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Supplemental Information

Methylome Analysis of Human Bone Marrow MSCs Reveals Extensive Age- and Culture-Induced Changes at Distal Regulatory Elements

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Figure S1

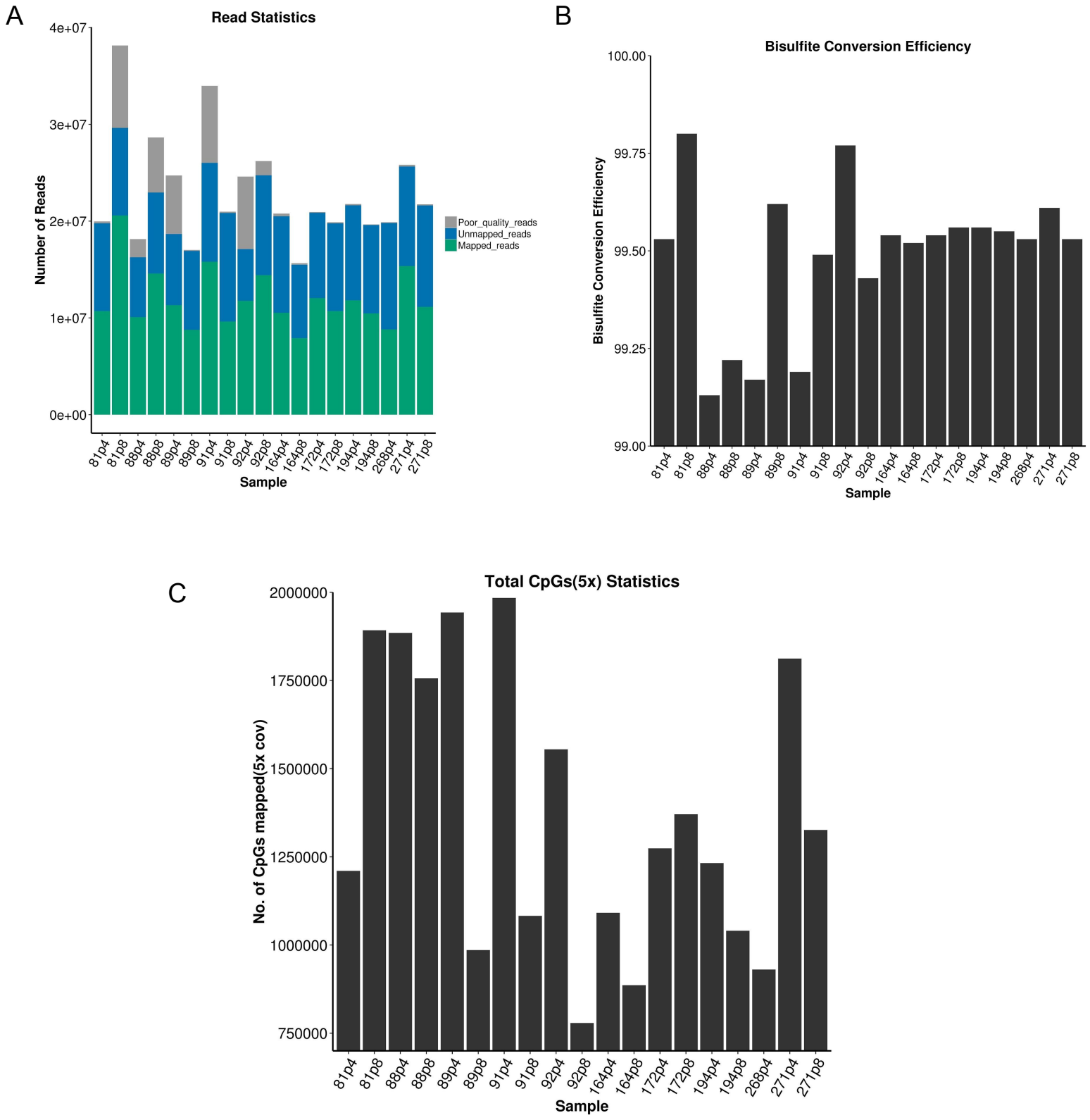

Figure S1. Enhanced RRBS stats. A) Methylation across the genome was captured using ERRBS (Illumina 1 x 50 bp single end sequencing). Around 50-60% of the reads were mapped to the hg19 version of the human genome. B) The data were examined for the efficiency of bisulfite conversion and is > 99% in all samples. C) Reads were mapped to hg19 and total CpGs were assessed. Number of CpGs captured varied from sample to sample and are in the range of 750,000 - 2,000,000. Related Figure 1.

Figure S2

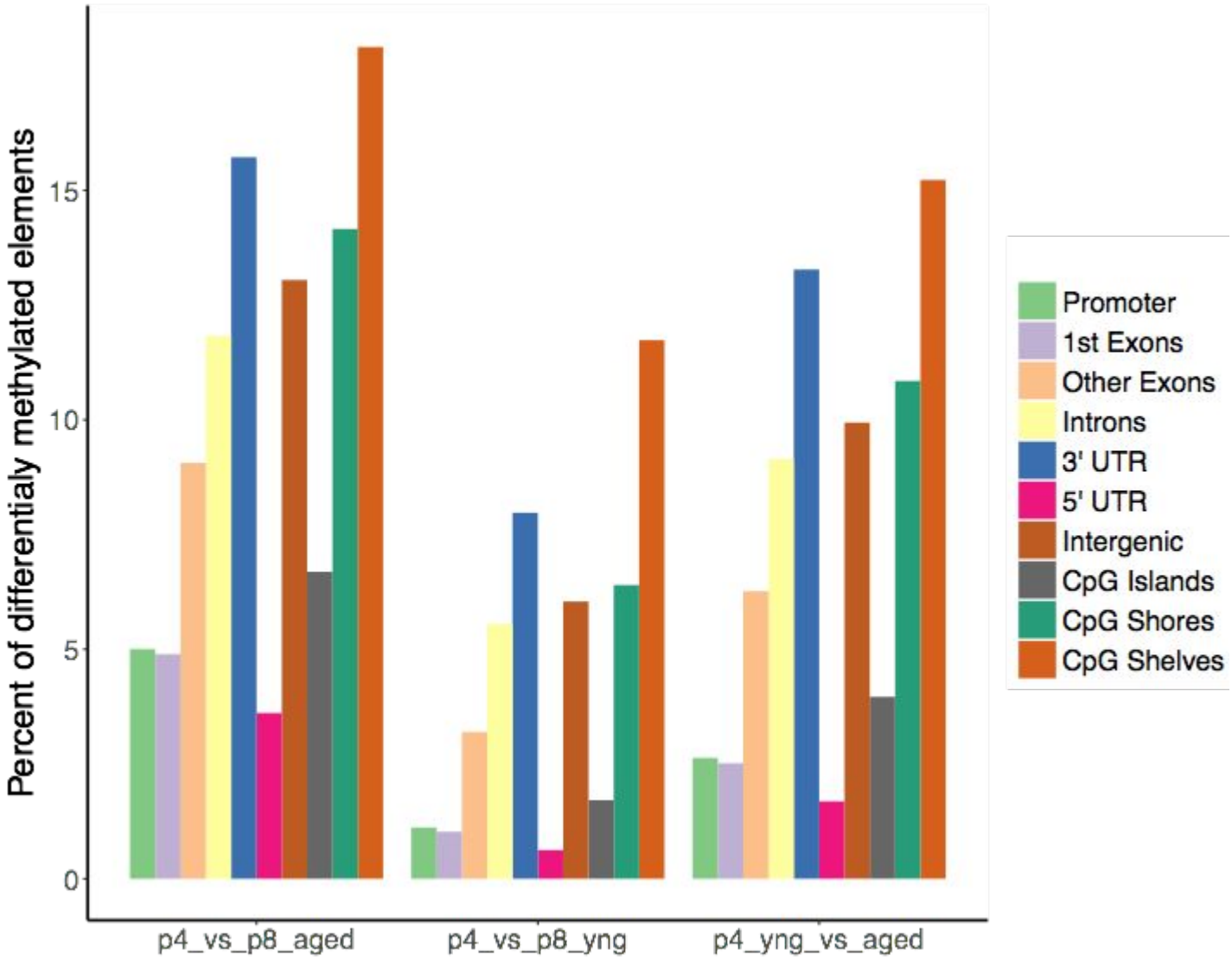

Figure S2. Percent of Genic and CpG annotated Elements with Differential Methylation. For each comparison (culture effect in aged - left; culture effect in young - center; age-associated - right) of ERRBS data, the percent of mapped elements that overlap at least one DMC are shown from left to right: promoters, 1st exons, all other exons, introns, 3' UTRs, 5' UTRs, intergenic regions, CpG islands, CG island shores, and CG island shelves. Related to Figure 2.

Figure S3

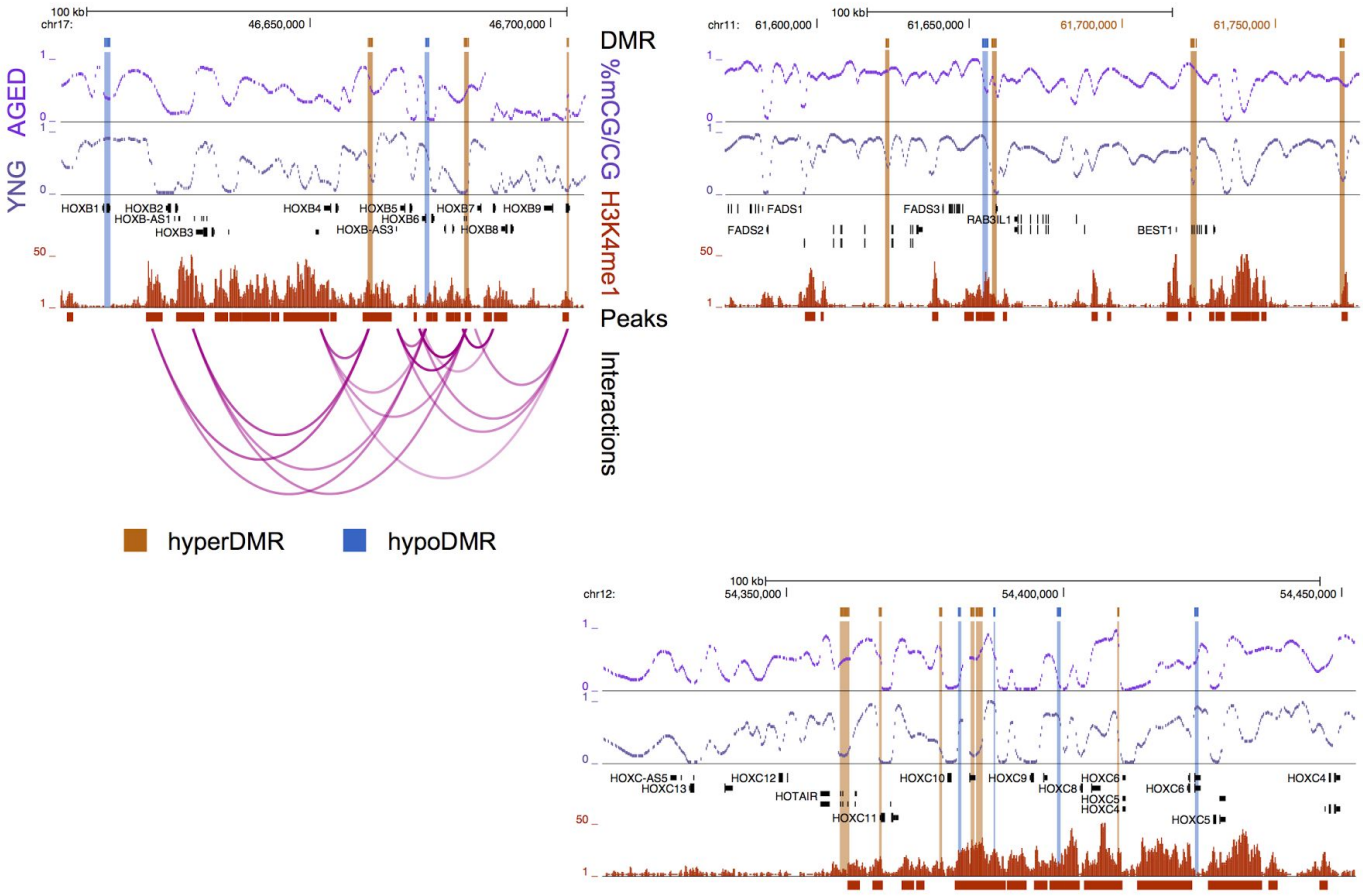

Figure S3. Browser shots of *HOXB*, *HOXC and FADS* gene cluster. This figure is as described in Figure 5 in the main text, and highlights DMRs at enhancer elements within the *HOXB*, *HOXC* and FADS gene clusters, which contains several genes down-regulated with age. Related to Figure 5.

Figure S4

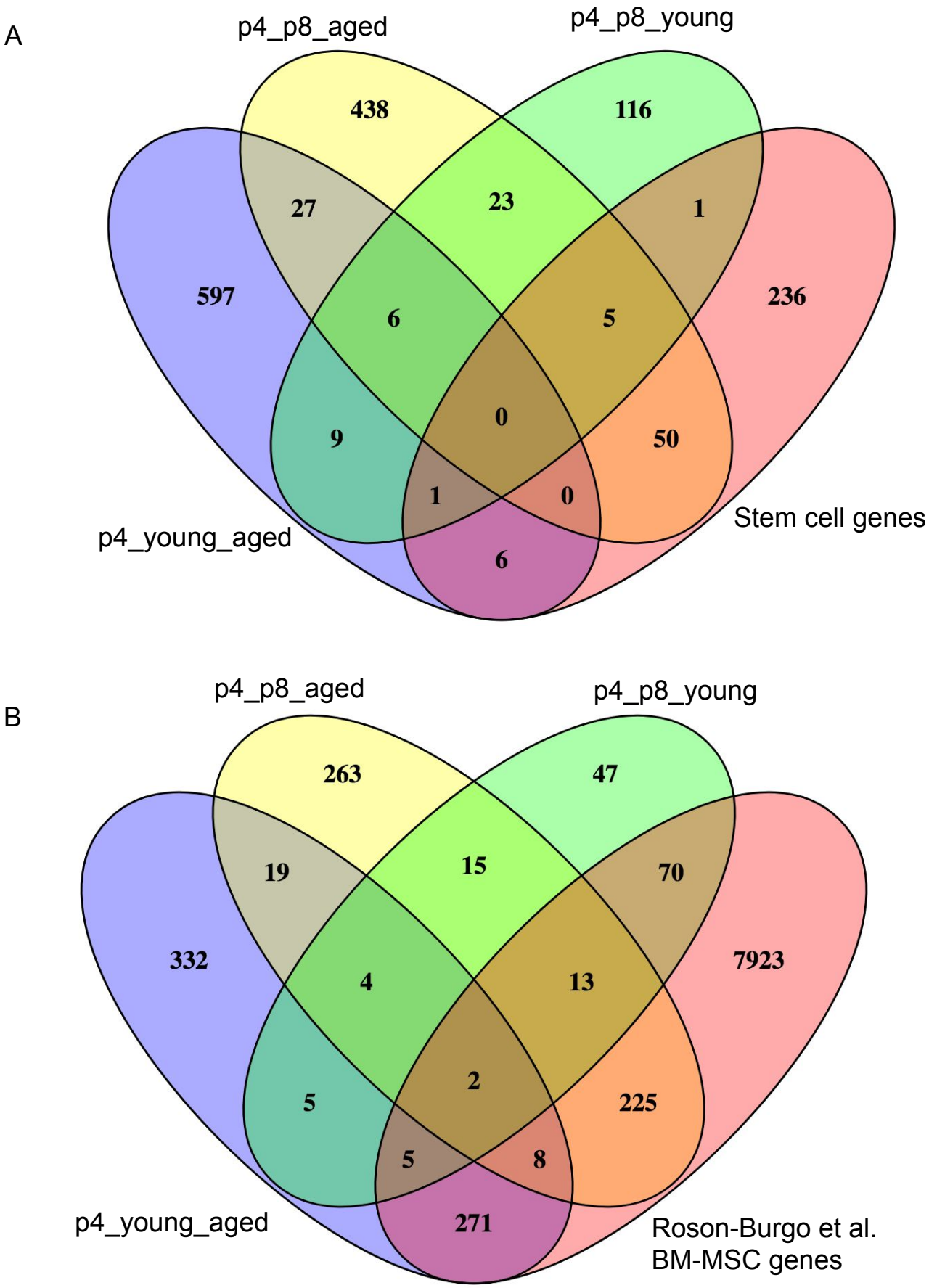

Figure S4. Overlap of BM-MSC genes. Overlap of BM-MSC genes from this study with stem cell genes from Muller et al., 2008 (A) and BM-MSC genes from Roson-Burgo et al., 2014 (B) comparisons. Related to Figure 6.

Figure S5

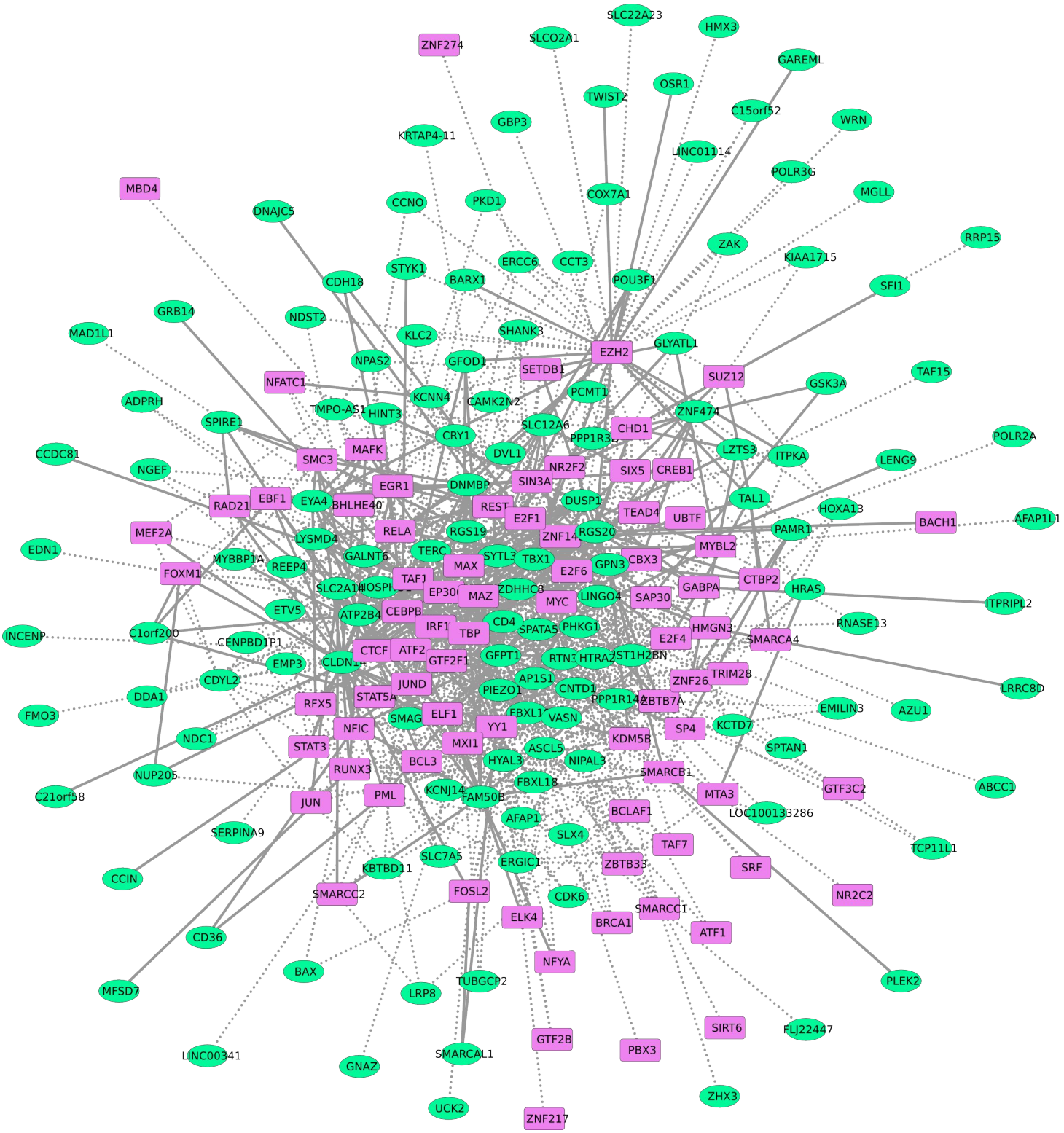

Figure S5. The *in silico* Hypomethylation Transcriptional Regulatory Network. The TFs (represented in magenta color) are enriched with hypomethylated CpGs and genes are activated DEGs (represented in green color), or increase with age. Here, solid lines denote direct target of TFs and dotted lines denote indirect targets. Related to Figure 7.

Figure S6

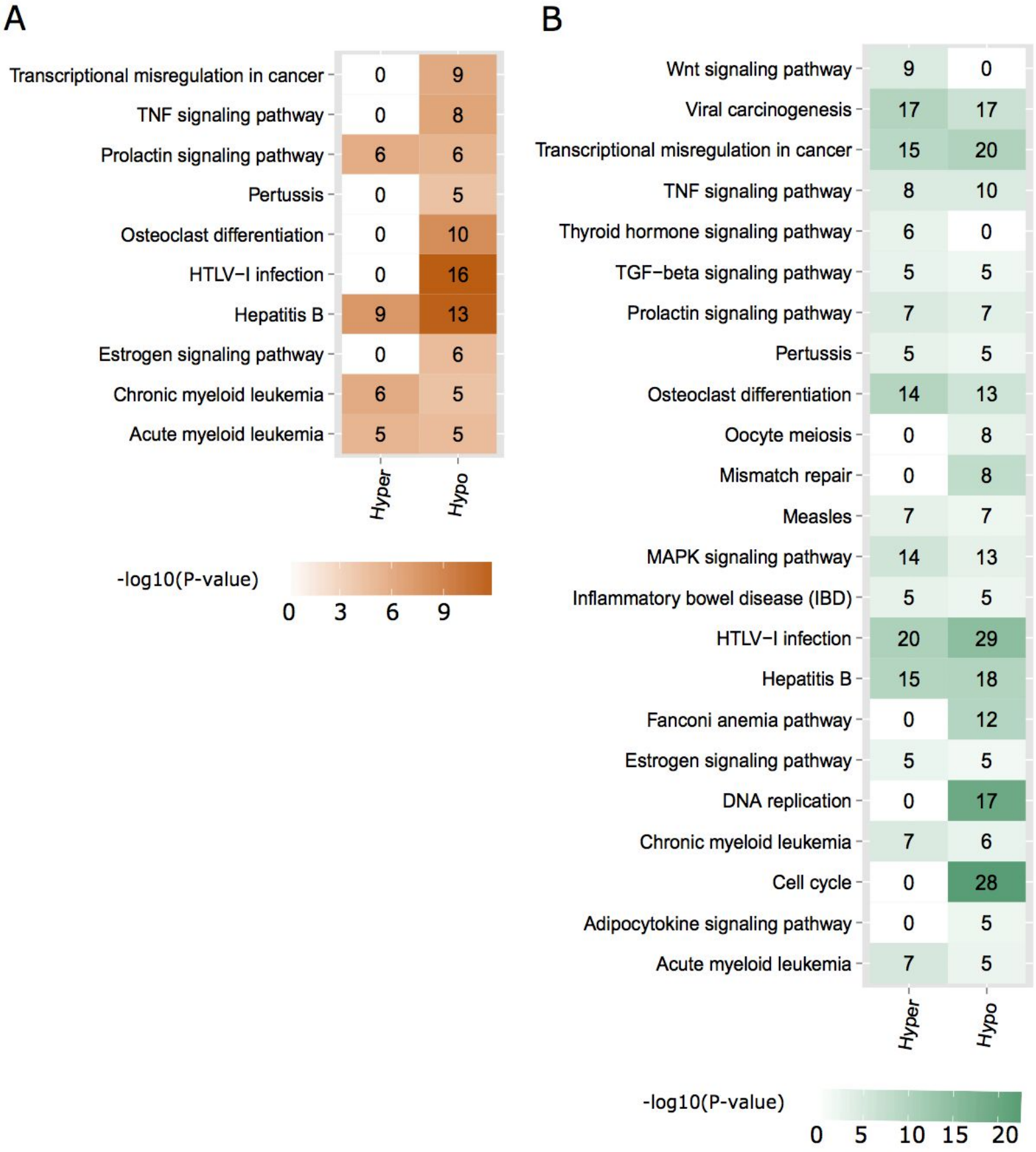

Figure S6. Overrepresented Pathways for Culture Induced Changes. Overrepresented pathways are identified with the list of transcription factors and differentially expressed genes (DEGs) from the p4_vs_p8_yng (A) and p4_vs_p8_aged (B) comparisons. The numbers indicate the number of TFs and/or DEGs involved in the pathway. Related to Figure 7.

Table S1

Table 1.1

	Number (Percent) of CpGs mapped to genic elements					
File_name	**Promoters**	**First Exons**	**Other exons**	**Introns**	**3' UTRs**	**5' UTRs**
p4_vs_p8_aged	108097 (17%)	88329 (13.89%)	65514 (10.3%)	254317 (40%)	10597 (1.67%)	55061 (8.66%)
p4_vs_p8_yng	108476 (21.94%)	91837 (18.58%)	38650 (7.82%)	187858 (38%)	6571 (1.33%)	57593 (11.65%)
p4_yng_vs_aged	105402 (18.24%)	88712 (15.35%)	53699 (9.29%)	228591 (39.56%)	9017 (1.56%)	55004 (9.52%)

Table 1.2

	Number (Percent) of DMCs mapped to genic elements					
File_name	**Promoters**	**First Exons**	**Other exons**	**Introns**	**3' UTRs**	**5' UTRs**
p4_vs_p8_aged	5409 (6.78%)	4323 (5.42%)	9617 (12.05%)	30085 (37.7%)	1666 (2.09%)	1988 (2.49%)
p4_vs_p8_yng	1210 (4.32%)	941 (3.36%)	3233 (11.54%)	10420 (37.2%)	524 (1.87%)	359 (1.28%)
p4_yng_vs_aged	2774 (5.07%)	2232 (4.08%)	6690 (12.22%)	20896 (38.17%)	1197 (2.19%)	927 (1.69%)

Table 1.3

	Number (Percent) of genic elements mapped with CpGs					
File_name	**Promoters**	**First Exons**	**Other exons**	**Introns**	**3' UTRs**	**5' UTRs**
p4_vs_p8_aged	13917(48.63%)	11929(39.92%)	15260 (7.85%)	45861 (22.17%)	2608 (11.97%)	9,738 (26.28%)
p4_vs_p8_yng	13578 (48.63%)	11927 (39.91%)	10328 (5.32%)	33880 (16.38%)	1810 (8.31%)	9805 (26.46%)
p4_yng_vs_aged	13838 (48.63%)	11866 (39.71%)	13979 (7.19%)	42859 (20.72%)	2431 (11.61%)	9749 (26.31%)

Table 1.4

	Number (Percent) of genic elements mapped with DMCs					
File_name	**Promoters**	**First Exons**	**Other exons**	**Introns**	**3' UTRs**	**5' UTRs**
p4_vs_p8_aged	4662 (16.29%)	3183 (10.65%)	6727 (3.46%)	21721 (10.5%)	1256 (5.76%)	1787 (4.82%)
p4_vs_p8_yng	1076 (3.76%)	720 (2.41%)	2522 (1.3%)	8425 (4.07%)	456 (2.09%)	312 (0.84%)
p4_yng_vs_aged	2188 (7.64%)	1502 (5.03%)	4504 (2.32%)	14822 (7.17%)	875 (4.02%)	786 (2.12%)

Table S1. DNA methylation statistics across genic elements. Related to Figure 2.

Table S2

Table 2.1

Number (Percent) of CpGs mapped to CpG annotation elements				
File_name	**Islands**	**Shores**	**Shelves**	**Outside**
p4_vs_p8_aged	290641 (45.72%)	88934 (13.99%)	26623 (4.19%)	229558 (36.11%)
p4_vs_p8_yng	285771 (57.80%)	63889 (12.92%)	14342 (2.90%)	130391 (26.37%)
p4_yng_vs_aged	280207 (48.50%)	78138 (13.52%)	21836 (3.78%)	197588 (3.78%)

Table 2.2

Number (Percent) of DMCs mapped to CpG annotation elements				
File_name	**Islands**	**Shores**	**Shelves**	**Outside**
p4_vs_p8_aged	19435 (24.36%)	12592 (15.78%)	4825 (6.05%)	42944 (53.82%)
p4_vs_p8_yng	4891 (17.46%)	4088 (14.59%)	1683 (6.01%)	17351 (61.94%)
p4_yng_vs_aged	11107 (20.29%)	8475 (15.48%)	3325 (6.07%)	31843 (58.16%)

Table 2.3

Number (Percent) of CpG annotation elements mapped with all CpGs			
File_name	**Islands**	**Shores**	**Shelves**
p4_vs_p8_aged	19169 (71.95%)	19885 (41.25%)	7388 (18.19%)
p4_vs_p8_yng	18265 (68.56%)	16645 (34.53%)	4796 (11.81%)
p4_yng_vs_aged	18864 (70.81%)	19006 (39.42%)	6663 (16.4%)

Table 2.4

Number (Percent) of CpG annotation elements mapped with DMCs			
File_name	**Islands**	**Shores**	**Shelves**
p4_vs_p8_aged	10670 (55.66%)	9015 (45.34%)	3520 (47.64%)
p4_vs_p8_yng	3033 (16.61%)	3239 (19.46%)	1355 (28.25%)
p4_yng_vs_aged	5397 (28.61%)	5899 (31.04%)	2479 (37.21%)

Table S2. DNA methylation statistics across CpG annotation elements. Related to Figure 2.

Table S5

Table 5.1

	Number (Percent) of DMCs mapped to genic elements					
File_name	**Promoters**	**First Exons**	**Other exons**	**Introns**	**3' UTRs**	**5' UTRs**
all_early_vs_late	577 (3.98%)	400 (2.76%)	1433 (9.90%)	4973 (34.34%)	249 (1.72%)	164(1.13%)

Table 5.2

Distribution of DMCs across CpG annotation elements during expansion				
File_name	**Islands**	**Shores**	**Shelves**	**Outside**
all_early_vs_late	2593 (17.9%)	2242 (15.4%)	877(6.05%)	8770 (60.55%)

Table 5.3

File_name	**Total DMCs**
p4_vs_p8_aged	79796
p4_vs_p8_yng	28013
p4_yng_vs_aged	54750
all_early _vs_late	14482

Table S5. DNA methylation statistics for culture changes irrespective of age.

As excel files:
Table S3. Statistical significance of DMC overlap analysis. Related to Figure 2.
Table S4. Methylation changes at TFBS (ERRBS). Related to Figure 3.
Table S6. Putative enhancer DMR-gene interactions. Related to Figure 5.
Table S7. Biological aging induced DNA methylation changes at TFBS (WGBS). Related to Figure 7.

Supplemental Experimental Procedures

Isolation and Cell Culture of BM-MSCs

Human BM-MSCs were obtained from bone marrow aspirates taken from the iliac crest or upper femur metaphysis of adult patients after written informed consent. All patient protocols were approved by the Ethical Committee of Northern Ostrobothnia Hospital District or Ethical Committee of Hospital District of Helsinki and Uusimaa. Human BM-MSCs from five aged (from 62 to 82 years of age, mean 74.6 years) and five young adult donors (from 20 to 24, mean 22.2 years) were isolated and cultured as previously described (Leskelä et al. 2003; Peura et al. 2009; Kilpinen et al. 2013). Briefly, by growth in minimum essential alpha-medium (αMEM) supplemented with 20mM HEPES, 10% heat inactivated fetal bovine serum (FBS), 2mM L-glutamine and 100 units/mL penicillin and 100 µg/mL streptomycin (all from Gibco, Invitrogen, Paisley, UK). The same serum lot was used throughout the study. The cells were plated at a density of 1000 cells/cm^2, medium was renewed twice a week and the cells were harvested when 70-80% confluent. Only four of five aged samples were carried to passage 8 and therefore used in the aged p4_vs_p8 comparison (Figure 1).

ERRBS and WGBS Library Preparation

For ERRBS, DNA (10 ng) was digested with MspI (Thermo Fisher, Cat # FD0544). Digested DNA was subjected to end repair using NEBNext End repair module (New England Biolabs, Cat# E6050S) followed by clean-up with Ampure XP beads. Further subjected to A-tailing reaction using NEBNext-dA-tailing module (Cat# E6053) and clean-up with beads. Purified A-tailed unmethylated lambda DNA (Promega, Cat# D1521) was spiked-in at 0.1-0.5% and mixed with methylated oligo adapters (20uM). Ligation was performed with NEBNext Quick Ligation module (New England Biolabs, Cat# E6056S). Ligated DNA was purified with Ampure XP beads. Ligated and purified DNA was subjected to bisulfite conversion using MethylCode Bisulfite conversion kit (Thermo Fisher, Cat# MECOV-50) and purified as per protocol. Purified DNA was subjected to 18 cycles of PCR amplification with KAPA HiFi HotStart Uracil+ (KAPABiosystems Cat# KK2802) and purified. 1-2 ul of purified library subjected to second round of PCR amplification for 2-4 cycles of PCR and purified with Ampure XP beads. Purified library was subjected to sequencing with Illumina HiSeq 2500 single end 50bp sequencing. For WGBS, genomic DNA (30ng in a

volume of 130 ul) was subjected to sonication using Covaris sonicator until DNA is sheared into fragments of size 40bp to 600bp. Sheared DNA was purified using Ampure XP beads. WGBS libraries were prepared similar to ERRBS library.

ChIP-seq Data Analysis

H3K4me1 data from four human BM-MSC donors was obtained from the Roadmap Epigenome Consortium. Peaks were called using MACS version 1.4.2 on individual H3K4me1 ChIP-seq replicates normalized to input to assess reproducibility (Zhang et al. 2008). Peaks were recalled on merged replicates and used for further analysis.

Gene Expression

Gene expression data from donor samples at each passage was previously described (Kilpinen et al. 2013). Data from this publication were utilized in this work to assess the correlation between gene expression and the methylation changes on the genome.

Pathway Analysis

ClueGO (Bindea et al. 2009) was used to identify the enriched pathways with the data from KEGG pathways. The statistically significant pathways were filtered with P-value < 0.05 and P-values are adjusted with Benjamini Hochberg method for multiple hypothesis correction.

Transcriptional Regulatory Network Construction

First we identified the TFs at significantly enriched with DMCs. Next, we checked whether their TFBS are overlapping within promoter regions of DEGs and termed these DEGs as direct targets. Next, we identified the nearest neighboring gene (NNG) for these TFBS and termed as their indirect targets. In addition, we included only only down- and up-regulated DEGs for hyper and hypomethylated TFs as targets respectively. Cytoscape software (Shannon et al. 2003) was used for visualization of the networks.

DMR-Gene Interaction Data

We downloaded the DHS elements and promoter interaction data from (Sheffield et al. 2013). These interactions are based on correlation between DHS signal intensity and gene expression pattern across numerous human cell types. Only positive correlation interactions are included for further analysis. We overlapped identified DMRs with DHS elements to find DMR-gene interactions further filtered interactions with only differentially expressed genes (DEGs).

SUPPLEMENTARY REFERENCES

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