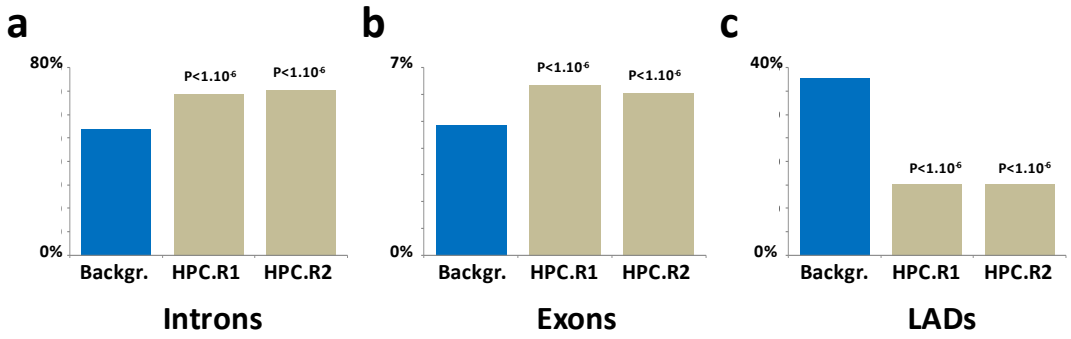
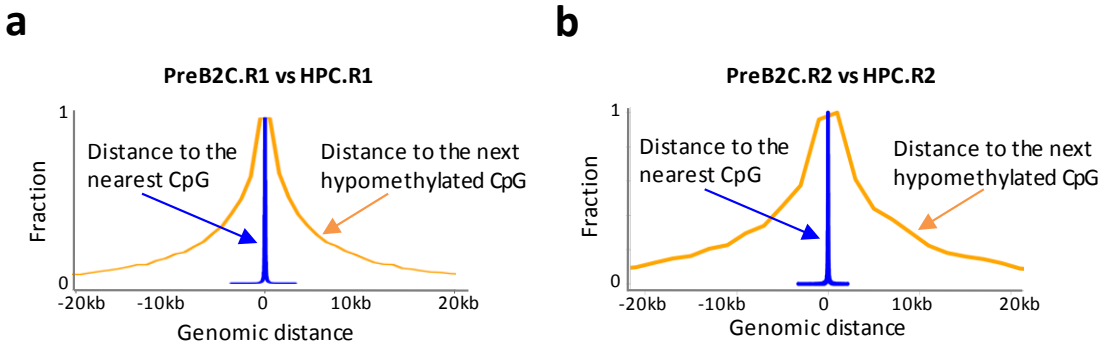


Supplementary Fig. 5

Genomic location of methylated non-CpGs sites. The non-CpG sites methylated in hematopoietic precursor cells (HPC) are significantly enriched in (a) introns and (b) exons, and depleted in (c) lamina-associated domains (LADs). As background (Backgr.), we used the percentage of cytosines in non-CpG context located within each feature in the whole genome.



Supplementary Fig. 6

Distribution of the distance of hypomethylated non-CpGs and CpGs in preB2Cs as compared to HPCs. The genomic distance from hypomethylated non-CpGs in preB2C vs. HPC to the nearest CpG (shown by a blue line) is much closer than the distance to the nearest hypomethylated CpG (shown by an orange line) ($P < 2.2x10^{-16}$). Data from the first (a) and second (b) set of biological replicates are shown. HPC: hematopoietic progenitor cell. preB2C: pre-B-II cell.

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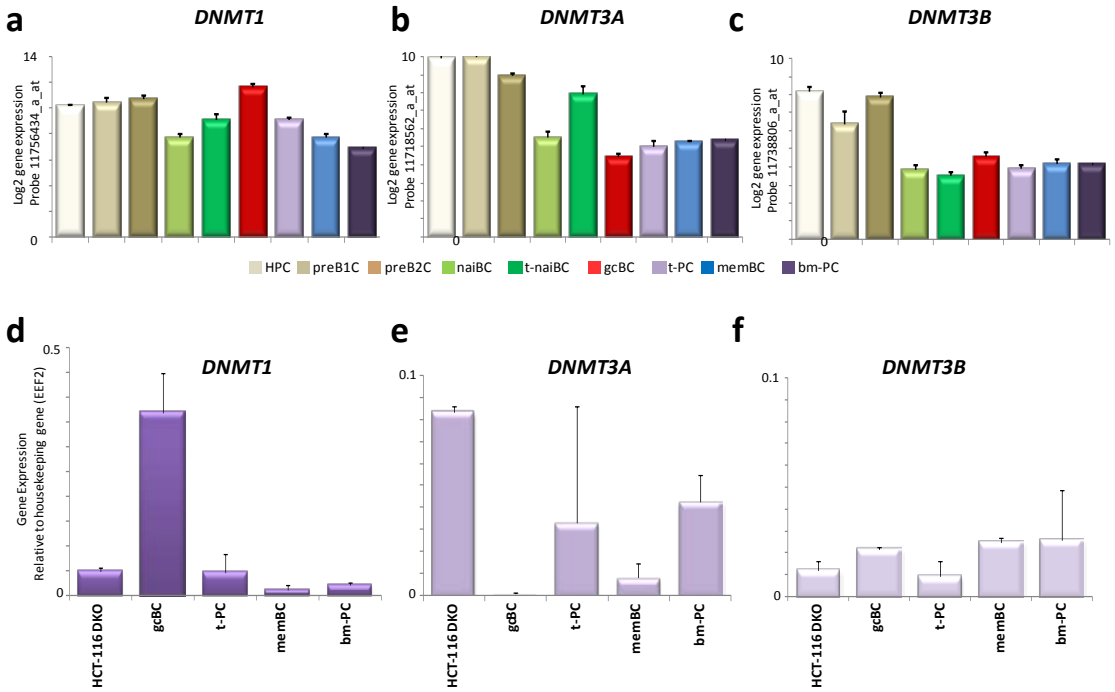

Supplementary Fig. 7

Expression of DNMTs in B-cell differentiation stages. Expression of *DNMT1* (a), *DNMT3A* (b) and *DNMT3B* (c) throughout the B-cell differentiation was measured by microarrays (a-c). Additionally, in late B-cell differentiation stages, we also measured expression of *DNMT1* (d), *DNMT3A* (e) and *DNMT3B* (f) respect to housekeeping gene (*EEF2*) by real-time qPCR.

HCT-116 DKO: *DNMT1* and *DNMT3B* double knock-out cell line. HPC: hematopoietic progenitor cell. preB1C: pre-B-I cell. preB2C: pre-B-II cell. naiBCs: naive B cell from peripheral blood. t-naiBCs: naive B cell from tonsil. gcBC: germinal center B cell. t-PC: plasma cell from tonsil. memBC: memory B cell from peripheral blood. bm-PCs: plasma cell from bone marrow

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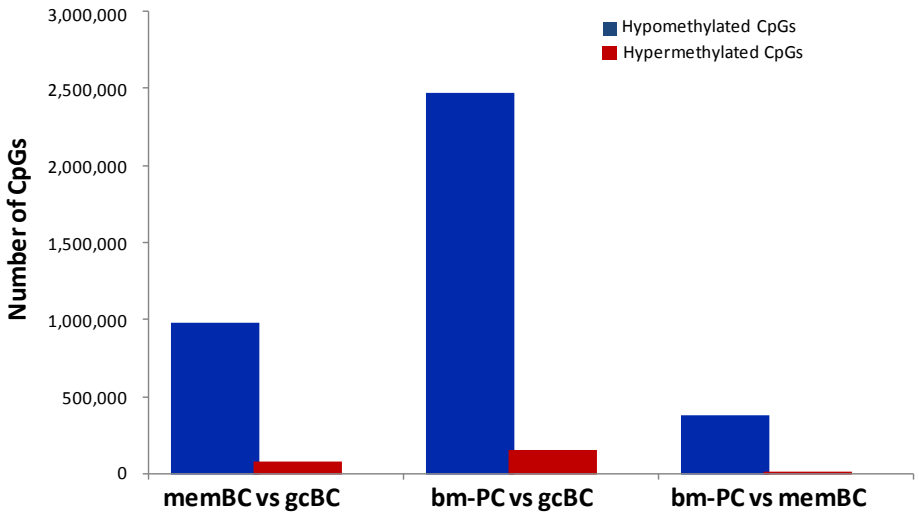

Supplementary Fig. 8

Analysis of the DNA methylation changes in gcBCs, memBCs and bm-PCs. Bars represent the number of hypo- and hypermethylated CpGs detected by WGBS for each comparison using data from two independent replicates per cell subpopulatation. gcBC: germinal center B cell. memBC: memory B cell from peripheral blood. bm-PC: plasma cell from bone marrow.

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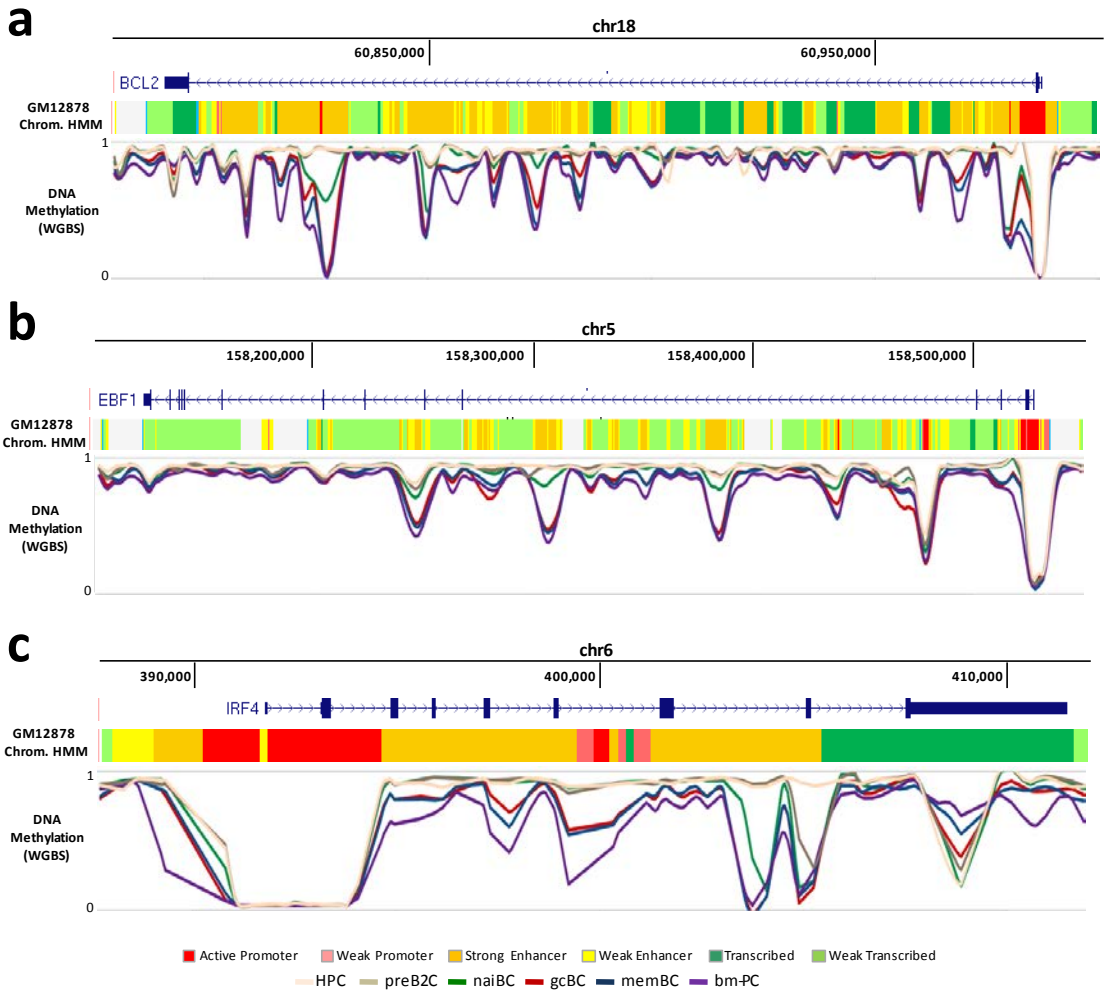

Supplementary Fig. 9

Modulation of the DNA methylation pattern of key B-cell genes during the differentiation process. This analysis shows smoothed DNA methylation data generated by WGBS across the promoter region and gene body of (a) *BCL2*, (b) *EBF1* and (c) *IRF4*. The DNA methylation pattern of these genes is widely modulated in different B-cell subpopulations, especially in enhancer regions. HPC: hematopoietic progenitor cell. preB2C: pre-B-II cell. naiBCs: naive B cell from peripheral blood. gcBC: germinal center B cell. memBC: memory B cell from peripheral blood. bm-PCs: plasma cell from bone marrow

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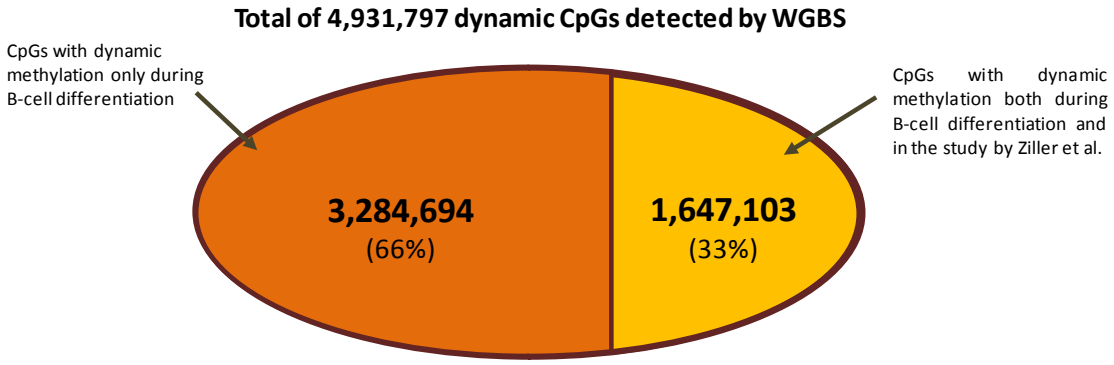
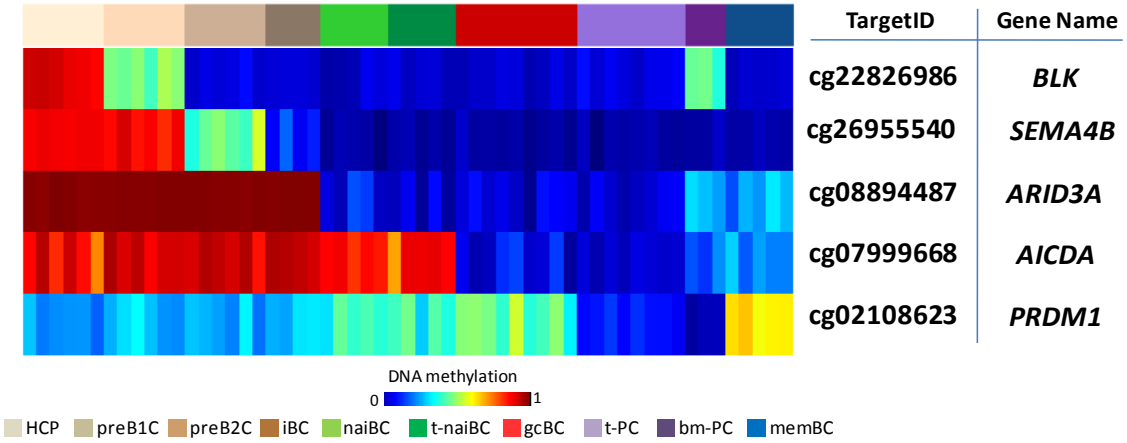


Supplementary Fig. 10

Comparison between the number of dynamically methylated CpGs during B-cell differentiation and in a wide range of human cells and tissues. Diagram showing the number of dynamically methylated CpGs in our study (4.93 million) that were also detected as dynamic by Ziller et al. (Ref. 1) in a DNA methylome study of multiple human cells and tissues.



Supplementary Fig. 11

Selection of five epigenetic biomarkers to identify each B-cell subpopulation. A selection of 5 CpGs in genes important for B-cell differentiation (*BLK*, *SEMA4B*, *ARID3A*, *AICDA*, and *PRDM1*) with different methylation patterns across B-cell differentiation is able to identify correctly each B-cell subpopulation (with the exception of naive B cells from tonsil and peripheral blood, which have virtually identical methylomes). We used the following procedure to identify these 5 CpGs: From each comparison of adjacent B-cell subsets, we selected two CpGs among those with the highest significance. Out of these comparisons, we ended up with a list of 16 unique CpGs. With those 16 CpGs, we calculated the misclassification rate of each combination of CpGs using the linear discriminant analysis (LDA) function (R software) and finally selected a combination of 5 CpGs that accurately classify all cell subpopulations.

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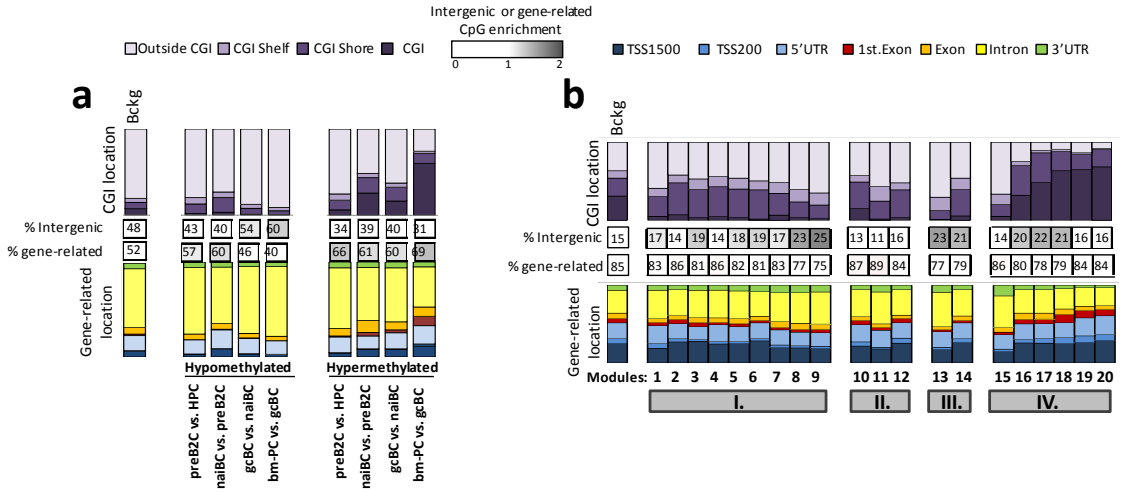

Supplementary Fig. 12

Genomic annotation of differentially methylated CpGs during B-cell differentiation. (a) Characterization of differentially methylated CpGs detected by WGBS. (B) Characterization of CpGs from the 20 modules defined by microarrays. (a-b) From upper to lower panel: Relative distribution of CpGs within CGI, in CGI shores, shelves and outside CGIs; Percentages of CpGs in intergenic and genic regions (gray color scale represents fold-change enrichment as compared to the background); Relative distribution of CpGs across different gene-related regions; Bckg – all CpGs included in each analysis (n = 16,117,712 for WGBS (a) and n = 475,030 in case of microarrays (b)).

CGI shores: 0–2 kb from island edge. CGI shelves: >2 to 4 kb from island edge. UTR: untranslated region. TSS200: 1–200 bp upstream of the transcription start site (TSS). TSS1500: 201–1500 bp upstream of TSS. CGI: CpG island. HPC: hematopoietic progenitor cell. preB2C: pre-B-II cell. naiBC: naive B cell from peripheral blood. gcBC: germinal center B cell. memBC: memory B cell from peripheral blood. bm-PC: plasma cell from bone marrow.

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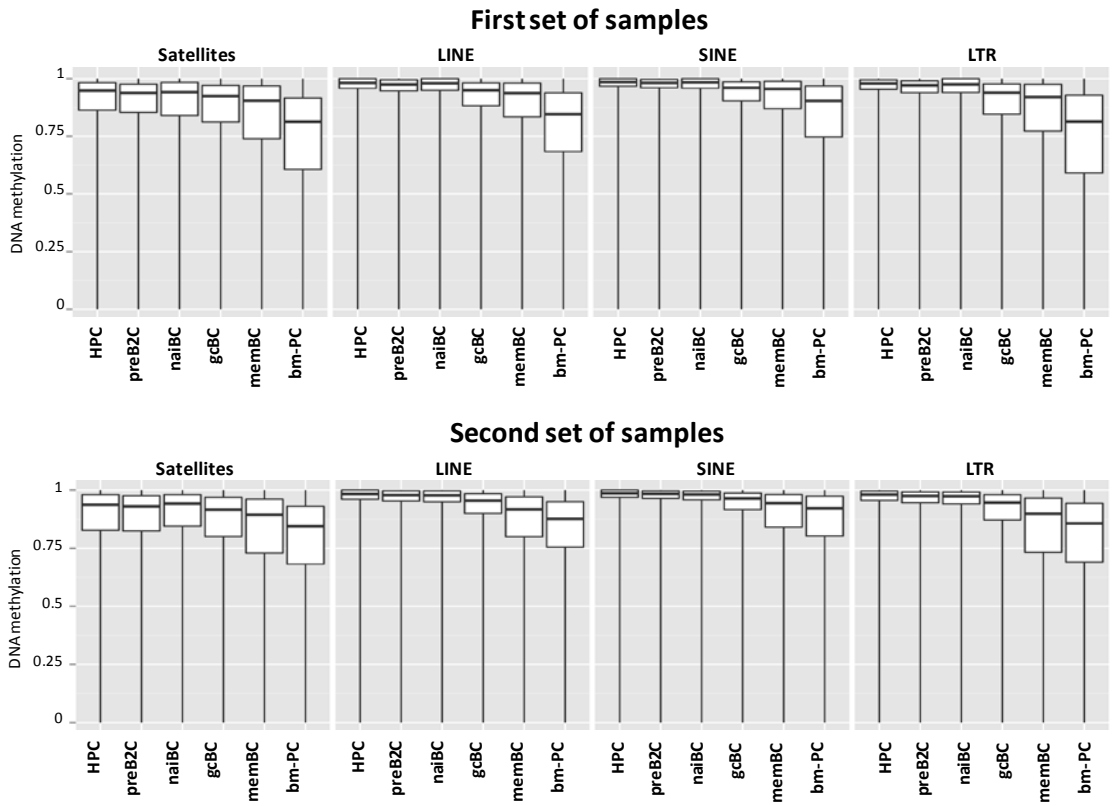

Supplementary Fig. 13

DNA methylation of major DNA repeat families in B-cell subpopulations sequenced by WGBS. Boxplot representation of DNA methylation values of CpGs associated with different repetitive elements. We show the data from the first set of replicates (upper panel) and second set of replicates (lower panel). HPC: hematopoietic progenitor cell. preB2C: pre-B-II cell. naiBC: naive B cell from peripheral blood. gcBC: germinal center B cell. memBC: memory B cell from peripheral blood. bm-PC: plasma cell from bone marrow.

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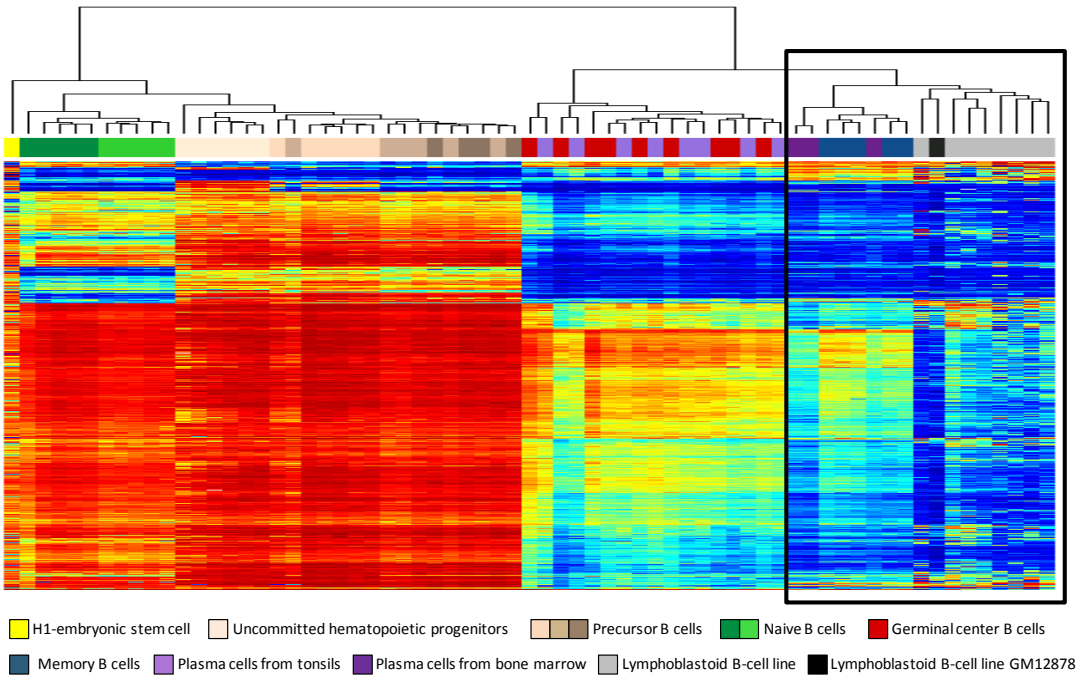
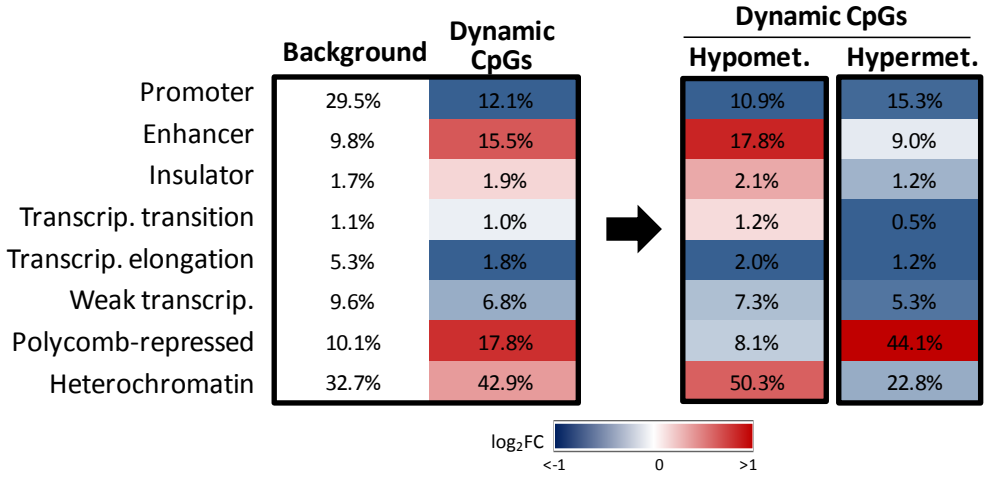


Supplementary Fig. 14

Immortalized mature B cells (i.e. lymphoblastoid B-cell lines) show a DNA methylation profile similar to normal memory B cells from peripheral blood and plasma cells from bone marrow (i.e. memBCs and bm-PCs, respectively). Unsupervised hierarchical clustering analysis of CpGs with variable DNA methylation levels (SD > 0.2) in lymphoblastoid B cell lines (including GM12878), ESC cell line (H1) and sorted cells from multiple B-cell differentiation stages. Based on this analysis, we decided to use the chromatin states categorization of GM12878 (ENCODE) as a representative model of mature B cells.



Supplementary Fig. 15

Epigenomic and transcriptional characterization of CpGs with dynamic methylation throughout B-cell differentiation. Characterization of all 106,562 dynamic CpGs detected by microarrays (left panel). On the right, the characterization of all dynamic CpGs separated into those losing or gaining methylation from HPC to bm-PCs. Each CpG site was classified into 8 different chromatin states. Numbers indicate the percentage of sites showing a particular feature and blue to red color scale represents log2 of enrichment values as compared to the background.

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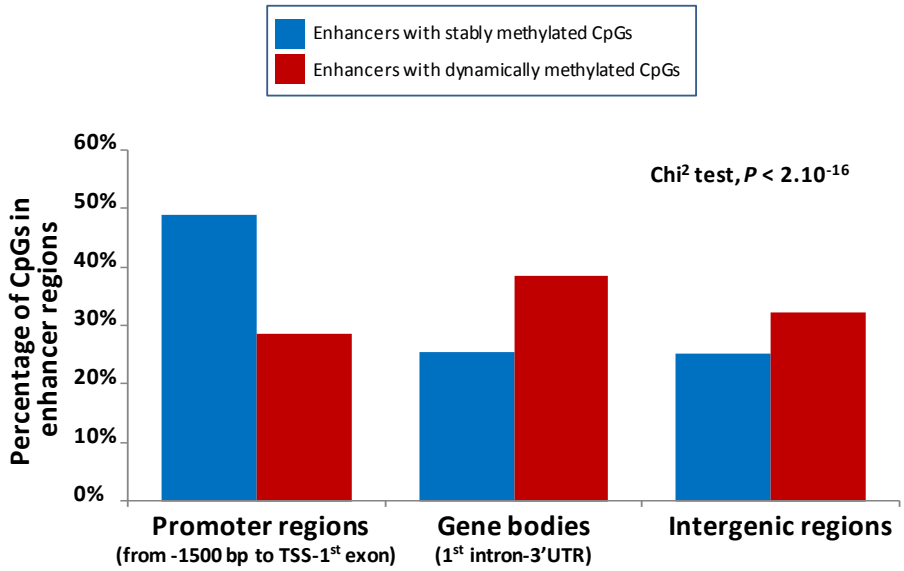

Supplementary Fig. 16

Location of hypomethylated enhancers. All the CpGs in enhancer regions were classified into three categories according to their gene-related location: promoter, gene body and intergenic. This analysis shows that enhancers with dynamic methylation are significantly enriched in gene bodies (and to a lesser extent also in intergenic regions) as compared to those with stable methylation during B-cell differentiation.

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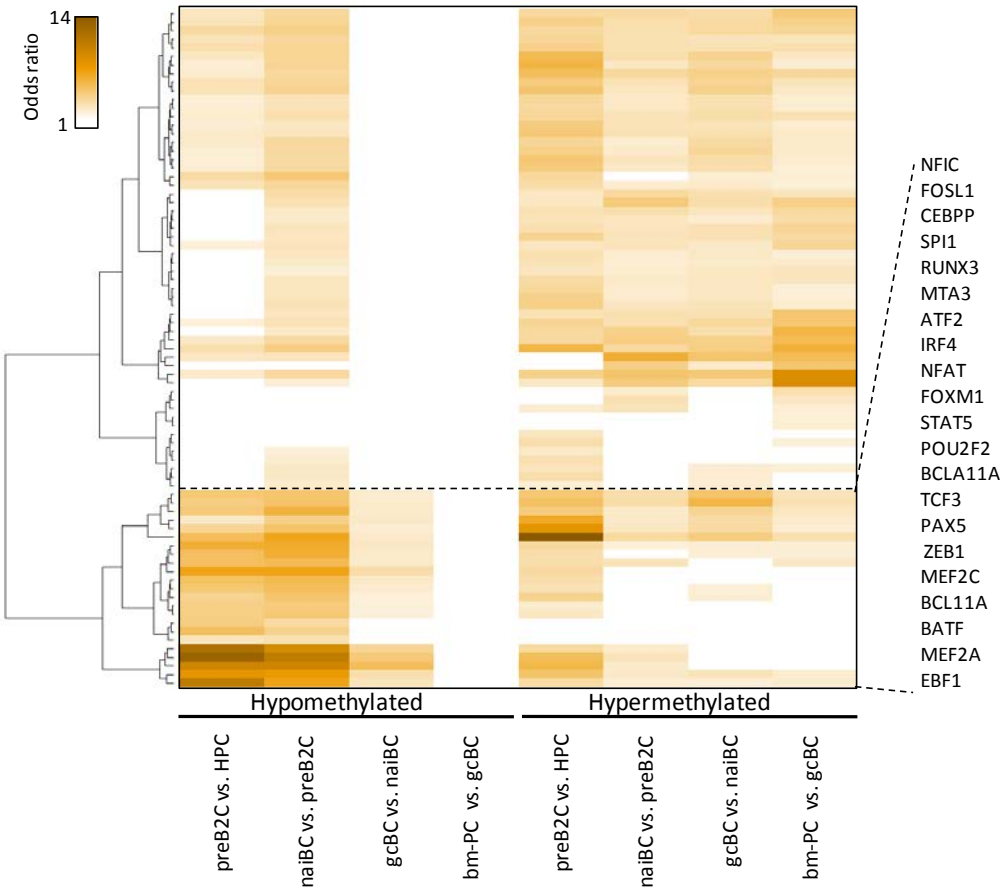

Supplementary Fig. 17

Association of differentially methylated CpGs, detected by WGBS, with transcription factor binding sites (TFBSs). Heatmap representing significant (P < 0.01) enrichments for TFBSs in different DMRs detected by WGBS. Demethylated CpGs are particularly enriched in TFBSs of key B-cell transcription factors such as BCL11A, EBF1, IRF4, MEF2A, MEF2C, PAX5 or TCF3 (E2A).

HPC: uncommitted hematopoietic progenitor. preB2C: pre-B-II cell. naiBC: naive B cell from peripheral blood. gcBC: germinal center B cell. bm-PC: plasma cell from bone marrow.

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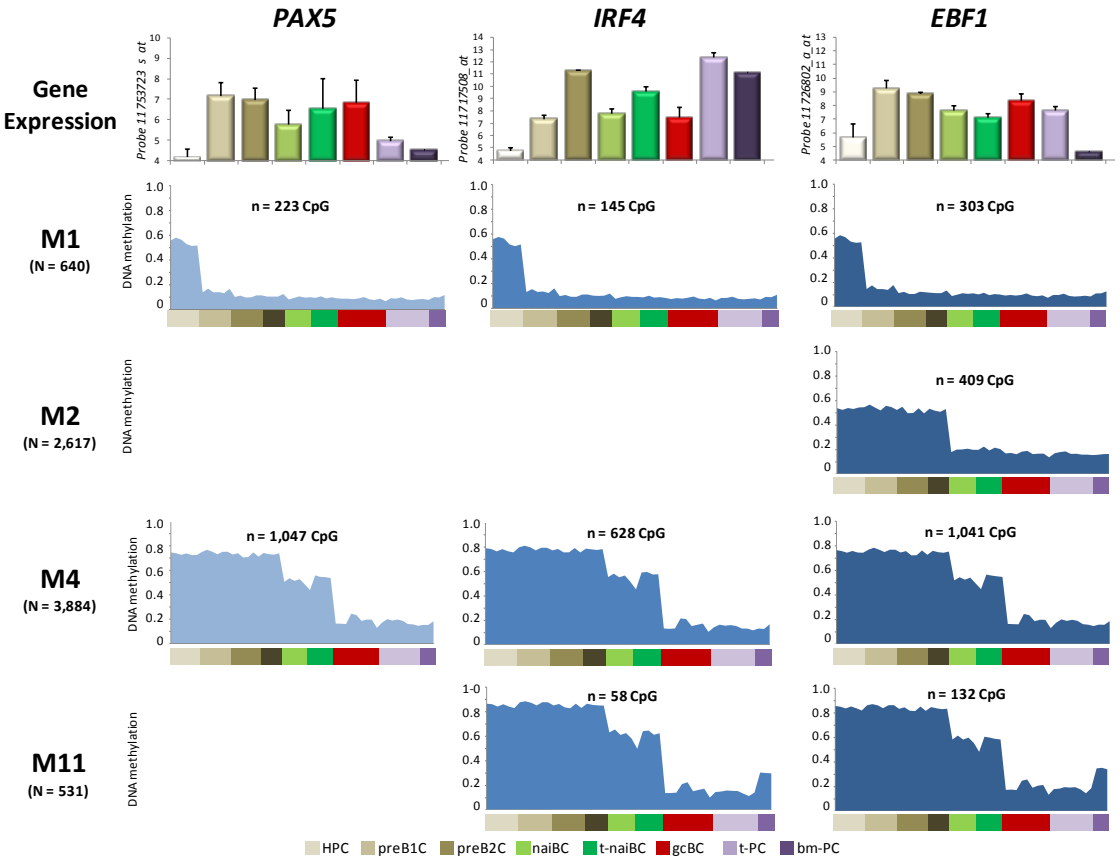

Supplementary Fig. 18

Analysis of *PAX5*, *IRF4* and *EBF1* expression and DNA methylation changes of their binding sites during B-cell differentiation. In the most upper panel, expression of three analyzed TFs in distinct B-cell differentiation stages is represented. Methylation patterns of CpGs associated with TF binding sites (TFBSs) is shown only for modules in which significant enrichment for these binding sites was observed.

These TFs showed different patterns of both expression and TFBSs methylation throughout B-cell maturation. We observed that EBF1 and PAX5 binding sites remained unmethylated in PCs, although these TFs become downregulated in this cell type. In the case of IRF4, its binding sites in early B-cells became demethylated, however, it seems that later overexpression in PCs does not induce demethylation of additional sites.

N: total number of CpGs belonging to each module; n: number of CpGs associated with PAX5, IRF4 or EBF1 binding sites. HPC: uncommitted hematopoietic progenitor. preB1C: pre-B-I cell. preB2C: pre-B-II cell. iBC: immature B cell. naiBCs: naive B cell from peripheral blood. t-naiBCs: naive B cell from tonsil. gcBC: germinal center B cell. t-PC: plasma cell from tonsil. memBC: memory B cell from peripheral blood. bm-PCs: plasma cell from bone marrow.

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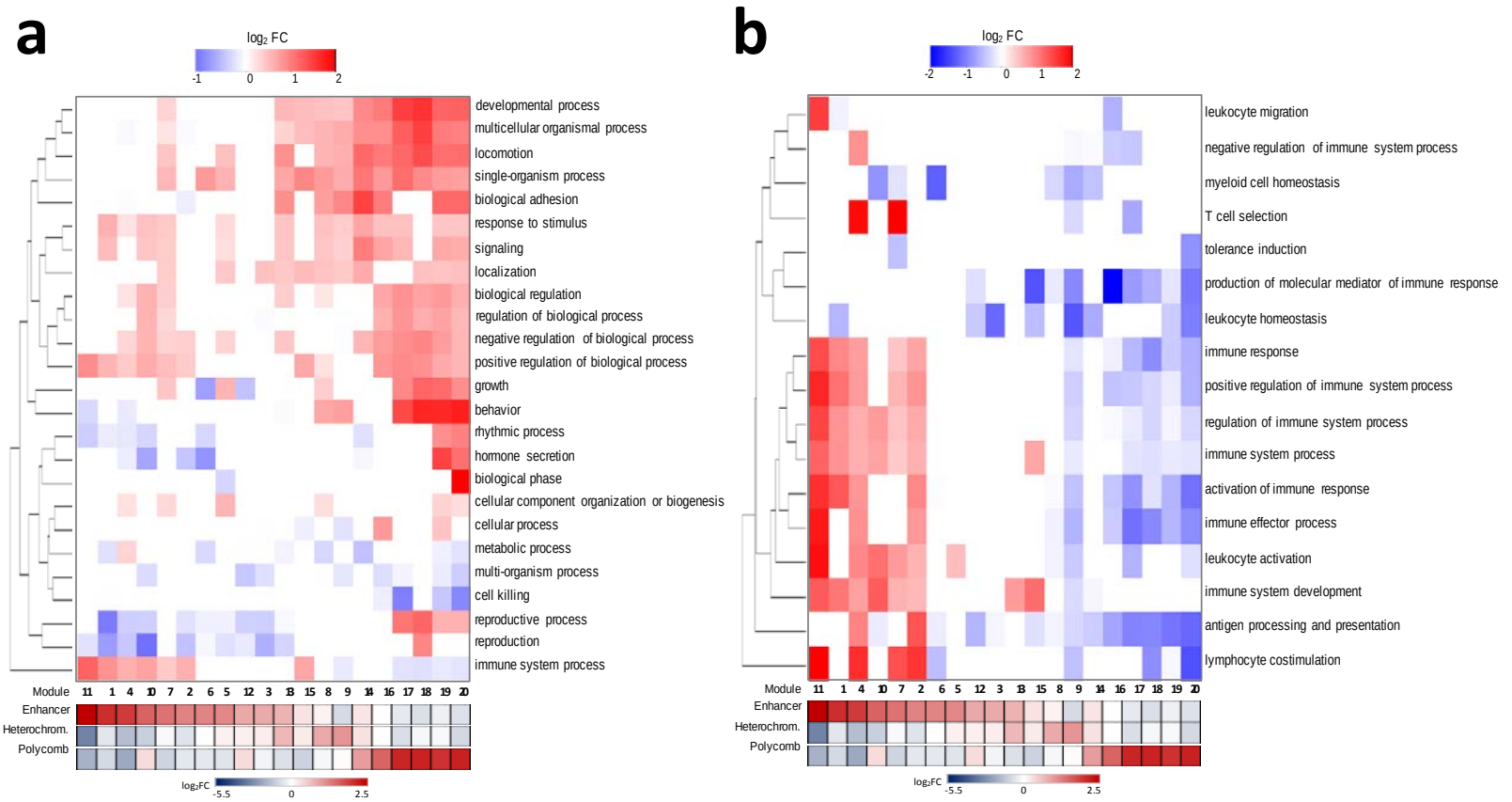

Supplementary Fig. 19

Main GO terms enriched in the 20 methylation modules detected by microarrays. Analysis of the child terms associated to the main GO term "Biological Process", which encompasses major cellular functions. This analysis shows that the methylation modules enriched for heterochromatin and polycomb-repressed regions were associated with terms not related to the immune system (e.g. development, locomotion or behavior) while the modules enriched for enhancer regions were the only ones associated with immune system-related functions (a). To further explore the immunological functions of particular modules, the child terms of the GO term "immune system process" were analyzed (b).

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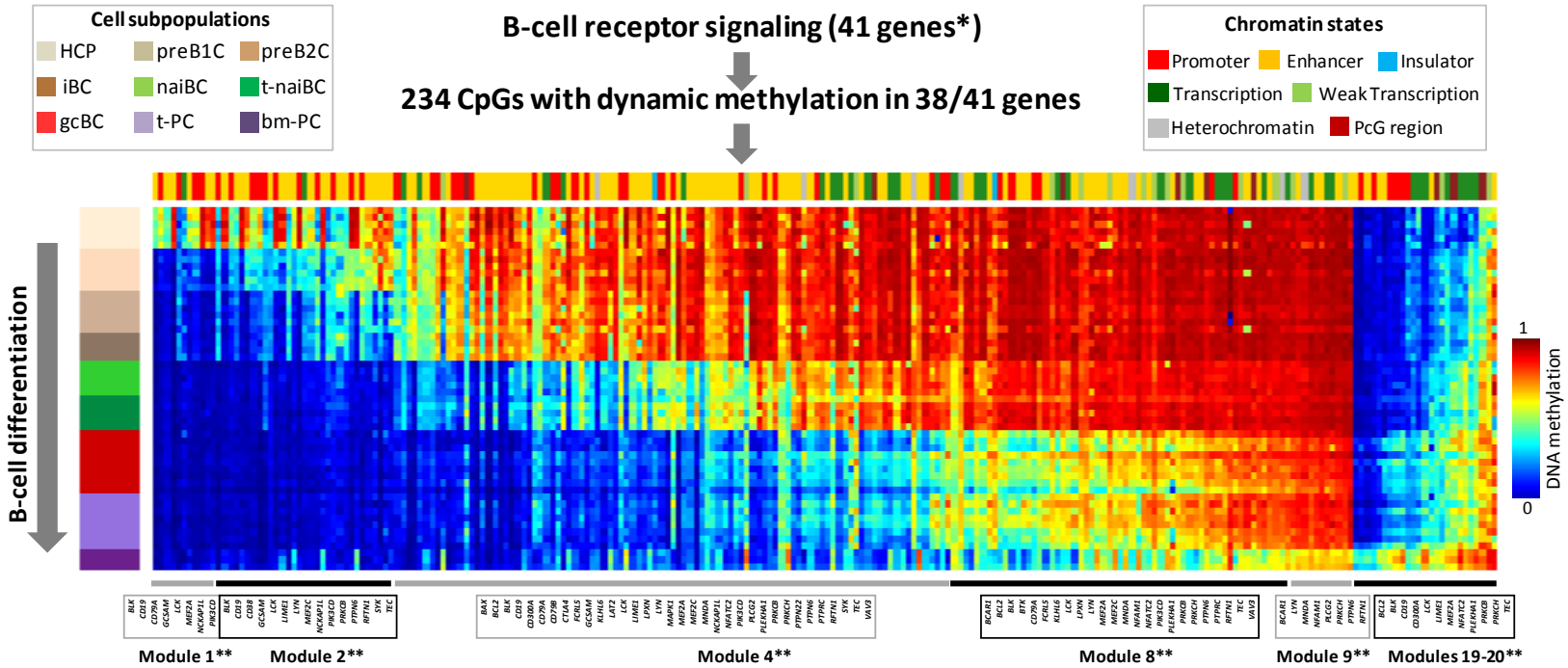

Supplementary Fig. 20

Analysis of the DNA methylation pattern of genes involved in B-cell receptor (BCR) signaling. 38 out of 41 genes (93%) involved in BCR signaling (identified by GO terms containing B-cell receptor signaling) had dynamic methylation during B-cell differentiation, as represented in the heatmap. The chromatin states associated with the displayed CpGs are shown in the upper part of the heatmap (48% of all CpGs are located in enhancers). Below the heatmap, we show the gene names associated with each of the different methylation patterns, which are enriched for particular modules. The reader can observe that CpGs associated with particular genes belong to distinct modules (e.g. *CD19* or *BLK*), demonstrating that genes gradually change their methylation during the differentiation program and not in one particular differentiation step. HPC: hematopoietic progenitor cell. preB1C: pre-B-I cell. preB2C: pre-B-II cell. iBC: immature B cell. naiBCs: naive B cell from peripheral blood. t-naiBCs: naive B cell from tonsil. gcBC: germinal center B cell. t-PC: plasma cell from tonsil. bm-PCs: plasma cell from bone marrow. * Identified by GO terms containing "*B cell receptor signaling*". ** Predominant module associated with a particular DNA methylation pattern of genes from the BCR signaling pathway.

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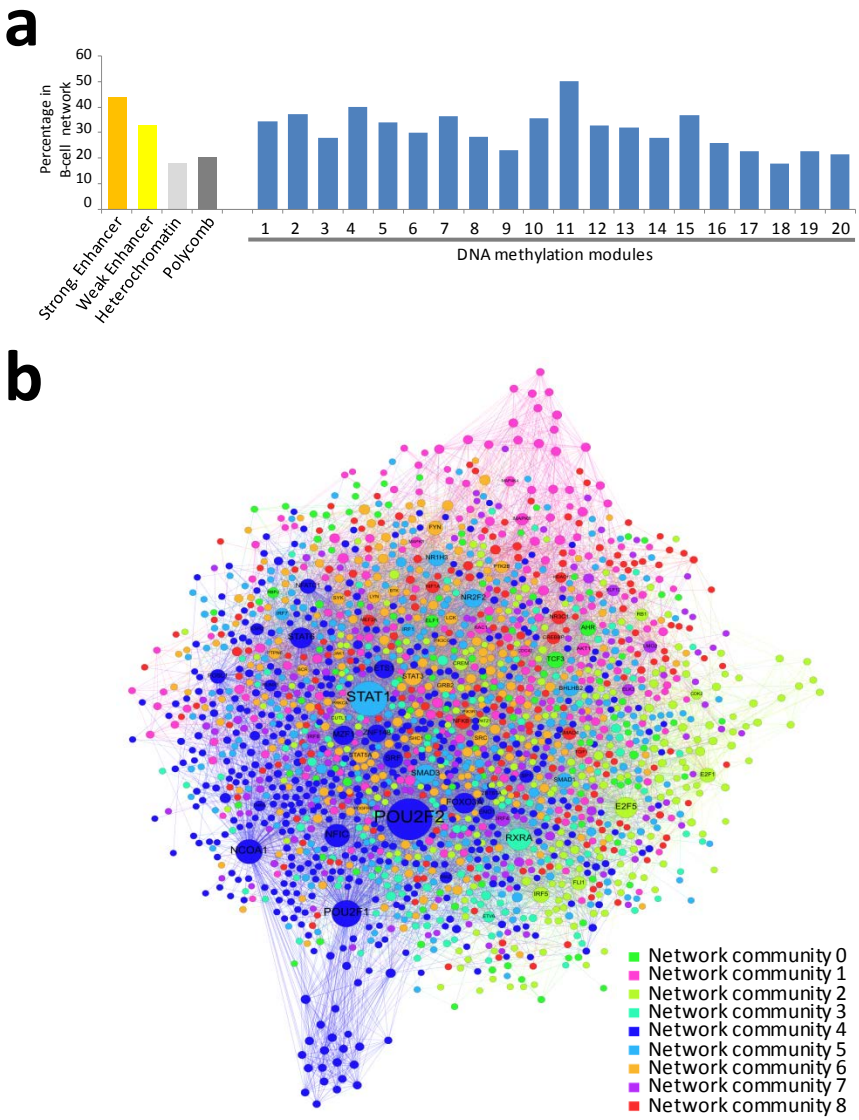

Supplementary Fig. 21

Association between dynamic methylated genes and a B-cell network. (a) Proportion of genes in different chromatin states and methylation modules overlapping with the B-cell network published by Lefebvre et al. (Ref. 29). Noteworthy, 44% of genes with active enhancers and 33% of genes with weak enhancers belonged to B-cell specific functional gene network and these percentages were significantly increased (P < 0.001) as compared to 18% and 21% for genes with dynamic methylation in heterochromatin and polycomb-repressed regions, respectively. (b) Network of genes with enhancers with dynamic methylation consisting of 1,993 genes connected via 11,741 interactions. The size of the nodes (= genes) corresponds to their degrees. The degrees in the network range from 1 to 449 and the gene names are only shown for nodes with a degree >= 50. The colors of the network represent the identified 9 functional communities, involved in functions modulated during B-cell development.

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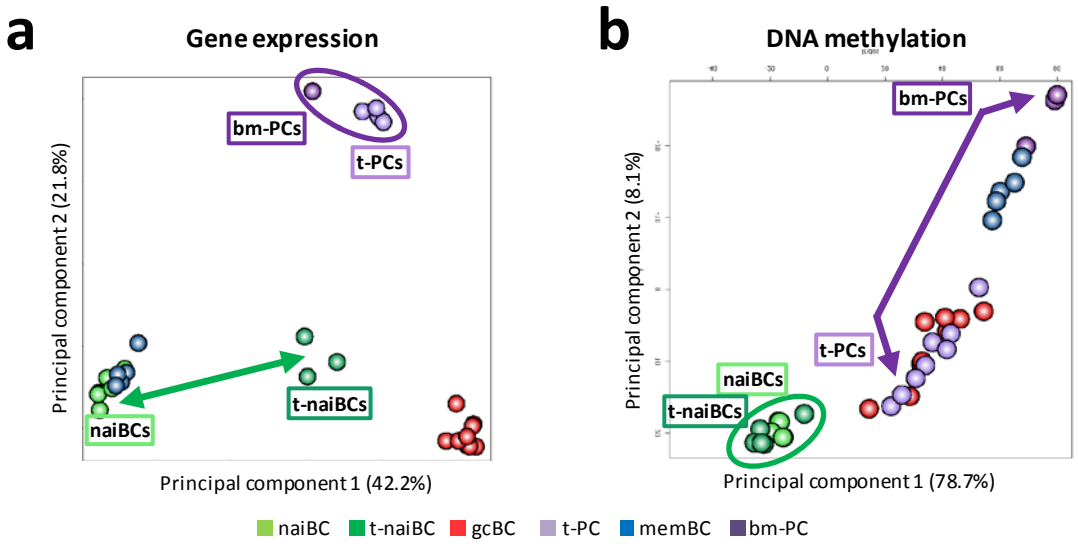

Supplementary Fig. 22

Differences in DNA methylomes and transcriptomes of naive B cells and plasma cells isolated from distinct anatomical locations. (a-b) Unsupervised principal component analyses (PCA) of microarray-based gene expression data (a) and microarray-based DNA methylation data (b) of all samples used in our study. t-naiBCs isolated from tonsils have clearly different transcriptomes as compared to naiBCs isolated from peripheral blood (marked with green arrow), while their methylomes are similar (marked with green circle). On the contrary, PCs isolated both from bone marrow and peripheral blood show a comparable gene expression pattern (marked with violet circle) but they widely differ in their DNA methylation profile (marked with violet arrow). naiBCs: naive B cell from peripheral blood. t-naiBCs: naive B cell from tonsil. gcBC: germinal center B cell. t-PC: plasma cell from tonsil. memBC: memory B cell from peripheral blood. bm-PCs: plasma cell from bone marrow.

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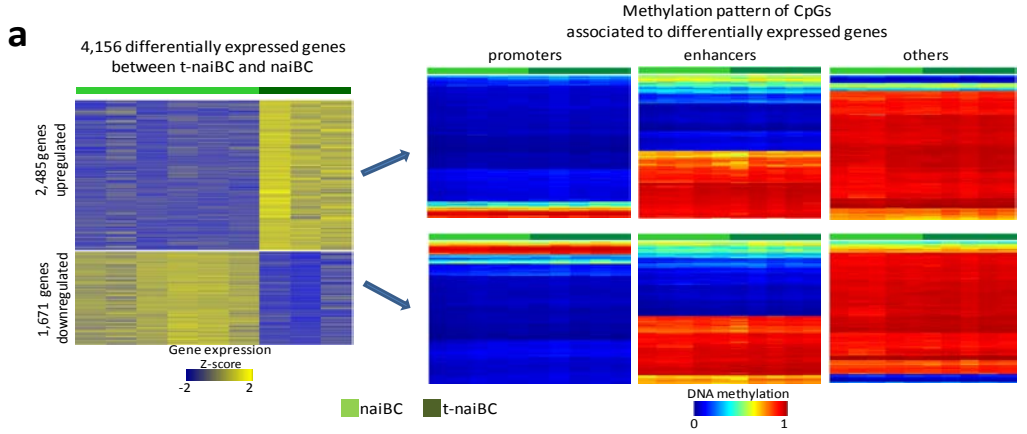

b

t-naiBC vs naiBC

	Genes without differential expression n=13,108			Upregulated genes n=2,485			Downregulated genes n=1,671		
Region	Promoter	Enhancer	Others	Promoter	Enhancer	Others	Promoter	Enhancer	Others
Total CpGs	83,635	25,142	157,774	29,140	6,461	16,487	20,326	5,160	16,558
Hypermethylated CpGs	368	266	876	115	73	71	83	63	60
(% of total)	(0.4%)	(1.1%)	(0.6%)	(0.4%)	(1.1%)	(0.4%)	(0.4%)	(1.2%)	(0.4%)
number of related genes	407	277	735	127	79	65	97	61	59
(% of total)	(3.1%)	(2.1%)	(5.6%)	(5.1%)	(3.2%)	(2.6%)	(5.8%)	(3.7%)	(3.5%)
Hypomethylated CpGs	77	129	588	26	19	41	22	21	33
(% of total)	(0.1%)	(0.5%)	(0.4%)	(0.1%)	(0.3%)	(0.2%)	(0.1%)	(0.4%)	(0.2%)
number of related genes	72	128	725	27	18	65	21	19	28
(% of total)	(0.5%)	(1.0%)	(5.5%)	(1.1%)	(0.7%)	(2.6%)	(1.3%)	(1.1%)	(1.7%)

Supplementary Fig. 23

Uncoupling of gene expression and DNA methylation in naive B cells isolated from tonsil and peripheral blood. (a) Heatmap representation of differentially expressed genes in t-naiBC vs naiBC (left panel), as well as methylation status of all the CpGs associated with these genes (right panels). CpGs were divided according to their location in the promoters, enhancer or other regions of the gene. (b) Numbers of differentially methylated CpGs in t-naiBC vs naiBC (at least 10% difference, FDR < 0.1), associated either with genes without changes in gene expression (left column), upregulated (center) or downregulated (right) in t-naiBC respect to naiBC. By means of this analysis, only a minor fraction of genes with differential methylation could be detected, which was similar to the changes observed in genes without any expression change. It should be noted that using our standard criteria (methylation difference at least 25%, FDR < 0.01) we did not identify any change between these two subpopulations. Therefore, the extensive transcriptional modification in t-naiBCs vs. naiBCs does not seem to be associated with any significant DNA methylation change. naiBC: naive B cell from peripheral blood. t-naiBC: naive B cell from tonsil.

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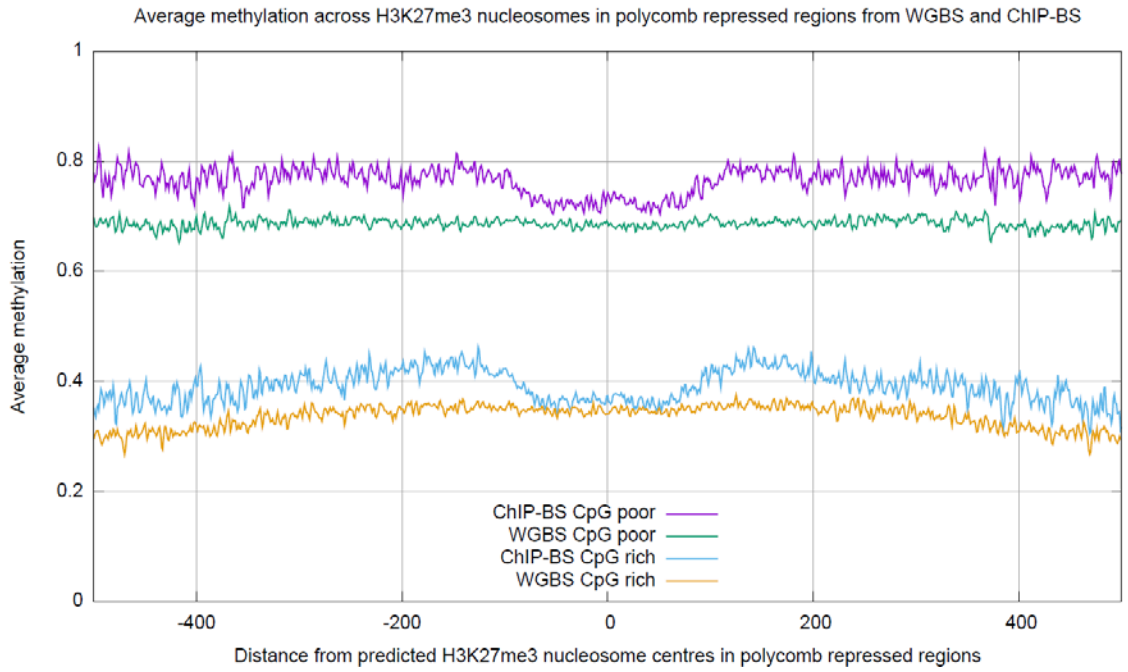

Supplementary Fig. 24

Association between DNA methylation levels and H3K27me3. Chromatin from purified memBCs was immunoprecipated with an antibody against H3K27me3, followed by bisulfite treatment and sequencing (ChIP-BS). As a comparison, the whole-genome bisulfite sequencing (WGBS) data of memBCs was used. Next, all polycomb-repressed regions as defined in the lymphoblastoid cell line GM12878 were selected and within these regions, the H3K27me3 nucleosome positioning was estimated by NucHunter using the ChIP-BS data. Average DNA methylation levels in CpG-rich and CpG-poor regions in relation to the distance of the H3K27me3 containing nucleosome (within a window of 500 bp up- and downstream) were calculated for both the ChIP-BS and WGBS data. The overall levels of DNA methylation of the H3K27me3-immunoprecipitated fraction were slightly higher than those in the non-immunoprecipitated DNA and DNA methylation levels within nucleosomes containing H3K27me3 were lower than regions outside such nucleosomes, both in CpG-rich and CpG-poor areas. This finding suggests that the regions positioned exactly at the H3K27me3-containing nucleosomes could be slightly protected from DNA methylation in comparison with the surrounding nucleosome-free areas.

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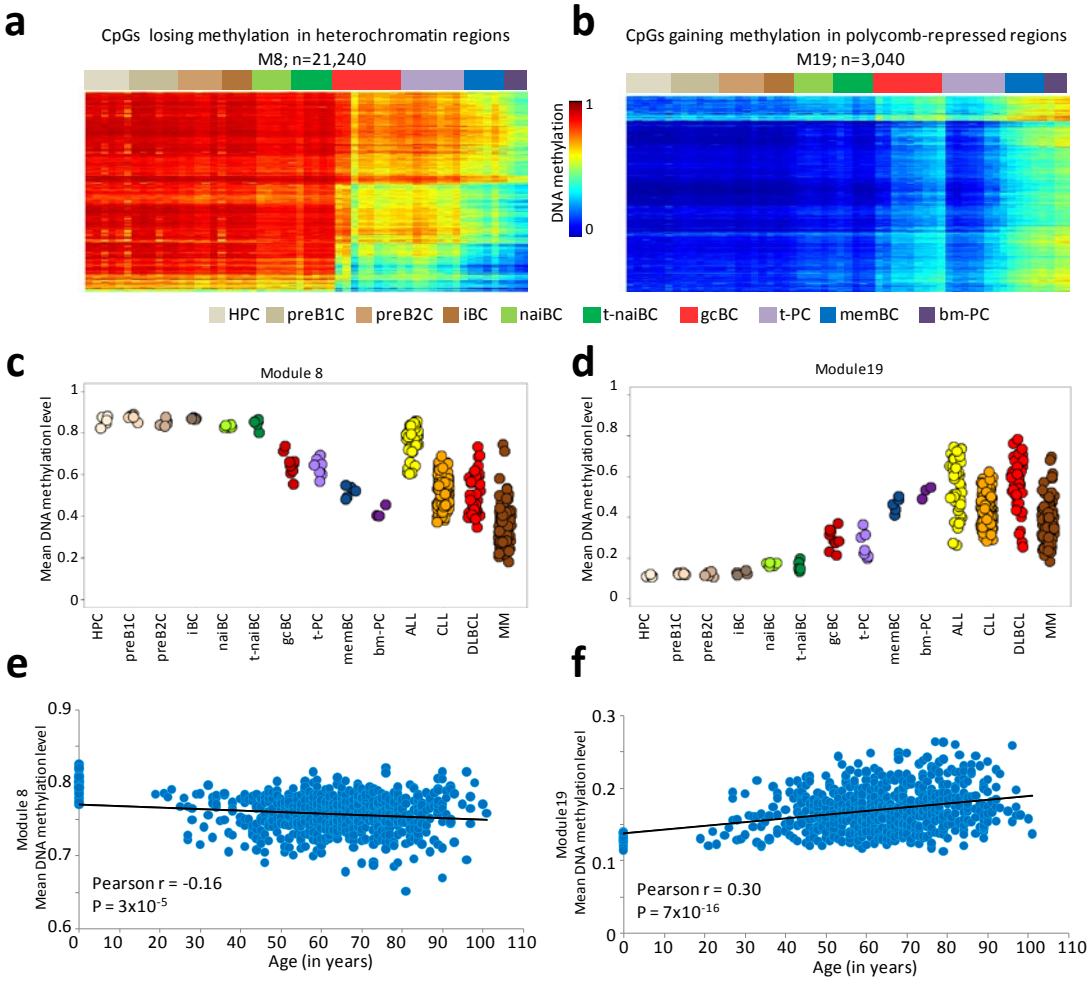

Supplementary Fig. 25

DNA methylation changes during B-cell differentiation in the context of cancer and aging. (a) Heatmap of subset of CpGs from M8 module that lose methylation in heterochromatin regions. (b) Heatmap of subset of CpGs from M19 module that gain methylation in polycomb-repressed regions. (c) Scatter plots representing mean methylation levels of CpGs in heterochromatin from M8 in different B-cell subsets and four types of hematological neoplasms. (d) Scatter plots representing mean methylation levels of CpGs in polycomb-repressed regions from M19 in different B-cell subsets and four types of hematological neoplasms. (e) Mean methylation levels of CpGs in heterochromatin from M8 in whole blood samples from donors of different age. (f) Mean methylation levels of CpGs in polycomb-repressed regions from M19 in whole blood samples from donors of different age.

HPC: uncommitted hematopoietic progenitor. preB1C: pre-B-I cell. preB2C: pre-B-II cell. iBC: immature B cell. naiBCs: naive B cell from peripheral blood. t-naiBCs: naive B cell from tonsil. gcBC: germinal center B cell. t-PC: plasma cell from tonsil. memBC: memory B cell from peripheral blood. bm-PC: plasma cell from bone marrow. ALL: acute lymphoblastic leukemia. CLL: chronic lymphocytic leukemia. DLBCL: diffuse large B-cell lymphoma. MM: multiple myeloma.

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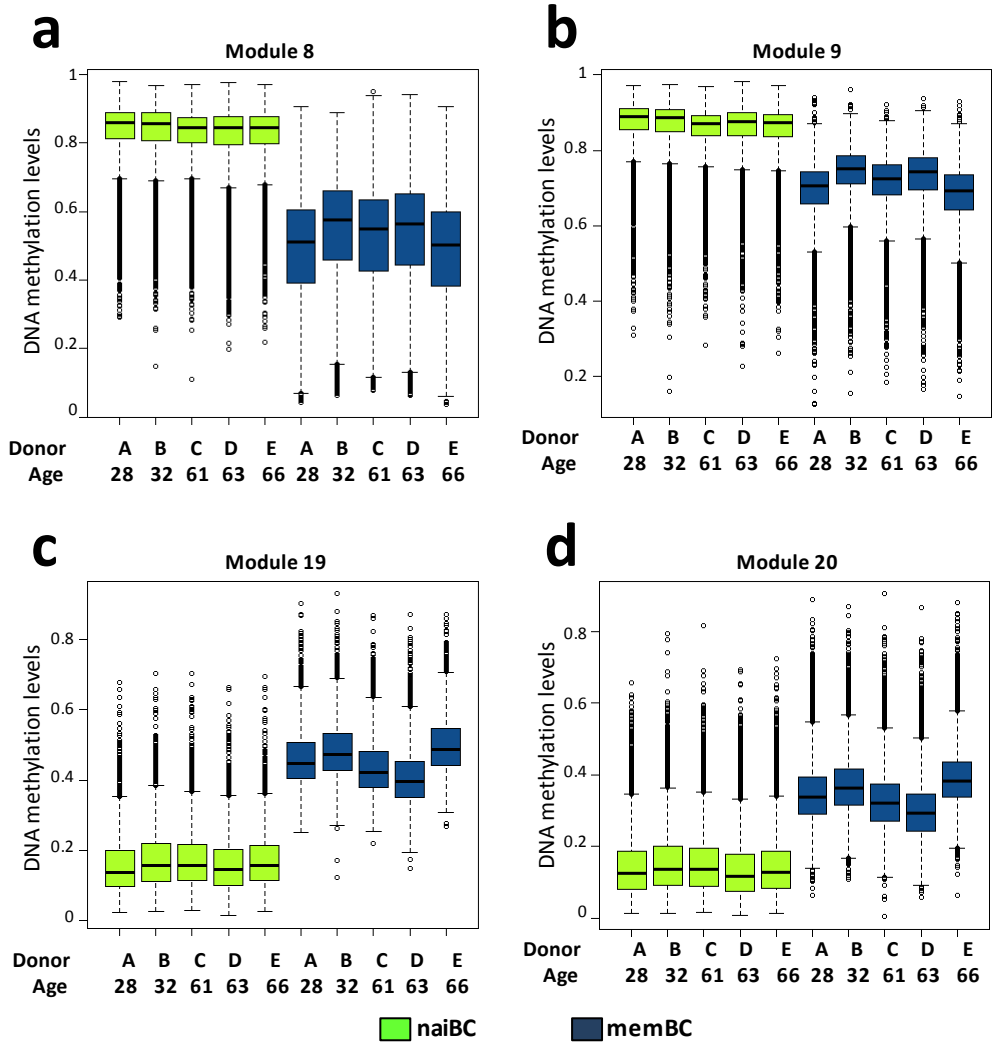

Supplementary Fig. 26

Comparison of DNA methylation levels in B-cell subpopulations with short and long lifespan (naiBCs and memBCs, respectively), isolated from individuals of different age (ranging from 28 to 66 years). (a-b) Boxplot showing methylation levels of CpGs in heterochromatic regions from module M8 (a) or M9 (b). (c-d) Boxplot representation of methylation levels of CpGs in polycomb-repressed regions from module M19 (c) or M20 (d).

naiBCs: naive B cell from peripheral blood. memBC: memory B cell from peripheral blood

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Supplementary Tables

Supplementary Table 1. Normal B-cell samples used in the present study (I).

Sample ID	Cell subtype	Sample Name	Source	Age	Sex	Selection markers	450k	WGBS	GE array U219	GE array 1.0 ST	Bis. Pyroseq	Reference	Comments
1	HPC	HPC_1	fetal bone marrow	22 weeks	NA	CD34hi/CD19-	Yes			Yes		Lee et al (*17*)	
2	HPC	HPC_2	fetal bone marrow	22 weeks	NA	CD34hi/CD19-	Yes			Yes		Lee et al (*17*)	
3	HPC	HPC_3	fetal bone marrow	22 weeks	NA	CD34hi/CD19-	Yes			Yes		Lee et al (*17*)	
4	HPC	HPC_4	fetal bone marrow	22 weeks	NA	CD34hi/CD19-	Yes			Yes		Lee et al (*17*)	
5	HPC	HPC_5	fetal bone marrow	22 weeks	NA	CD34hi/CD19-	Yes			Yes		Lee et al (*17*)	
6	HPC	HPC_6	fetal bone marrow	22 weeks	NA	CD34hi/CD19-	Yes			Yes		Lee et al (*17*)	
7	HPC	HPC_7	fetal bone marrow	22 weeks	NA	CD34hi/CD19-		Yes				Lee et al (*17*)	
8	HPC	HPC_8	fetal bone marrow	22 weeks	NA	CD34hi/CD19-		Yes	Yes				
9	HPC	HPC_9	fetal bone marrow	22 weeks	NA	CD34hi/CD19-			Yes				
10	HPC	HPC_10	fetal bone marrow	22 weeks	NA	CD34hi/CD19-					Yes		
11	HPC	HPC_11	fetal bone marrow	22 weeks	NA	CD34hi/CD19-			Yes		Yes		
12	preB1C	preB1C_12	fetal bone marrow	22 weeks	NA	CD34+/CD19+	Yes			Yes		Lee et al (*17*)	
13	preB1C	preB1C_13	fetal bone marrow	22 weeks	NA	CD34+/CD19+	Yes			Yes		Lee et al (*17*)	
14	preB1C	preB1C_14	fetal bone marrow	22 weeks	NA	CD34+/CD19+	Yes			Yes		Lee et al (*17*)	
15	preB1C	preB1C_15	fetal bone marrow	22 weeks	NA	CD34+/CD19+	Yes			Yes		Lee et al (*17*)	
16	preB1C	preB1C_16	fetal bone marrow	22 weeks	NA	CD34+/CD19+	Yes			Yes		Lee et al (*17*)	
17	preB1C	preB1C_17	fetal bone marrow	22 weeks	NA	CD34+/CD19+	Yes			Yes		Lee et al (*17*)	
18	preB1C	preB1C_18	fetal bone marrow	22 weeks	NA	CD34+/CD19+			Yes				
19	preB1C	preB1C_19	fetal bone marrow	22 weeks	NA	CD34+/CD19+			Yes		Yes		
20	preB1C	preB1C_20	fetal bone marrow	22 weeks	NA	CD34+/CD19+			Yes		Yes		
21	preB2C	preB2C_21	fetal bone marrow	22 weeks	NA	CD34-/CD19+/sIgM-	Yes			Yes		Lee et al (*17*)	
22	preB2C	preB2C_22	fetal bone marrow	22 weeks	NA	CD34-/CD19+/sIgM-	Yes			Yes		Lee et al (*17*)	
23	preB2C	preB2C_23	fetal bone marrow	22 weeks	NA	CD34-/CD19+/sIgM-	Yes			Yes		Lee et al (*17*)	
24	preB2C	preB2C_24	fetal bone marrow	22 weeks	NA	CD34-/CD19+/sIgM-	Yes			Yes		Lee et al (*17*)	
25	preB2C	preB2C_25	fetal bone marrow	22 weeks	NA	CD34-/CD19+/sIgM-	Yes			Yes		Lee et al (*17*)	
26	preB2C	preB2C_26	fetal bone marrow	22 weeks	NA	CD34-/CD19+/sIgM-	Yes			Yes		Lee et al (*17*)	
27	preB2C	preB2C_27	fetal bone marrow	22 weeks	NA	CD34-/CD19+/sIgM-		Yes				Lee et al (*17*)	
28	preB2C	preB2C_28	fetal bone marrow	22 weeks	NA	CD34-/CD19+/sIgM-			Yes				
29	preB2C	preB2C_29	fetal bone marrow	22 weeks	NA	CD34-/CD19+/sIgM-		Yes	Yes				
30	preB2C	preB2C_30	fetal bone marrow	22 weeks	NA	CD34-/CD19+/sIgM-			Yes		Yes		
31	preB2C	preB2C_31	fetal bone marrow	22 weeks	NA	CD34-/CD19+/sIgM-			Yes		Yes		
32	iBC	iBC_32	fetal bone marrow	22 weeks	NA	CD34-/CD19+/sIgM+	Yes			Yes		Lee et al (*17*)	
33	iBC	iBC_33	fetal bone marrow	22 weeks	NA	CD34-/CD19+/sIgM+	Yes			Yes		Lee et al (*17*)	
34	iBC	iBC_34	fetal bone marrow	22 weeks	NA	CD34-/CD19+/sIgM+	Yes			Yes		Lee et al (*17*)	
35	iBC	iBC_35	fetal bone marrow	22 weeks	NA	CD34-/CD19+/sIgM+	Yes					Lee et al (*17*)	
36	iBC	iBC_36	fetal bone marrow	22 weeks	NA	CD34-/CD19+/sIgM+					Yes		
37	iBC	iBC_37	fetal bone marrow	22 weeks	NA	CD34-/CD19+/sIgM+					Yes		

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Supplementary Table 1. Normal B-cell samples used in the present study (II).

Sample ID	Cell subtype	Sample Name	Source	Age	Sex	Selection markers	450k	WGBS	GE array U219	GE array 1.0 ST	Bis. Pyroseq	Reference	Comments
38	naiBC	naiBC_38	peripheral blood	63	M	CD19+/CD27-/IgD+	Yes	Yes				Kulis et al (*10*)	
39	naiBC	naiBC_39	peripheral blood	65	F	CD19+/CD27-/IgD+	Yes					Kulis et al (*10*)	
40	naiBC	naiBC_40	peripheral blood	61	F	CD19+/CD27-/IgD+	Yes					Kulis et al (*10*)	
41	naiBC	naiBC_41	peripheral blood	32	M	CD19+/CD27-/IgD+	Yes		Yes				
42	naiBC	naiBC_42	peripheral blood	28	F	CD19+/CD27-/IgD+	Yes						
43	naiBC	naiBC_43	peripheral blood	56	M	CD19+/CD27-/IgD+			Yes				
44	naiBC	naiBC_44	peripheral blood	57	M	CD19+/CD27-/IgD+			yes				
45	naiBC	naiBC_45	peripheral blood	56	F	CD19+/CD27-/IgD+			yes				
46	naiBC	naiBC_46	peripheral blood	61	M	CD19+/CD27-/IgD+			yes				
47	naiBC	naiBC_47	peripheral blood	57	M	CD19+/CD27-/IgD+			yes				
48	naiBC	naiBC_48	peripheral blood	61	F	CD19+/CD27-/IgD+		Yes					
49	naiBC	naiBC_49	peripheral blood	50	M	CD19+/CD27-/IgD+					Yes		
50	naiBC	naiBC_50	peripheral blood	45	F	CD19+/CD27-/IgD+					Yes		
51	t-naiBC	t-naiBC_51	tonsil	5	M	CD20+/CD38lo/CD23+	Yes						
52	t-naiBC	t-naiBC_52	tonsil	4	F	CD19+/CD27-/IgD+	Yes						
53	t-naiBC	t-naiBC_53	tonsil	4	F	CD20+/CD38lo/CD23+	Yes						
54	t-naiBC	t-naiBC_54	tonsil	4	M	CD20+/CD38lo/CD23+			Yes				
55	t-naiBC	t-naiBC_55	tonsil	9	F	CD20+/CD38lo/CD23+			Yes				
56	t-naiBC	t-naiBC_56	tonsil	2-6	F	IgD+/CD38low/CD27-	Yes		Yes				
57	t-naiBC	t-naiBC_57	tonsil	2-6	F	IgD+/CD38low/CD27-	Yes		Yes				
58	t-naiBC	t-naiBC_58	tonsil	3	M	CD20+/CD38lo/CD23+					Yes		
59	t-naiBC	t-naiBC_59	tonsil	3	F	CD20+/CD38lo/CD23+					Yes		
60	memBC	memBC_60	peripheral blood	63	M	CD19+/CD27+/IgA+ or IgG+	Yes	Yes				Kulis et al (*10*)	
61	memBC	memBC_61	peripheral blood	65	F	CD19+/CD27+/IgA+ or IgG+	Yes					Kulis et al (*10*)	
62	memBC	memBC_62	peripheral blood	61	F	CD19+/CD27+/IgA+ or IgG+	Yes					Kulis et al (*10*)	
63	memBC	memBC_63	peripheral blood	32	M	CD19+/CD27+/IgA+ or IgG+	Yes		Yes				
64	memBC	memBC_64	peripheral blood	28	F	CD19+/CD27+/IgA+ or IgG+	Yes						
65	memBC	memBC_65	peripheral blood	57	M	CD19+/CD27+/IgA+ or IgG+			yes				
66	memBC	memBC_66	peripheral blood	56	F	CD19+/CD27+/IgA+ or IgG+			yes				
67	memBC	memBC_67	peripheral blood	61	M	CD19+/CD27+/IgA+ or IgG+			yes				
68	memBC	memBC_68	peripheral blood	57	M	CD19+/CD27+/IgA+ or IgG+			yes				
69	memBC	memBC_69	peripheral blood	61	F	CD19+/CD27+/IgA+ or IgG+		Yes					
70	memBC	memBC_70	peripheral blood	50	M	CD19+/CD27+/IgA+ or IgG+					Yes		
71	memBC	memBC_71	peripheral blood	45	F	CD19+/CD27+/IgA+ or IgG+					Yes		

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Supplementary Table 1. Normal B-cell samples used in the present study (III).

Sample ID	Cell subtype	Sample Name	Source	Age	Sex	Selection markers	450k	WGBS	GE array U219	GE array 1.0 ST	Bis. Pyroseq	Reference	Comments
72	gcBC	gcBC_72	tonsil	3	M	CD20hi/CD38mid			Yes				
73	gcBC	gcBC_73	tonsil	5	M	CD20hi/CD38mid	Yes						
74	gcBC	gcBC_74	tonsil	5	F	CD20hi/CD38mid	Yes						
75	gcBC	gcBC_75	tonsil	5	F	CD20hi/CD38mid	Yes						
76	gcBC	gcBC_76	tonsil	6	F	CD20hi/CD38mid	Yes						
77	gcBC	gcBC_77	tonsil	4	F	CD20hi/CD38mid	Yes						
78	gcBC	gcBC_78	tonsil	2	M	CD20hi/CD38mid	Yes		Yes				
79	gcBC	gcBC_79	tonsil	4	F	CD20hi/CD38mid	Yes	Yes	Yes				
80	gcBC	gcBC_80	tonsil	4	M	CD20hi/CD38mid			Yes				
81	gcBC	gcBC_81	tonsil	4	M	CD20hi/CD38mid			yes				
82	gcBC	gcBC_82	tonsil	4	M	CD20hi/CD38mid			yes				
83	gcBC	gcBC_83	tonsil	4	M	CD20hi/CD38mid			yes				
84	gcBC	gcBC_84	tonsil	5	M	CD20hi/CD38mid			yes				
85	gcBC	gcBC_85	tonsil	3	F	CD20hi/CD38mid			yes		Yes		
86	gcBC	gcBC_86	tonsil	5	F	CD20hi/CD38mid		Yes					
87	gcBC	gcBC_87	tonsil	5	F	CD20hi/CD38mid					Yes		
88	gcBC	gcBC_88	tonsil	2-6	F	IgD-/CD38hi/CD10+/CXCR4+	Yes		Yes				
89	gcBC	gcBC_89	tonsil	2-6	F	IgD-/CD38hi/CD10+/CXCR4+	Yes		Yes				
90	t-PC	t-PC_90	tonsil	5	M	CD20lo/CD38hi	Yes						
91	t-PC	t-PC_91	tonsil	5	F	CD20lo/CD38hi	Yes						
92	t-PC	t-PC_92	tonsil	5	F	CD20lo/CD38hi	Yes						
93	t-PC	t-PC_93	tonsil	6	F	CD20lo/CD38hi	Yes						
94	t-PC	t-PC_94	tonsil	4	F	CD20lo/CD38hi	Yes						
95	t-PC	t-PC_95	tonsil	2	M	CD20lo/CD38hi	Yes						
96	t-PC	t-PC_96	tonsil	4	F	CD20lo/CD38hi	Yes						
97	t-PC	t-PC_97	tonsil	13	M	CD20lo/CD38hi	Yes						
98	t-PC	t-PC_98	tonsil	4	M	CD20lo/CD38hi			Yes				
99	t-PC	t-PC_99	tonsil	4	M	CD20lo/CD38hi			yes				
100	t-PC	t-PC_100	tonsil	4	M	CD20lo/CD38hi			yes				
101	t-PC	t-PC_101	tonsil	4	M	CD20lo/CD38hi			yes				
102	t-PC	t-PC_102	tonsil	3	F	CD20lo/CD38hi			Yes				
103	t-PC	t-PC_103	tonsil	5	F	CD20lo/CD38hi					Yes		
104	t-PC	t-PC_104	tonsil	5	F	CD20lo/CD38hi					Yes		
105	bm-PC	bm-PC_105	bone marrow	20-30	NA	CD138+	Yes						pooled from 4 different donors
106	bm-PC	bm-PC_106	bone marrow	20-30	NA	CD138+	Yes						pooled from 4 different donors
107	bm-PC	bm-PC_107	bone marrow	20-30	NA	CD138+	Yes						pooled from 4 different donors
108	bm-PC	bm-PC_108	bone marrow	20-30	NA	CD138+		Yes					pooled from 4 different donors
109	bm-PC	bm-PC_109	bone marrow	20-30	NA	CD138+			Yes				pooled from 4 different donors
110	bm-PC	bm-PC_110	bone marrow	20-30	NA	CD138+		Yes					pooled from 4 different donors
111	bm-PC	bm-PC_111	bone marrow	20-30	NA	CD138+					Yes		pooled from 4 different donors

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Supplementary Table 2. Sequencing amounts in each of the 12 samples analyzed by WGBS.

Sample name	Yield passing filter (Gb)	Mapping (%)	Unique mapping (%)	Mean coverage
HPC-R1	178.3	94.0	79.7	53.4
HPC-R2	194.4	95.5	81.6	56.8
preB2C-R1	180.0	94.2	80.3	54.1
preB2C-R2	208.6	95.3	81.6	59.3
naiBC-R1*	147.2	89.2	76.9	41.9
naiBC-R2	190.6	94.8	82.0	55.6
gcBC-R1	177.8	92.2	77.5	52.2
gcBC-R2	198.4	84.7	81.5	56.9
memBC-R1*	152.4	88.9	77.4	43.2
memBC-R2	203.6	95.0	82.5	59.0
bm-PC-R1	183.5	95.0	82.1	55.5
bm-PC-R2	202.5	94.3	81.9	59.5
Total	**2217.3**			
Mean	**184.8**	**92.8**	**80.4**	**54.0**

* These two samples were previously published by Kulis et al. (Ref. 10)

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Supplementary Table 3. Primers used for validation experiments using bisulfite pyrosequencing and quantitative PCR.

Gene Name	Primer	Sequence	Comments
Validation by pyrosequencing			
BLNK (cg02980499, cg20540372)	Forward Reversed (biotinylated) Sequencing	GGAATTGTATTGTTGTGAAATTGTTAG TCAAATATACAACCTCCTTATTACC ATTTTGGTTTGTTTGAAAGTA	
HOXA1 (cg07659054)	Forward Reversed (biotinylated) Sequencing	AGATTTAAGTGAAGATTTGGTTTTAGAA AAATCCCAACCCAAAAAAATACC GTGAAGATTTGGTTTTAGAAT	
KLF6 (cg06048750)	Forward Reversed (biotinylated) Sequencing	GGTTTTTTTTAGGGTTGGTGTAATG ACACCAAAAACTCCCACTTAAA GTGGGTATTGATTTG	
LYN (cg21441674)	Forward (biotinylated) Reversed Sequencing	TTTTTTTTTGGTAAGGTATAATGGTTTA ACCCAAAATAAAATACAATAATACCATCA CCTAAACTCAAATAATCCTC	
nonCpG methylation measurements by pyrosequencing			
CAC.1	Forward Reversed (biotinylated) Sequencing	TGTTTAGGTGTTATTATTGGGATGAA ACAATCTTAATAAAAAATAAACCCAACATC ATTGGGATGAATGAGTTT	
CAC.2	Forward Reversed (biotinylated) Sequencing	GAAAAAAATTTGGAGTATATGGGGAAAGT AAAACCAAAAAATCTACCTACATCTT GGAGAGATTTTAGGGTTG	
CAC.3	Forward Reversed (biotinylated) Sequencing	TTGGTTTATTTATTTTTTGAGTATGTGAAA ATCTATAACAACCCCAATATCCTC TTGAGTTGGAGAGTTTATGG	this assay permits to measure also both flanking CpGs
CAC.4	Forward (biotinylated) Reversed Sequencing	GGATTGAGATTTTATATTATTTGGGTTGAA ACCTCCTTAAACACACACAA CTAAAACACAATCCTCA	this assay permits to measure also both flanking CpGs
Methylation of CpGs that flank nonCpGs			
CAC.1_CpG_5'	Forward Reversed (biotinylated) Sequencing	ATAGATAGGGGTTAGGTAGTTTAGAT ACTTTCCTCTCCCCATCTTACAACA AGATGGTTTTGGAAGTAG	
CAC.1_CpG_3'	Forward Reversed (biotinylated) Sequencing	GTTTAGGTGTTATTATTGGGATGAATG ATACCCTACCCCTACCTAACTTTCCTC GTTTTTGGTTGTTGTAAGAT	
CAC.2_CpG_5'	Forward Reversed (biotinylated) Sequencing	TGAGATTTTTTTAGGGTGGAATTTGT ACACCCTCCCACTAACTTT AATTTGTGTTTTTAGTTATGTAG	
CAC.2_CpG_3'	Forward (biotinylated) Reversed Sequencing	TTGGTTGTGTTTATATTTAGGGGTATGG CATTTAACACACCCTCCCACTAAC CTCAAAATATAAACAAATCCCTT	
quantitative PCR for DNMTs expression			
DNMT1 **DNMT3A** **DNMT3B**			primers taken from Fang et al. [Ref. 71]
EEF2	Forward Reversed	TGGAGATCTGCCTGAAGGAC GACTTGGAGAGGCAGAGCAC	

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