**Supplementary Figure 1: Inference of TGF-β signaling from the aging HSC transcriptome.**

Shown is the TGF-β1 mechanistic network from Ingenuity Pathway Analysis (IPA) consisting of network regulators (Tgfb1, Sp1, Egr1, Smad3) and the subset of HSC aging differentially expressed downstream genes that function in the regulation of transcription. TGF-β1 downstream genes not associated with the network are grouped in the upper left. Symbolic representations of genes, expression changes, and regulatory relationships are shown in the upper right. Grey arrows indicate the Ingenuity database contains evidence of a regulatory relationship but an effect could not be predicted based on available evidence. Further details of the analysis are in Extended Experimental Procedures. Full lists of TGF-β1-regulated genes and network memberships are included in Table S3.

See also Figure 1.
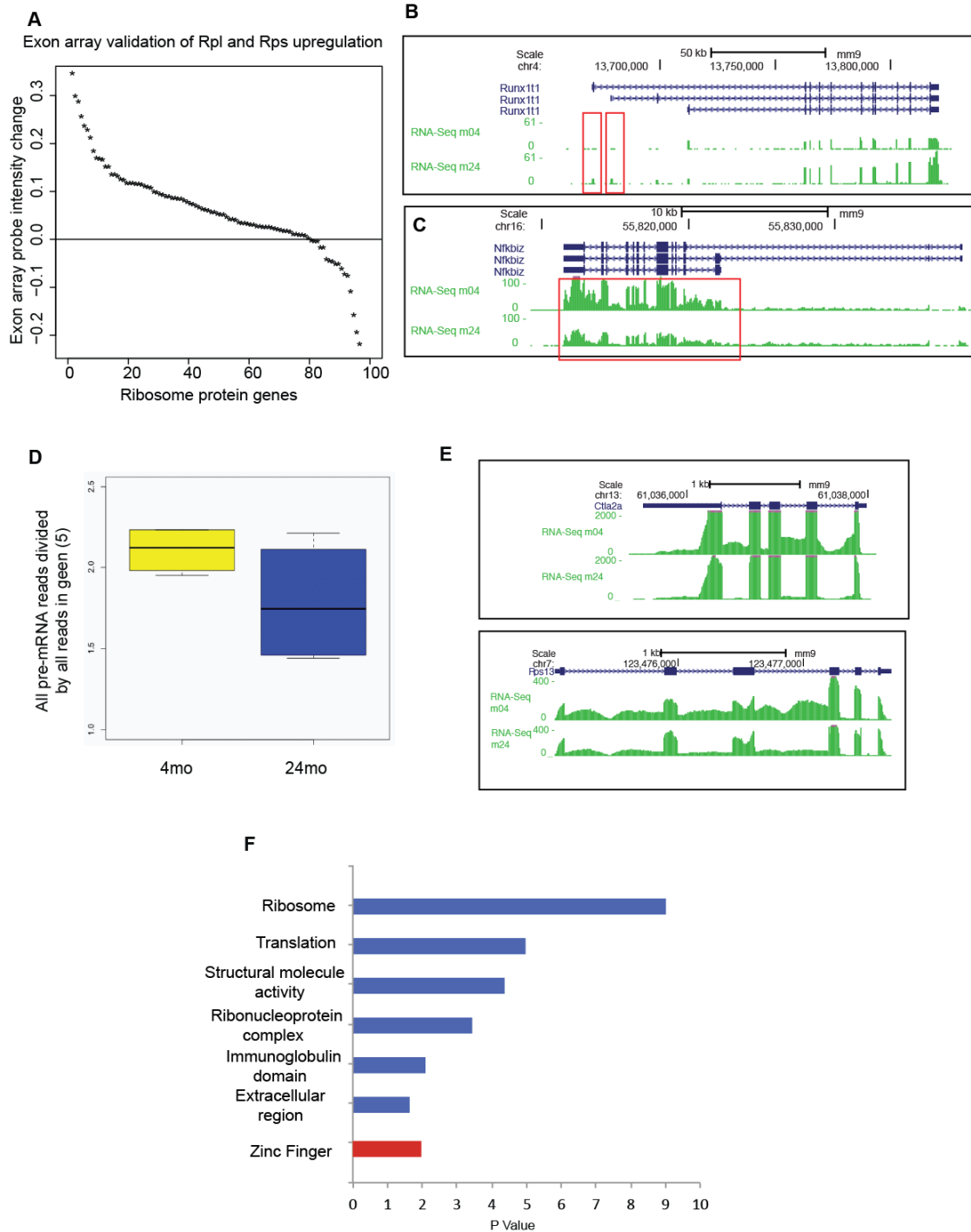
Supplementary Figure 2: RNA-seq revealed altered alternative splicing outcomes with aging.

(A) Exon array validation of Rpl and Rps gene expression changes with HSC aging. The Y-axis shows probe intensity changes.

(B) UCSC browser tracks showing alternative promoter usage of the *Runx1t1* gene in 4mo and 24mo HSC.

(C) UCSC browser tracks showing Nfkbiz gene expression in 4mo and 24mo HSC.

(D) Bar graph showing percentage of pre-mRNA reads divided by all reads in 4mo and 24mo HSCs.

(E) UCSC browser tracks showing Ctla2a and Rps13 genes expression in 4mo and 24mo HSC.

(F) GO analysis for genes with decreased (blue) and increased (red) pre-mRNA levels with aging.

See also Figure 1.
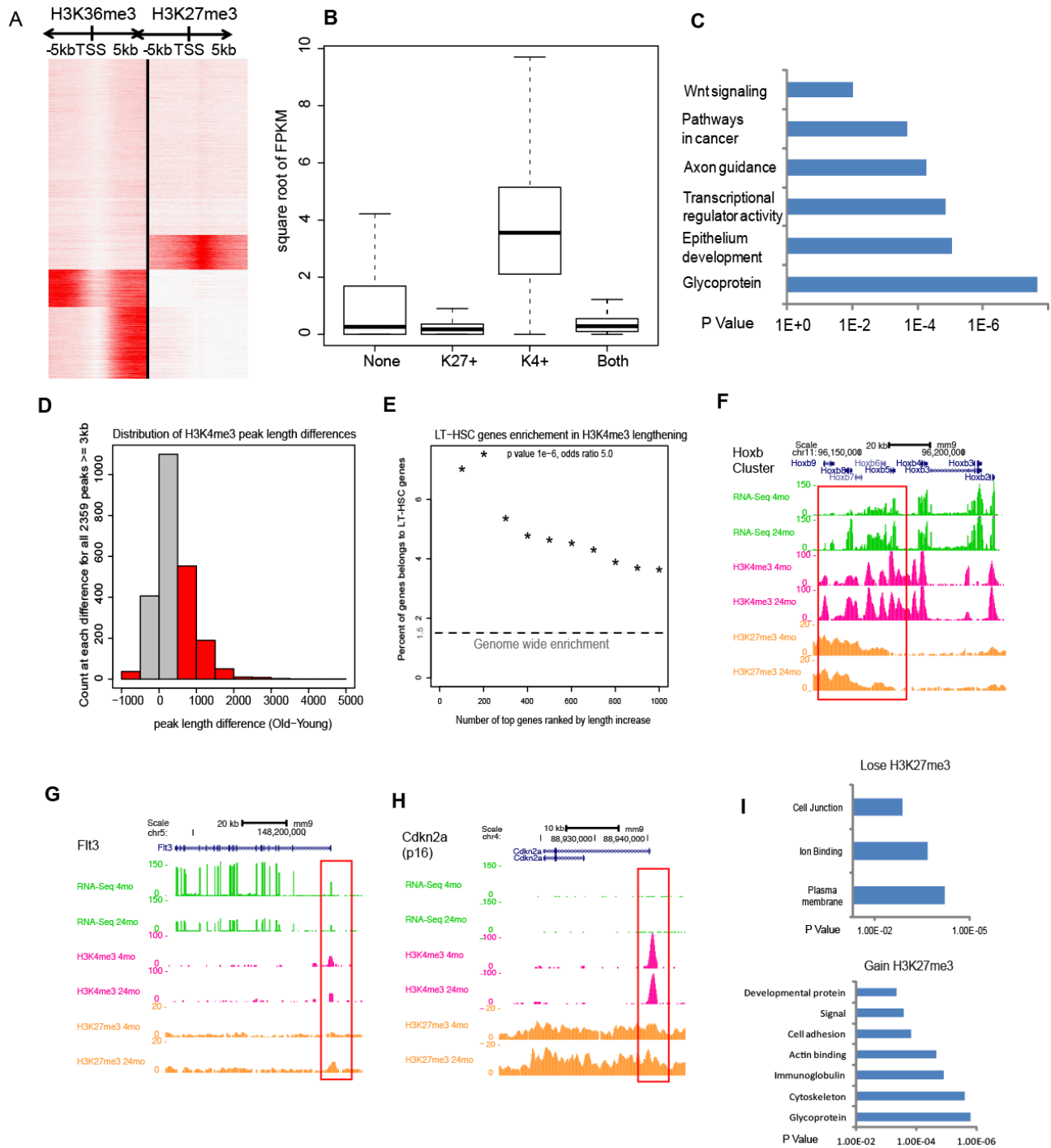
Supplementary Figure 3: Chromatin alterations with aging, and their correlation with gene expression changes.

(A) Heatmap comparing H3K36me3 and H3K27me3 signals on the TSS±5 kb of the Refseq genes.

(B) Box blot showing the expression of bivalent genes compared with H3K4me3 or H3K27me3 only marked genes. K27+ indicates H3K27me3, and K4+ indicates H3K4me3.

(C) GO analysis of 240 genes marked only by H3K4me3 in HSC, bivalently in ESC, and H3K27me3-only in differentiated B-cells and granulocytes. The X-axis shows the p-value.

(D) Bar graph for the H3K4me3 peak length differences between old and young. Only the peaks longer than 3kb are considered. The grey bars represent peaks with length differences smaller than 500bps, while the red bars show the number of peaks longer or less than 500 base pairs in old HSC.

(E) The running enrichment of the group of LT-HSC genes among genes that are ranked at the top of those that exhibit an increase in H3K4me3 peak length with age. For each star, the abscissa denotes the selection of X top genes from the gene list ranked by H3K4me3 peak length increase and the ordinate denotes the percent of selection belonging to LT-HSC fingerprint genes defined by (Chambers et al., 2007a). The enrichment is maxed at selection of the top 200 genes, among which 8% are LT-HSC genes. The Fisher's exact test gives a p-value 4E-6 and a 4.5-fold enrichment against genome wide background, marked by the grey horizontal line.

(F) UCSC browser tracks showing the expression, H3K4me3 and H3K27me3 profiles across the Hoxb cluster in 4mo and 24mo HSC. Red box highlights the Hoxb genes with increased H3K4me3.

(G) UCSC browser tracks showing the expression, H3K4me3 and H3K27me3 profiles across the Flt3 gene in 4mo and 24mo HSC. Red box highlights the region with increased H3K27me3.

(H) UCSC browser tracks showing the expression, H3K4me3 and H3K27me3 profiles across the Cdkn2a (p16) gene in 4mo and 24mo HSC.

(I) Functional enrichment for the genes gaining or losing H3K27me3 with HSC aging.

See also Figure 2 and 3
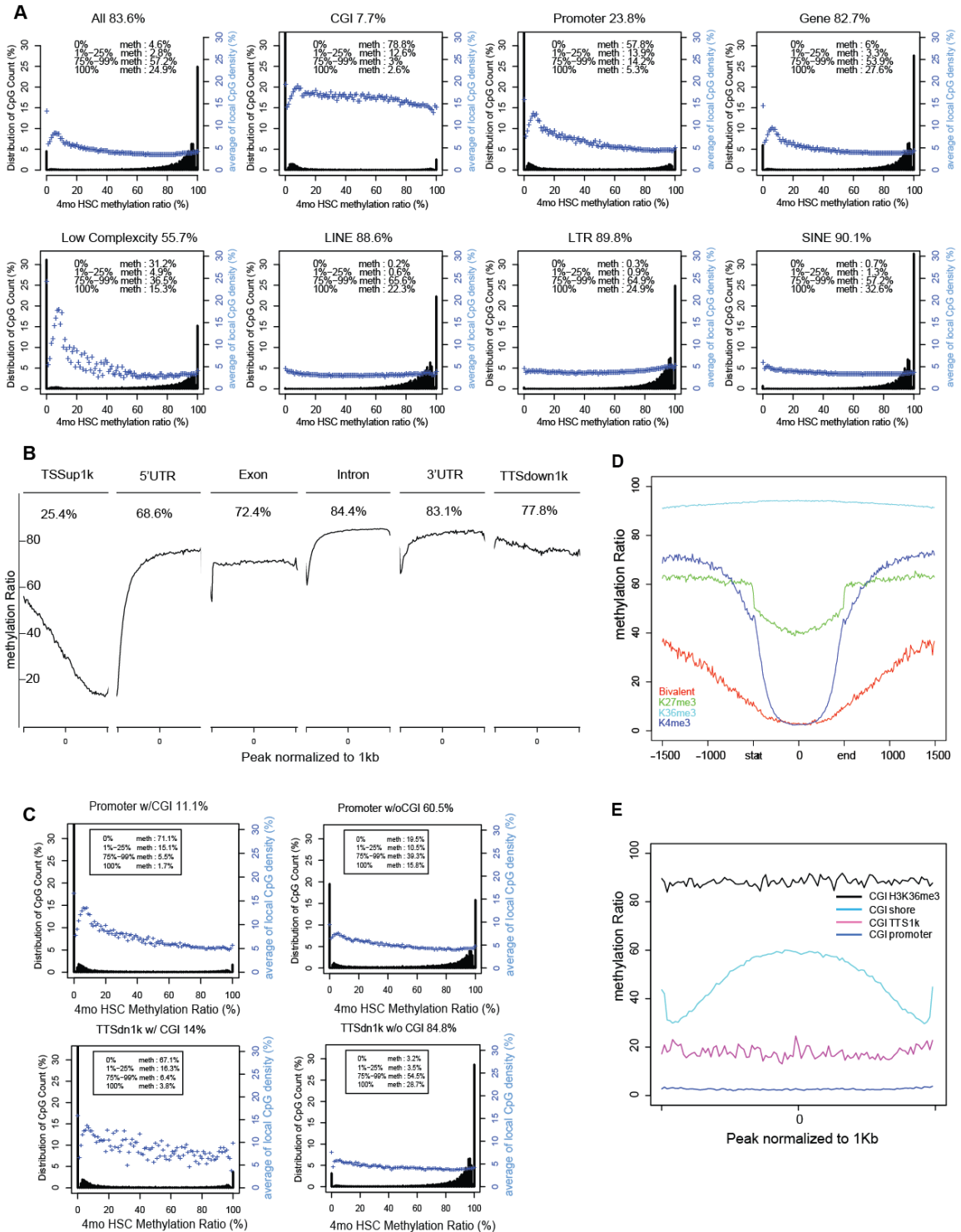
Supplementary Figure 4: HSC specific methylome.

(A) Distribution of methylation ratios for different genomic regions in 4mo HSC and their relationship to local CpG density (blue dots). The X-axis shows the methylation ratio (0-100%), and Y-axis shows the percent of CpG counts at each x-axis methylation ratio.

(B) Average methylation ratio based on gene structure. TSSup1k: the region from Transcription start site to a limit of 1kb upstream. TSSdown1k: the region from the transcription termination site to a limit of 1 kb downstream. The x-axis is the coordinate from -500 bp to 500 bp and the y-axis is the methylation ratio. Methylation for each feature is average of all refseq genes.

(C) Distribution of the methylation ratio for promoter regions and regions from the transcriptional termination site to its downstream 1kb (TTSdn1k) with or without CGIs. Blue dots show the local CpG density. The X-axis shows the methylation ratio (0-100%), and the Y-axis shows the percent of CpG counts at each x-axis methylation ratio.

(D) Position-dependent profile of the methylation ratio for different histone modifications binding regions, including H3K4me3 binding sites, H3K27me3 binding sites, bivalent domains (both H3K4me3 and H3K27me3), and H3K36me3 binding sites. The X-axis is the coordinate - 1500 bp to 1500 bp and the Y-axis is the average methylation ratio. All binding sites are centered at an X-axis coordinate of 0.

(E) Position-dependent profiles of methylation ratios for CGIs located at different genomic regions, including CGI marked by H3K36me3 binding sites, CGI shore, and CGI overlapping with TTS downstream 1k region and CGI overlapping promoter regions. The X-axis is the coordinate from -500 bp to 500 bp and the Y-axis is the methylation ratio. Methylation is average of all CpG islands in each category.

See also Figure 4
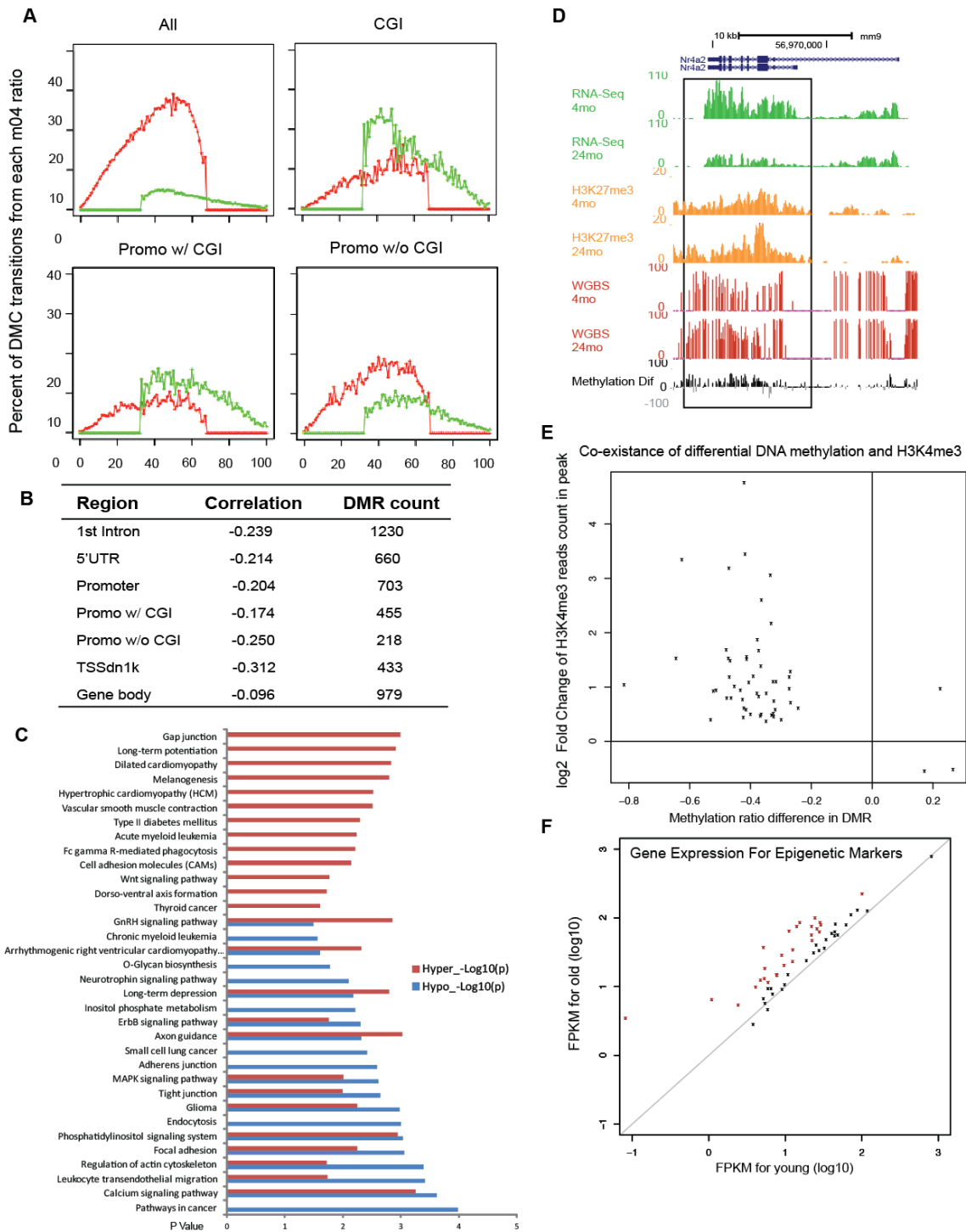
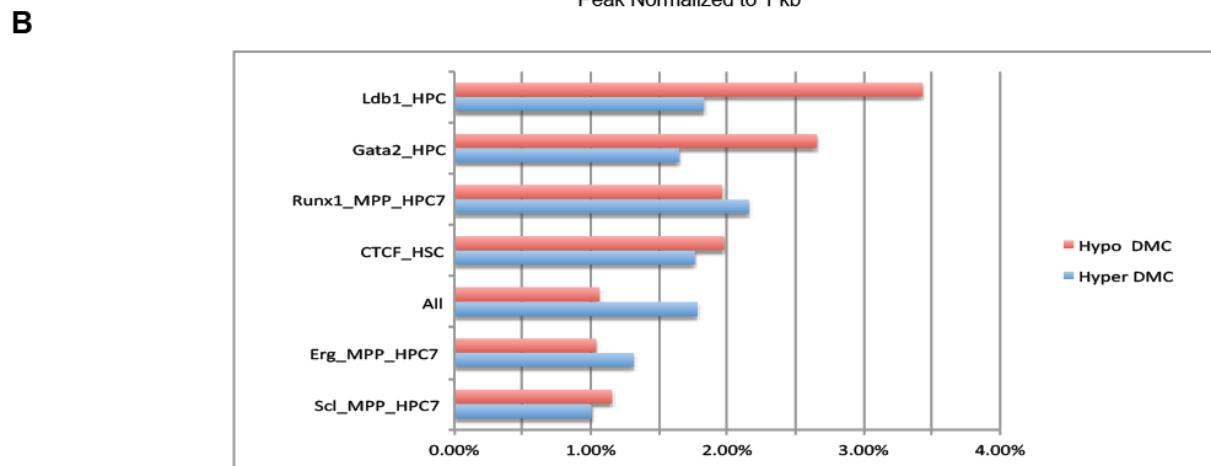**Supplementary Figure 5: DNA methylation alterations with HSC aging.**

(A) CpGs with intermediate methylation ratio in young HSC are more susceptible for alterations with aging. The x-axis is the methylation ratio in 4mo HSC and the y-axis is % of DMCs relative to all CpGs at each specified 4-month ratio. The red line represents % of hyper-

DMCs and the green line represents % of hypo-DMCs. The four sub-figures are for subset of CpGs that are located in the different regions of the genome, including All, CGI, non-CGI promoters and CGI promoters.

(B) The correlation of DMRs at different regions with gene expression. DMRs at TSSdn1K show the highest anti-correlation with gene expression.

(C) Gene ontology analysis of hyper- and hypo-DMR genes. X-axis shows the p-value.

(D) UCSC browser tracks showing the expression, methylation and histone profiles across the Nr4a2 gene in 4mo and 24mo HSC. The boxed region indicates the shorter isoform that becomes hypermethylated with age, with downregulation.

(E) Scatter plot showing co-existence of differential DNA methylation and H3K4me3. X-axis shows methylation ratio differences in DMR. Y-axis shows log2 fold changes of H3K4me3 reads count in peaks.

(F) Scatter plot showing gene expression changes between young and old HSCs. X-axis log10 shows FPKM for young HSCs. Y-axis shows log10 FPKM for old HSCs.
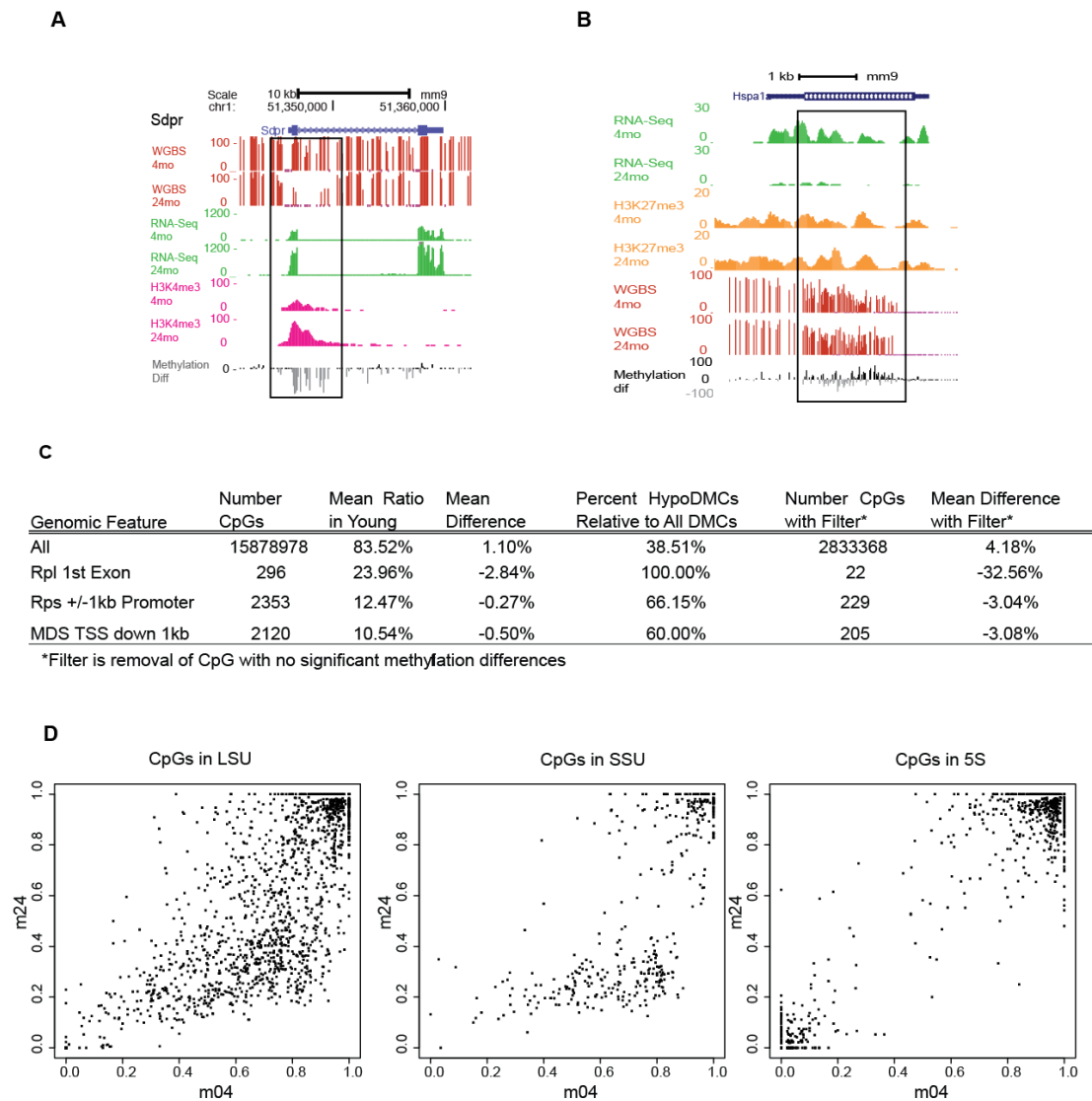
See also Figure 5
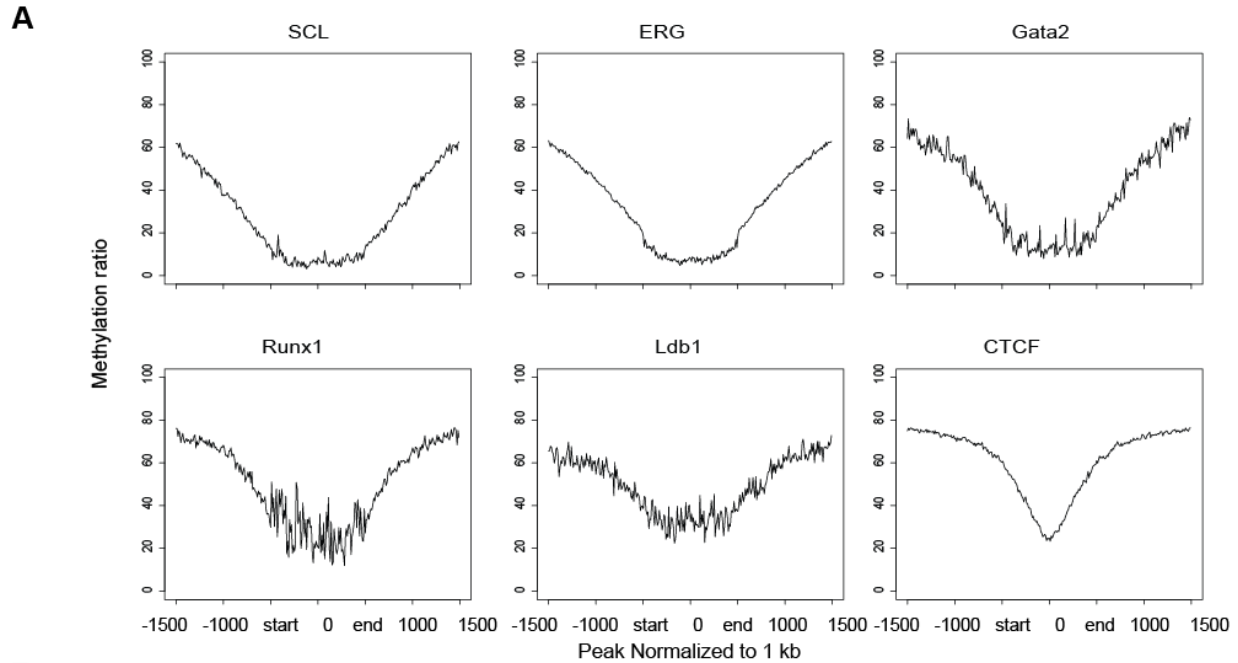
Supplementary Figure 6: DMRs are enriched for transcription factor (TF) binding sites.

(A) Average methylation ratios for different TF binding sites in HSC. Scl, Erg, Gata2, Runx1 and Ldb1 binding sites were collected from a previous ChIP-seq study in the HPC7 cell line (Wilson et al., 2010). CTCF binding sites were obtained by ChIP-seq using old HSC (24 mo) in this study. Peaks are normalized to 1 kb. The X-axis shows the relative distance to the core binding sites.

(B) The enrichment of hyper and hypo-DMCs for different TF binding sites in the indicated cell types or cell lines. The X-axis shows the % of hyper or hypo-DMCs among the total CpGs in different bindings sites. "All" represents the genome background.

See also Figure 6

C

Genomic Feature	Number CpGs	Mean Ratio in Young	Mean Difference	Percent HypoDMCs Relative to All DMCs	Number CpGs with Filter*	Mean Difference with Filter*
All	15878978	83.52%	1.10%	38.51%	2833368	4.18%
Rpl 1st Exon	296	23.96%	-2.84%	100.00%	22	-32.56%
Rps +/-1kb Promoter	2353	12.47%	-0.27%	66.15%	229	-3.04%
MDS TSS down 1kb	2120	10.54%	-0.50%	60.00%	205	-3.08%

*Filter is removal of CpG with no significant methylation differences

Supplementary Figure 7: Genome stability with HSC aging.

(A) Genome browser tracks showing methylation ratio, expression and H3K4me3 for one example of an LT-HSC gene, Sdpr, in 4mo HSC and 24mo HSC. The boxed area indicates the promoter region that shows loss of DNA methylation, increased H3K4me3 marking, and increased gene expression with age.

(B) Genome browser tracks showing methylation ratio, expression, H3K4me3 and H3K27me3 for one example of an MDS gene, Hspa1a, in 4mo HSC and 24mo HSC.

(C) Table of methylation ratio differences for ribosome Rpl genes first exon, Rps genes ± 1 kb promoter and MDS genes TSSdn1k with aging.

(D) Methylation ratio alterations for CpGs in ribosomal large subunit (LSU), ribosomal small subunit (SSU) and 5S subunit genes with aging.

See also Figure 6