Supplemental figure legends

**Figure S1. Overview of ATAC-seq signal and Alternative Flow Cytometry to Isolate Cell Types of HSCs.** (A) The distribution of open chromatin regions along each chromosome. The y-axis shows the abundance of open chromatin peak number in physical bins of 1 Mb for each chromosome (generated using ggbio R package). (B) Pearson correlation coefficient matrix of pairwise comparison among ATAC-seq samples. (C) Representative flow cytometry to assess the Sca1+c-Kit+ population of cells, then to further distinguish LT-HSCs, ST-HSCs and MPPs using the CD34 and Flk2/Flt3 combination of markers.

**Figure S2. Browser Track Plot of ATAC-seq signal (RPM) in the Genomic Regions Around CD48, CD150, Sca-1/Ly6a and Bmi1, respectively.** ATAC-seq signal (RPM) in the genomic regions around CD48 (A), CD150 (B), Sca-1/Ly6a (C) and Bmi1 (D) are shown.

**Figure S3. Browser Track Plot of ATAC-seq signal (RPM) in the Genomic Regions Around Gfi1, P21, P57 and c-Myc, respectively.** ATAC-seq signal (RPM) in the genomic regions around Gfi1 (A), P21 (B), P57 (C) and c-Myc (D) are shown.

**Figure S4. Relationship between open chromatin in gene promoter and gene expression activity.** (A-C) Boxplot of ATAC-seq signal (RPM) at 1kb gene promoter in different groups of gene expression activity in LT-HSCs (A), ST-HSCs (B) and MPPs (C). The gene expression activity from Gene Expression Commons (https://gexc.stanford.edu) were ordered from high and low, and were equally divided into 5 groups. e.g. 0-20% represented top 20% expressed genes. (D) Enrichment (-log_{10} (FDR)) of Gene ontology (GO) terms in top 3000 highly expressed genes in LT-HSCs.

**Figure S5. Chromatin Remodeling in the Promoter Region upon HSC Differentiation to MPPs.**
(A) Heat map of read density around all gene promoter-TSS regions (from -1000 bp to +1000 bp) in LT-HSCs, ST-HSCs and MPPs, respectively. (B) The average read density around promoter-TSS regions of all genes (from -1000 bp to +1000 bp) in LT-HSCs, ST-HSCs and MPPs, respectively. (C) The average read density (smoothed) around promoter-TSS regions of all genes (from -1000 bp to +1000 bp) in LT-HSCs, ST-HSCs and MPPs, respectively.

**Figure S6. Enrichment of DNA Cis-elements in Open Protein-coding Genes and Enhancer Regions.** (A) The overlap of enriched TF binding motifs in LT-HSCs, ST-HSCs and MPPs. (B) Heat map of the significant enrichment values (-log (p-value)) of transcription factor binding motifs found in enhancers that display an open chromatin state in LT-HSCs, ST-HSCs and MPPs. (C) The overlap of enriched TF binding DNA sequence motifs in LT-HSCs, ST-HSCs and MPPs. (D) The overlap of poised enhancers in LT-HSCs and active enhancers in ST-HSCs.

**Figure S7. Metaplot of ATAC-seq Signal Around the Flanking 1kb of Specific TF Motifs.** Metaplots of ATAC-seq signal around the flanking 1kb of ERG (A), ETS1 (B), EHF (C), GABPA (D), FLI1 (E), ETV1 (F) motifs.