Supplemental Materials

Regeneration Alters Open Chromatin and *Cis*-Regulatory Landscape of Erythroid Precursors (Zhou et al.)

File Contains:

Supplemental Methods

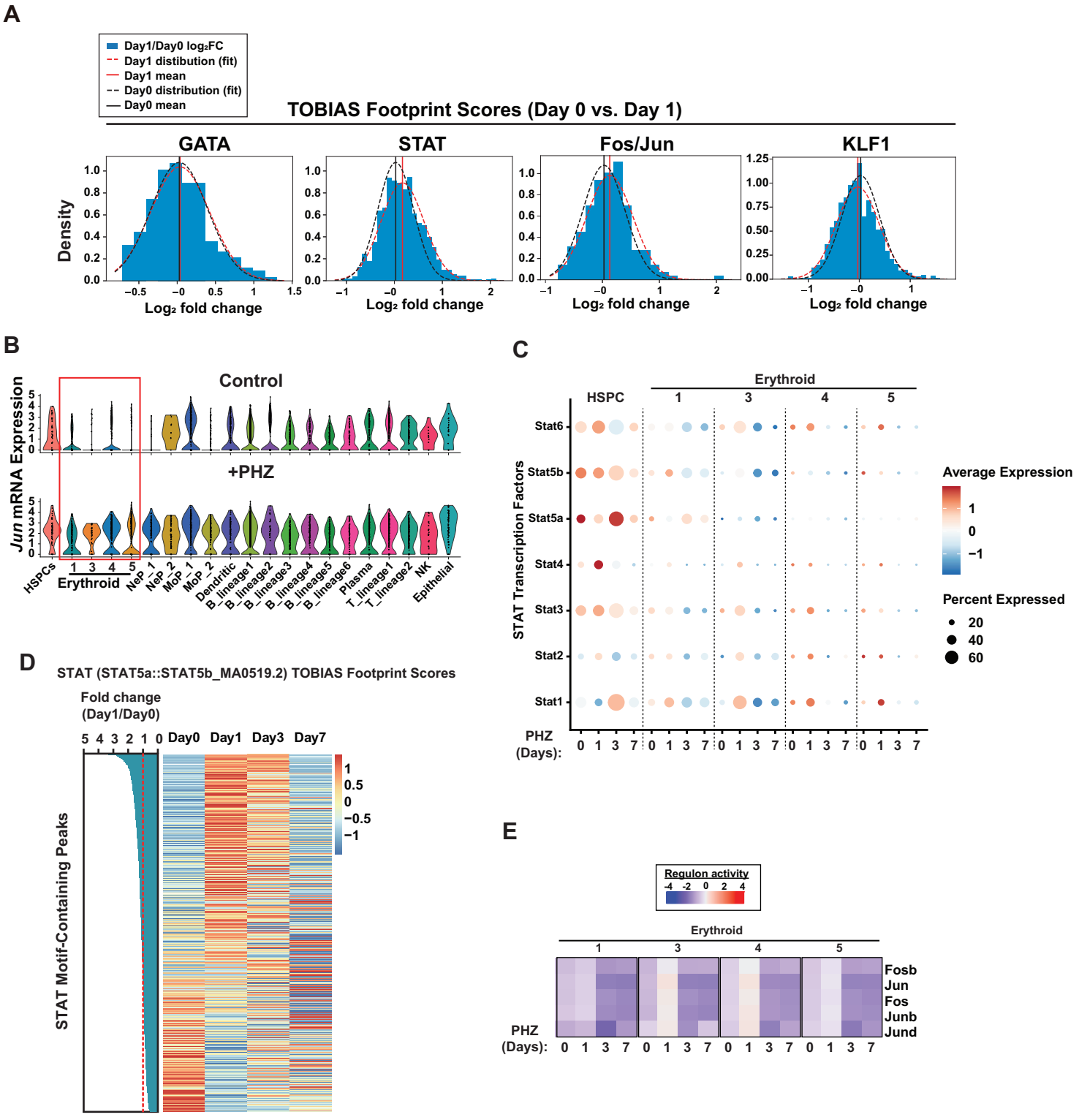
Supplemental Figures and Figure Legends

Supplemental References

Supplemental Methods

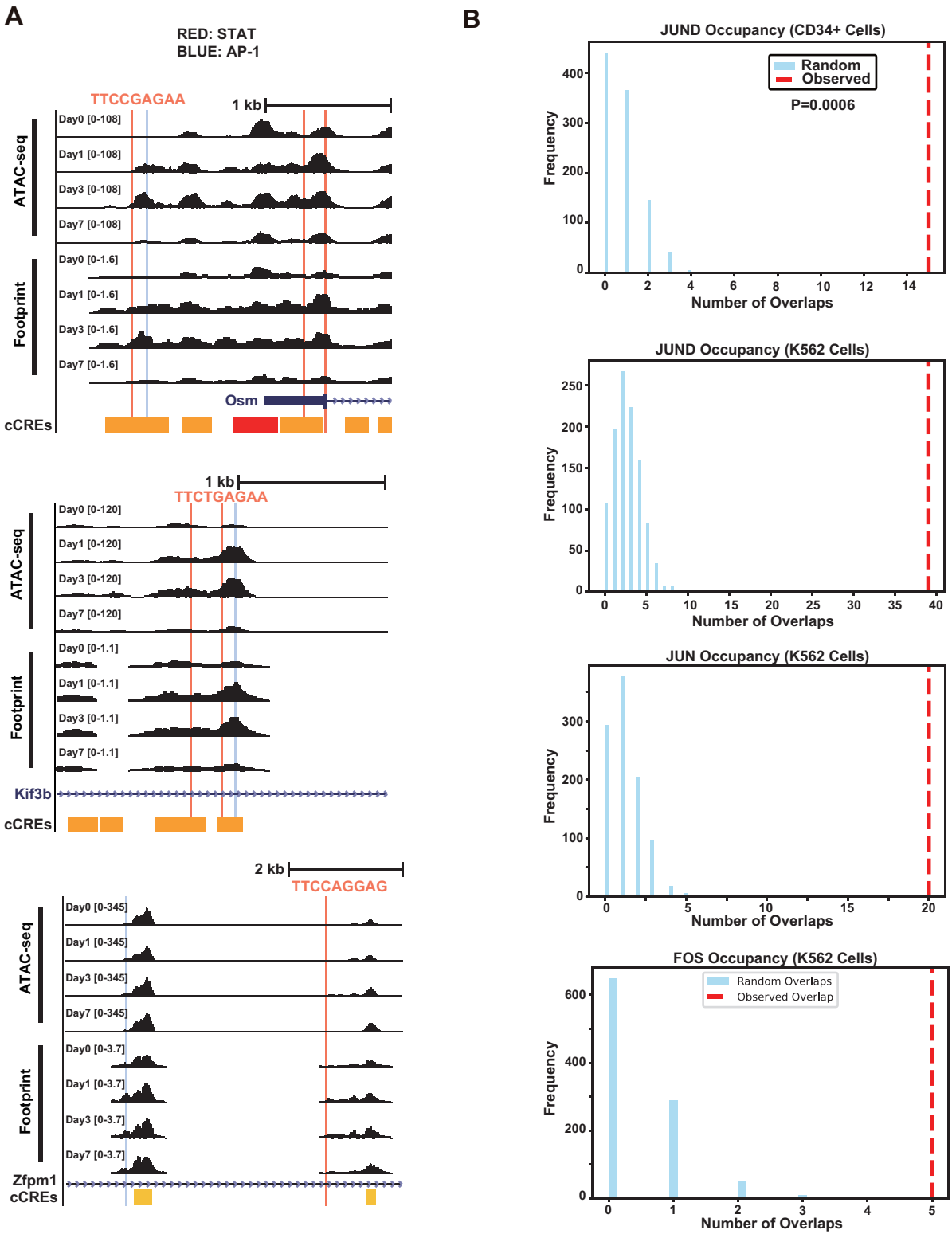
ATAC-seq Data Analysis

To analyze acute and long-lasting peaks, we used MEME-ChIP. Differential ATAC-seq peaks were called using Manorm (1,2). Acute peaks were defined as differential between Day 0 and Day 7 but resolved by Day 35. Long-lasting peaks were peaks that were differential between Day 0 and Day 7 and remained changed by Day 35. To compare PHZ-induced changes with ATAC-seq changes that occur during haematopoiesis we downloaded the publicly available data at the GEO accession GSE59992. The fastq files were downloaded and remapped to mm10 using bowtie2 (3). Samtools was used to quality filter (q=10) and sort reads (4). PCR duplicates were removed with picard MarkDuplicates (5). Finally biological replicates were merged, and bigwig files were generated using deeptools bamCoverage –normalizeUsing BPM (6). Log2 fold changes in signal between CMP and MEP as well as between MEP and ErA were then calculated for loci corresponding to all differential peaks found in PHZ timepoints. Pearson correlation was then used to compare these changes to that of day 7 vs day 0 PHZ for these loci.

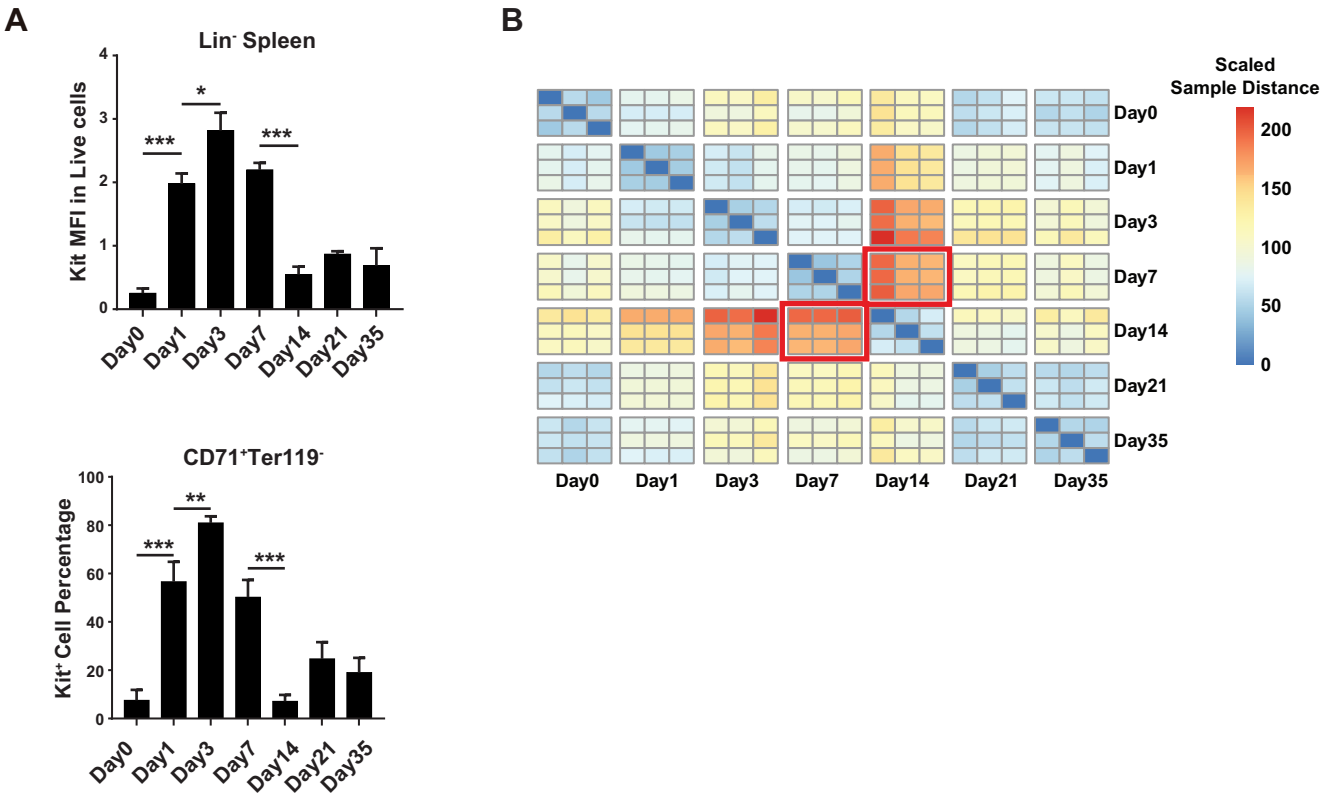

A
Day1/Day0 log2FC
Day1 distibution (fit)
Day1 mean
Day0 distribution (fit)
Day0 mean
TOBIAS Footprint Scores (Day 0 vs. Day 1)
GATA
STAT
Fos/Jun
KLF1
Density
Log2 fold change
B
Control
+PHZ
Jun mRNA Expression
HSPCs
Erythroid
NeP_1
NeP_2
MoP_1
MoP_2
Dendritic
B_lineage1
B_lineage2
B_lineage3
B_lineage4
B_lineage5
B_lineage6
Plasma
T_lineage1
T_lineage2
NK
Epithelial
C
HSPC
Erythroid
STAT Transcription Factors
Stat6
Stat5b
Stat5a
Stat4
Stat3
Stat2
Stat1
PHZ (Days):
Average Expression
Percent Expressed
D
STAT (STAT5a::STAT5b_MA0519.2) TOBIAS Footprint Scores
Fold change (Day1/Day0)
Day0
Day1
Day3
Day7
STAT Motif-Containing Peaks
E
Regulon activity
Erythroid
Fosb
Jun
Fos
Junb
Jund
PHZ (Days):

SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1. Early onset changes to chromatin post-anemia in erythroid cells are associated with increased AP-1 footprint, gene expression, and activity scores. A) Density plots represent a HOMER motif analysis at regions where ATAC-seq peaks were gained 24 hours post-PHZ. TF motifs corresponding to ATAC footprinting (TOBIAS) analysis: GATA1 (MA0035.5), Stat5a/Stat5b (MA0519.2), Fos/Jun (MA1126.2), and KLF1 (MA0493.3). B) Jun mRNA expression plots from single cell RNA-seq progenitor data (all Kit^+ cells) annotated as HSPCs, erythroid clusters, neutrophils (NeP), monocytes (MoP), Dendritic, B cells, T cells, Natural Killer (NK) plasma and epithelial cells. Units of expression are relative log normalized. Error bars represent SD. ***p<0.001 (Wilcoxon signed rank test). C) Dot plot depicting single cell RNA-seq analysis of HSPC and erythroid-specific expression of all detectable components of the AP-1 transcription factor family at 0, 1, 3, and 7 days post-PHZ. D) Heat map of genomic regions containing Stat5a/5b motifs (MA0519.2) with changes to TOBIAS footprint scores between 0- and 1-day post-PHZ, ranked by fold change. E) SCENIC analysis comparing the regulon activity of AP-1 components in erythroid cells at 0, 1, 3, and 7 days post-PHZ.

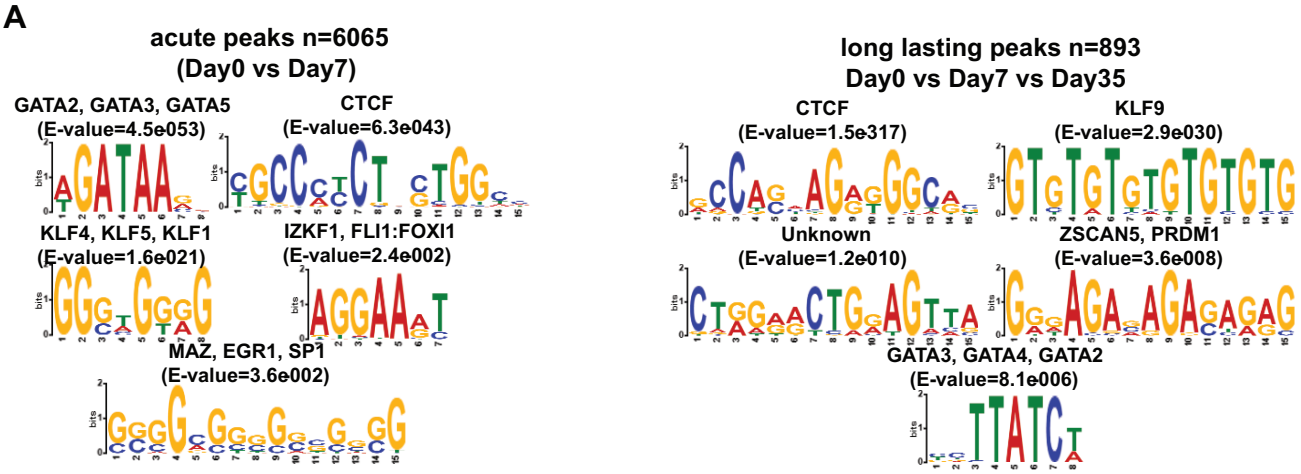

A
RED: STAT
BLUE: AP-1
TTCCGAGAAA
1 kb
ATAC-seq
Day0 [0-108]
Day1 [0-108]
Day3 [0-108]
Day7 [0-108]
Footprint
Day0 [0-1.6]
Day1 [0-1.6]
Day3 [0-1.6]
Day7 [0-1.6]
Osm
cCREs
1 kb
TTCTGAGAAA
ATAC-seq
Day0 [0-120]
Day1 [0-120]
Day3 [0-120]
Day7 [0-120]
Footprint
Day0 [0-1.1]
Day1 [0-1.1]
Day3 [0-1.1]
Day7 [0-1.1]
Kif3b
cCREs
2 kb
TTCCAGGAG
ATAC-seq
Day0 [0-345]
Day1 [0-345]
Day3 [0-345]
Day7 [0-345]
Footprint
Day0 [0-3.7]
Day1 [0-3.7]
Day3 [0-3.7]
Day7 [0-3.7]
Zfpm1
cCREs
B
JUND Occupancy (CD34+ Cells)
Random
Observed
P=0.0006
Frequency
Number of Overlaps
JUND Occupancy (K562 Cells)
Frequency
Number of Overlaps
JUN Occupancy (K562 Cells)
Frequency
Number of Overlaps
FOS Occupancy (K562 Cells)
Random Overlaps
Observed Overlap
Frequency
Number of Overlaps

Supplemental Figure 2. Anemia recovery alters ATAC footprints at sites associated with signal-responsive AP-1 and STAT sites and known AP-1 occupancy sites. (A) ATAC-seq and TOBIAS Footprint scores at the Osm, *Kif3b, and Zfpm1* loci. Blue line demarcates region with changing AP-1 footprint score. Red line demarcates region with changing STAT footprint score. cCREs were classified by ENCODE. Red=promoter, orange=proximal enhancer-like signature. (B) Plots depict the overlap in ChiP-seq observed (dotted red line) and random (blue lines) occupancy of JUND (CD34+), JUND (K562), JUN (K562), and FOS (K562) at 178 sites with AP-1 footprints changing at day 1 post-PHZ. Blue lines were calculated by randomly assigning 178 ATAC-seq peaks and comparing against ChIP-seq data.

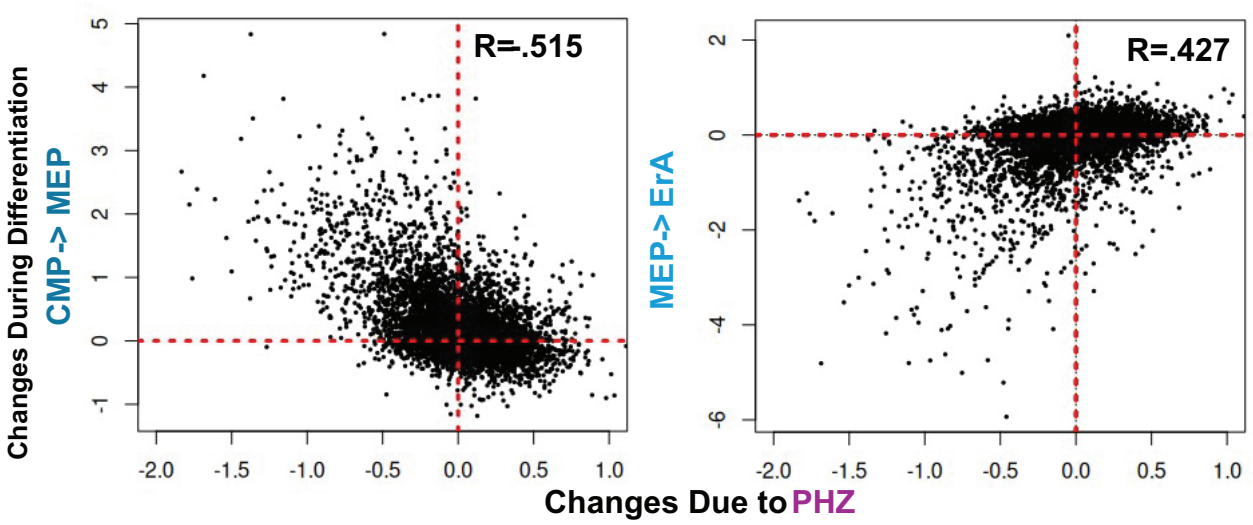

A
Lin- Spleen
Kit MFI in Live cells
0
1
2
3
4
***
*
***
Day0
Day1
Day3
Day7
Day14
Day21
Day35
CD71+Ter119-
Kit+ Cell Percentage
0
20
40
60
80
100
***
**
***
Day0
Day1
Day3
Day7
Day14
Day21
Day35
B
Scaled
Sample Distance
200
150
100
50
0
Day0
Day1
Day3
Day7
Day14
Day21
Day35
Day0
Day1
Day3
Day7
Day14
Day21
Day35

Supplemental Figure 3. Changes in the frequency of Kit+ and erythroid precursors at time points post-PHZ and chromatin occupancy. A) (top) Flow cytometry analysis of the Kit median fluorescence intensity (MFI) in live (DAPI-negative) Lineage-negative spleen cells. (bottom) Flow cytometry analysis of the percentage of Kit-positive cells in live (DAPI-negative) Lineage-negative CD71-positive, Ter119-negative spleen cells. B) Scaled sample distance depicting ATAC-seq comparisons of individual replicates between each time point post-PHZ (days 0, 1, 3, 7, 14,21, and 35 (N=3). Statistical significance was determined by two-tailed unpaired Student's t test. *p<0.05, **p<0.01, ***p<0.001.

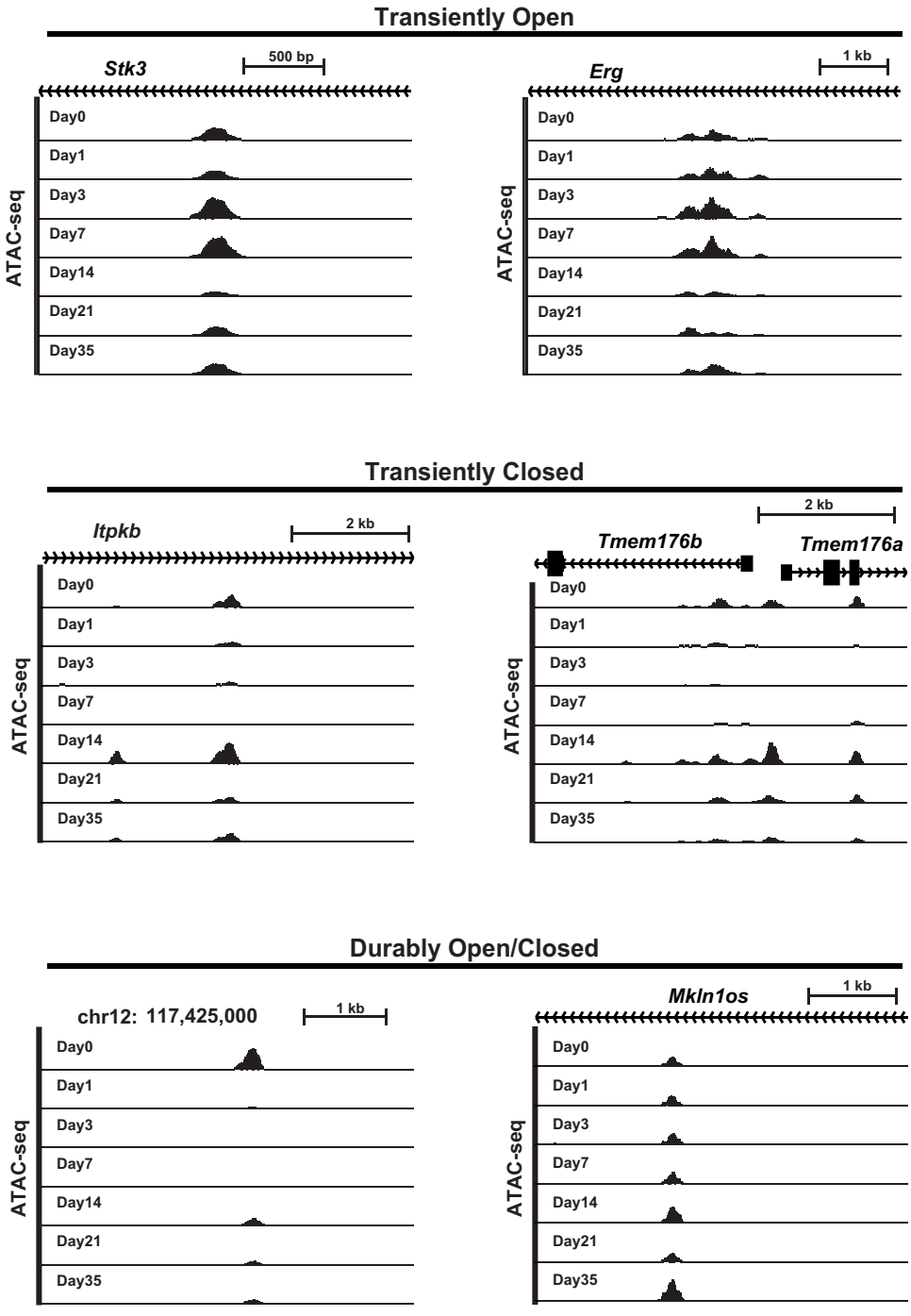
A


acute peaks n=6065
(Day0 vs Day7)
GATA2, GATA3, GATA5
(E-value=4.5e053)
CTCF
(E-value=6.3e043)
KLF4, KLF5, KLF1
(E-value=1.6e021)
IZKF1, FLI1:FOXI1
(E-value=2.4e002)
MAZ, EGR1, SP1
(E-value=3.6e002)
long lasting peaks n=893
Day0 vs Day7 vs Day35
CTCF
(E-value=1.5e317)
KLF9
(E-value=2.9e030)
Unknown
(E-value=1.2e010)
ZSCAN5, PRDM1
(E-value=3.6e008)
GATA3, GATA4, GATA2
(E-value=8.1e006)

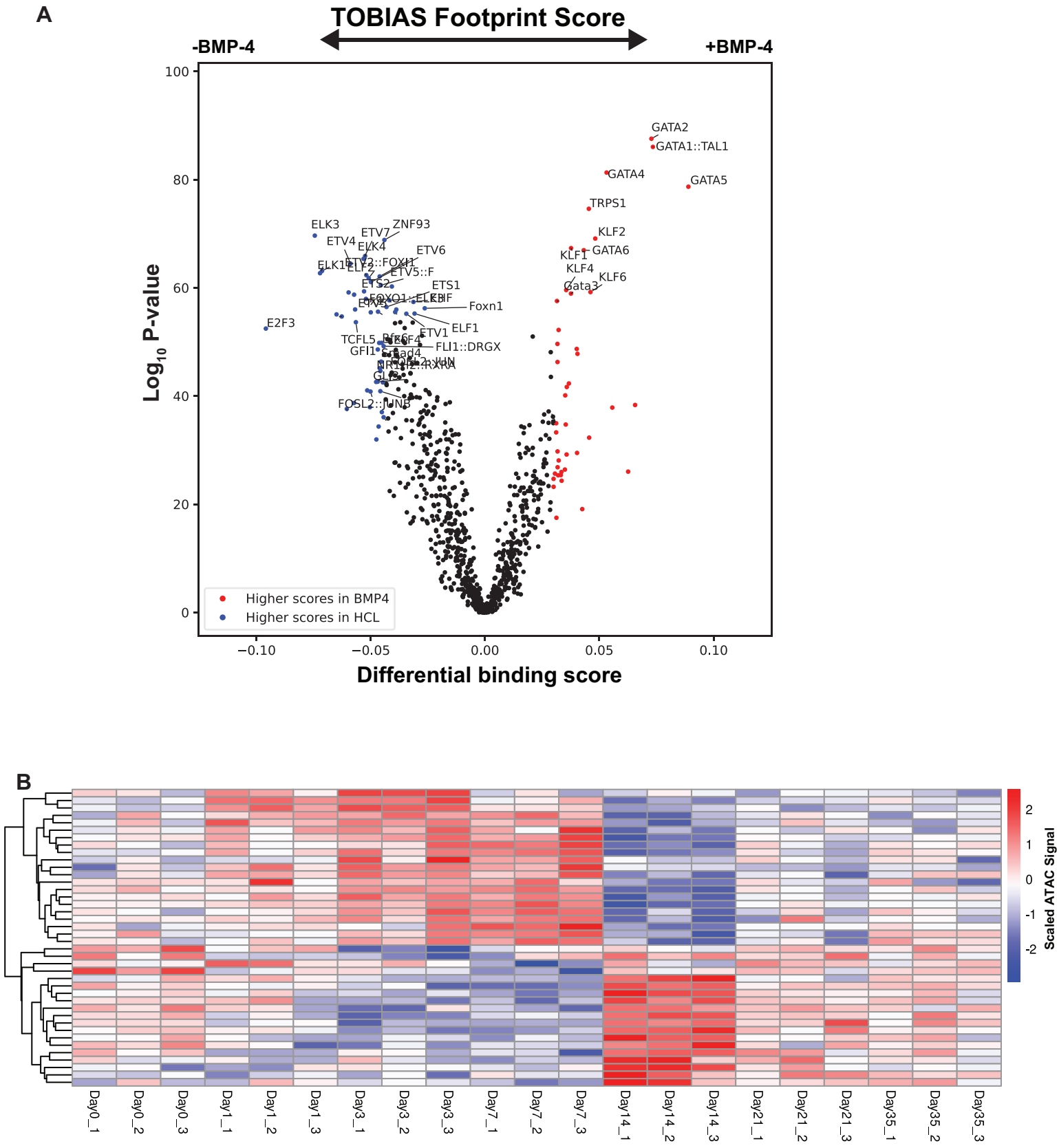
B


R=.515
R=.427
Changes During Differentiation
CMP-> MEP
MEP-> ErA
Changes Due to PHZ

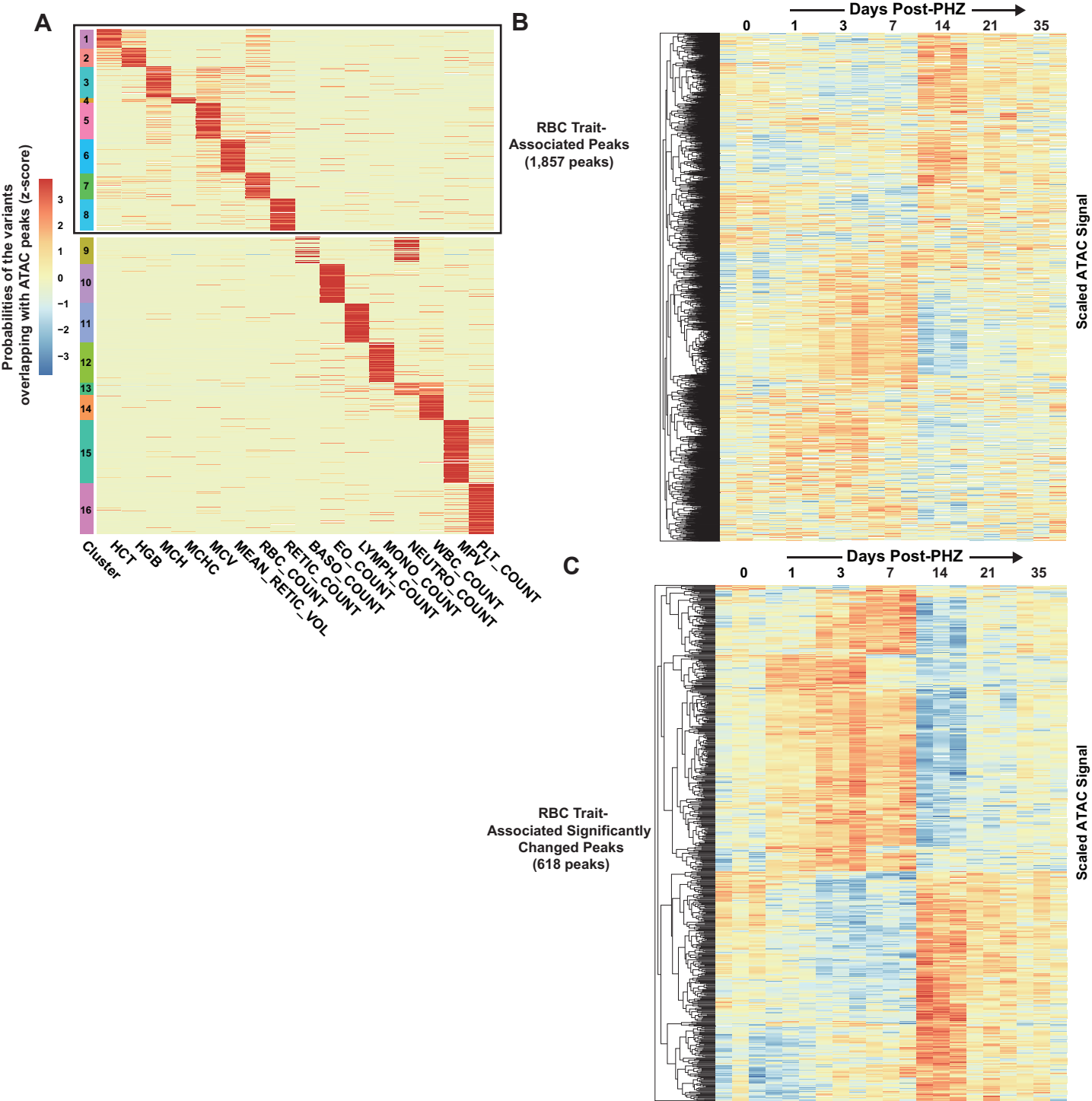
Supplemental Figure 4. Assigning temporal and durable chromatin changes in acute anemia to discrete functional cell types. A) Motif analysis (MEME-ChIP) at regions where ATAC-seq peaks were transiently gained or lost at 7 days post-PHZ, followed by restoration of the original state, and at regions where ATAC-peaks remained durably elevated at 35 days post-PHZ. B) Comparisons of PHZ-induced chromatin changes to chromatin changes which occur during transitions between common myeloid progenitor (CMP), megakaryocyte-erythrocyte progenitor (MEP), and committed erythroid precursors (EryA).


Transiently Open
Stk3
500 bp
Erg
1 kb
ATAC-seq
Day0
Day1
Day3
Day7
Day14
Day21
Day35
Transiently Closed
Itpkb
2 kb
Tmem176b
Tmem176a
Durably Open/Closed
chr12: 117,425,000
Mkln1os

Supplemental Figure 5. Transient and durable changes to chromatin accessibility over a 35-day anemia-recovery time course. UCSC genome browser tracks depict examples of ATAC-seq data from transiently open, transiently-closed and durably changed chromatin accessible regions.


A
TOBIAS Footprint Score
-BMP-4
+BMP-4
Log10 P-value
Differential binding score
Higher scores in BMP4
Higher scores in HCL
GATA2
GATA1::TAL1
GATA4
GATA5
TRPS1
KLF2
GATA6
KLF1
KLF4
KLF6
Gata3
ELK3
ETV7
ZNF93
ETV4
ELK4
ETV6
ELK1
ELF2
ETV5::F
ETS2
ETS1
Foxn1
E2F3
ELF1
ETV1
TCFL5
FLI1::DRGX
GFI1
FOSL2::JUNB
B
Scaled ATAC Signal
Day0_1
Day0_2
Day0_3
Day1_1
Day1_2
Day1_3
Day3_1
Day3_2
Day3_3
Day7_1
Day7_2
Day7_3
Day14_1
Day14_2
Day14_3
Day21_1
Day21_2
Day21_3
Day35_1
Day35_2
Day35_3

Supplemental Figure 6. BMP-4 induces chromatin accessibility changes at select sites genome wide. A) Volcano plot of transcription factor motif differential binding scores in control vs. BMP-4-stimulated human primary erythroid cultures on the x-axis (calculated using TOBIAS) and -log10 (p value) on the y-axis. Each dot represents one transcription factor assigned by consensus motif. B) Heat map depicts the ATAC signal (scaled based on each regions max signal) of 39 chromatin sites which are BMP4-sensitive and either activated or repressed in acute anemia induced by PHZ.


A
B
C
Probabilities of the variants overlapping with ATAC peaks (z-score)
3
2
1
0
−1
−2
−3
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
Cluster
HCT
HGB
MCH
MCHC
MCV
MEAN_RETIC_VOL
RBC_COUNT
RETIC_COUNT
BASO_COUNT
EO_COUNT
LYMPH_COUNT
MONO_COUNT
NEUTRO_COUNT
WBC_COUNT
MPV_COUNT
PLT_COUNT
RBC Trait-Associated Peaks (1,857 peaks)
Days Post-PHZ
0
1
3
7
14
21
35
Scaled ATAC Signal
3
2
1
0
−1
−2
−3
RBC Trait-Associated Significantly Changed Peaks (618 peaks)
Days Post-PHZ
0
1
3
7
14
21
35
Scaled ATAC Signal
2
1
0
−1
−2

Supplemental Figure 7. Evaluating SNPs linked to hematologic traits at anemia-sensitive cis-elements. A) After human (hg38) to mouse (mm10) lift-over, heat map depicts the probability that individual SNP variants overlap with peaks in the mouse genome. B) Heat map of scaled ATAC-seq signal at each of the trait-associated SNPs. C) Heat map of scaled ATAC-seq signal at SNPs associated with red blood cell traits.

SUPPLEMENTAL REFERENCES

1. Bailey, T.L., Johnson, J., Grant, C.E. and Noble, W.S. (2015) The MEME Suite. *Nucleic Acids Res*, **43**, W39-49.
2. Shao, Z., Zhang, Y., Yuan, G.C., Orkin, S.H. and Waxman, D.J. (2012) MAnorm: a robust model for quantitative comparison of ChIP-Seq data sets. *Genome Biol*, **13**, R16.
3. Langmead, B. and Salzberg, S.L. (2012) Fast gapped-read alignment with Bowtie 2. *Nat Methods*, **9**, 357-359.
4. Danecek, P., Bonfield, J.K., Liddle, J., Marshall, J., Ohan, V., Pollard, M.O., Whitwham, A., Keane, T., McCarthy, S.A., Davies, R.M. *et al.* (2021) Twelve years of SAMtools and BCFtools. *Gigascience*, **10**.
5. @misc{Picard2019toolkit, title = {Picard toolkit}, year = {2019}, publisher = {Broad Institute}, journal = {Broad Institute, G.r., {\url{https://broadinstitute.github.io/picard/}}, h. and }.
6. Ramirez, F., Dundar, F., Diehl, S., Gruning, B.A. and Manke, T. (2014) deepTools: a flexible platform for exploring deep-sequencing data. *Nucleic Acids Res*, **42**, W187-191.