Chromatin reconstruction during mouse terminal erythropoiesis

Honghao Bi, Ye Hou, Juan Wang, ..., Ermin Li, Feng Yue, Peng Ji

yue@northwestern.edu (F.Y.) peng-ji@lsm.northwestern.edu (P.J.)

Highlights
- 3D genomic organization changes during mouse terminal erythropoiesis are revealed.
- Late-stage terminal erythropoiesis involves global loss of TADs.
- Decreased intra- and increased inter-chromosomal interactions during erythropoiesis.

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Chromatin reconstruction during mouse terminal erythropoiesis

Honghao Bi,1,2 Ye Hou,2,3 Juan Wang,2,3 Zongjun Xia,1,2 Dongmei Wang,1,2 Yijie Liu,1,2 Haiyan Bao,1,4 Xu Han,1,2 Kehan Ren,1,2 Ermin Li,1,2 Feng Yue,2,3,* and Peng Ji1,2,5,*

SUMMARY
Mammalian terminal erythropoiesis involves chromatin and nuclear condensation followed by enucleation. Late-stage erythroblasts undergo caspase-mediated nuclear opening that is important for nuclear condensation through partial histone release. It remains unknown the dynamic changes of three-dimensional (3D) genomic organization during terminal erythropoiesis. Here, we used Hi-C to determine the chromatin structural change during primary mouse erythroblast terminal differentiation. We also performed RNA-sequencing and ATAC-sequencing under the same experimental setting to further reveal the genome accessibility and gene expression changes during this process. We found that late-stage terminal erythropoiesis involves global loss of topologically associating domains and establishment of inter-chromosomal interactions of the heterochromatin regions, which are associated with globally increased chromatin accessibility and upregulation of erythroid-related genes.

INTRODUCTION
Under constant replenishment, the development of mammalian erythrocytes can be broadly divided into two stages, commitment of hematopoietic stem and progenitor cells to erythroid lineage and terminal differentiation of erythroblasts to enucleated red blood cells. During late stages of terminal erythropoiesis, erythroblasts undergo a series of unique changes including nuclear condensation, enucleation, and loss of major organelles.1–4

Besides terminal erythropoiesis, nuclear and chromatin condensation also occurs during apoptosis. Apoptotic mechanisms including caspase activation are known to play an important role in erythroid nuclear condensation.1,2 We recently reported that late-stage erythroblasts undergo caspase-mediated transient nuclear opening that mediates partial histone release to the cytoplasm and nuclear condensation.7,9–13 Mechanistically, inactivation of caspases inhibits caspase-mediated lamin B cleavage and compromises nuclear condensation and enucleation. Loss of caspase-3 or ectopic expression of a caspase-3 non-cleavable lamin B mutant also inhibits nuclear opening and condensation. Same as mouse erythroblasts, disruption of caspase functions in human CD34+ cells leads to compromised nuclear opening and histone release.10

Histone cytoplasmic release during terminal erythropoiesis was also reported to be mediated by exportin 7, a highly erythroid specific exportin. Loss of exportin 7 inhibits histone and DNA release, as well as nuclear condensation.11 Moreover, a recent report showed that loss of Wdr, a gene essential for lamin B degradation, could induce anemia in zebrafish and disrupt erythroid nuclear condensation in mouse.12 Together, these studies demonstrate that histone release is essential in nuclear condensation and enucleation. However, the chromatin structural change during histone release and nuclear condensation remains unclear.

To address this question, we used a murine fetal liver primary erythroid culture system to investigate the changes in the three-dimensional (3D) genomic landscape during terminal erythropoiesis. Hi-C, ATAC-seq and RNA-seq experiments were performed to integrate the study of chromatin structural changes during terminal erythropoiesis. We found that terminal erythropoiesis involves compaction and establishment of inter-chromosomal interactions of the heterochromatin regions, which is associated with globally increased accessibility and upregulation of erythroid-related genes.

1Department of Pathology, Feinberg School of Medicine, Northwestern University, 303 East Chicago Avenue, Ward 3-230, Chicago, IL60611, USA
2Robert H. Lurie Comprehensive Cancer Center, Northwestern University, Chicago, IL, USA
3Department of Biochemistry and Molecular Genetics, Feinberg School of Medicine, Northwestern University, 303 East Superior, Simpson Querrey 7-518, Chicago, IL60611, USA
4Department of Hematology and Oncology, Children’s Hospital of Soochow University, Suzhou, China
5Lead contact
*Correspondence: yue@northwestern.edu (F.Y.), peng-ji@fsm.northwestern.edu (P.J.)
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To study the 3D chromatin architectural changes during terminal erythropoiesis, we used a well-established murine primary erythroid in vitro differentiation system in which Ter119 negative fetal liver erythroid precursors from E13.5 fetus were cultured in erythropoietin (EPO)-containing medium for 48 h. These cells undergo gradual chromatin and nuclear condensation, as well as 4 to 5 rounds of nuclear opening formation with associated histone cytoplasmic release, before the start of enucleation at around 30 h in culture (Figure 1A).

Our previous study demonstrated that mouse fetal liver erythroid cells cultured at 30 h exhibit dramatic chromatin condensation compared to the cells at the beginning of culture. Thus, to compare the dynamic
change of chromatin 3D structures during mouse terminal erythropoiesis, we performed Hi-C with cells at 0 and 30 h in culture. By comparing the difference of Hi-C contact map, we observed increased contacts in 30 h group at a higher resolution on certain chromosome regions (Figure 1B) and no detectable changes in others (data not shown), compared with 0 h group. Further quantitative analysis in the whole genome level revealed that cells at 30 h group show detectable shift of contact distance with less long-range (>10 million base pairs) interactions compared with those at 0 h group. Short (<1 million base pairs) and medium-range (1-10 million base pairs) interactions are indiscernible (Figure 1C).

To further investigate the chromatin structural change, we performed a Topologically Associating Domains (TADs) analysis. We found that TADs are decreased in the late stage of erythroid terminal differentiation (Figure 1D). The TAD lengths are predominantly less than 2 Mb in both groups (Figure 1E). The average profile of insulation score on TADs demonstrated weakened boundaries in late-stage cells (30 h) compared with those at 0 h (Figure 1F). We further aggregated the Hi-C contact map on the TADs and the surrounding regions. We observed decreased interactions within the TADs in 30 h group cells compared to cells at 0 h (Figure 1G). We also observed multiple regions with disrupted TADs in cells at 30 h group, exemplified at chr3:41-51Mb and chr4:74-83Mb regions (Figure 1H).

Increased inter-chromosomal interactions during erythroid chromatin condensation

Chromosome can be arranged into A and B compartments on a large scale in Hi-C analyses. A and B compartments are associated with open/active and closed/inactive chromatins, respectively. To further characterize the DNA structure during terminal erythropoiesis, we investigated the A/B compartment and compartment switches in these cells. Comparing cells at 0 versus 30 h, 2.73% of B compartments in cells at 0 h switch to A compartment at 30 h whereas 2.24% A switch to B. The rest of the compartments remain unchanged (Figure 2A).

To compare the genome-wide compartment interactions, we performed a saddle plots analysis. We determined fixed genes’ compartment identity at 0 h and applied for all group analysis for better picturing of the dynamic compartmental changes during development. We then generated saddle plots for cis and trans interactions.
trans interactions, representing relative intra-chromosomal and inter-chromosomal interactions, respectively (Figure 2B). The analysis demonstrates that the interaction patterns change significantly during development. The cis interactions show a slight decrease in AA, BB and AB interactions in cells at 30 h whereas the trans interactions increase in BB and AB interactions in these cells (Figure 2C). These results indicate increased inter-chromosomal interactions during chromatin condensation.

Changes of chromatin accessibility during terminal erythropoiesis

We next performed ATAC-seq analyses on these cells to determine the chromatin accessibility changes during terminal erythropoiesis. Cells from the same experimental conditions as above were used (0 and 30 h). In total, we identified 17,941 peaks, among which 1,518 peaks show differential accessibility (>2-fold change, adjusted p-value < 0.00001). Compared to cells at 0 h, 394 peaks were upregulated and 1,124 were downregulated in cells at 30 h group (Figure 3A).

We then grouped peaks in ATAC-seq into 12 clusters using seqMINER. Among these clusters, cluster 9 and 10 show decreased peak reads at 30 h, suggesting reduced accessibility (Figures 3B and 3C). Of interest, Gene Ontology (GO) analysis reveals that many genes in these clusters are involved in immune responses (Figure S1). Cluster 11 and 12 show increased accessibility at 30 h (Figures 3B and 3C). GO analysis shows that genes in these two clusters are closely related to terminal erythropoiesis including porphyrin metabolism and biosynthesis, heme metabolism and biosynthesis, erythrocyte differentiation, iron hemostasis, etc. (Figure S1).

Gene expression changes during terminal erythropoiesis

Under the same experimental settings, we then performed RNA-sequencing analyses to further determine the changes in gene expression during terminal erythropoiesis. In total, 23,564 genes were identified,
among which 3,076 genes were found differentially expressed between 0 and 30 h groups (fold change>4, p<0.01). Among the differentially expressed genes, 813 genes were upregulated in cells at 30 h compared to those at 0 h, whereas 2,263 were downregulated (Figure 4 A). We then generated a heatmap with most significantly differentially expressed genes. As expected, we observed that erythroid-related genes are among those that are most significantly upregulated (Figure 4 B). GO enrichment analysis showed that the upregulated genes are closely related to erythropoiesis, as well as cell cycle and autophagy that are also well known to be involved in terminal erythropoiesis (Figure S2).

Cross dataset analyses reveal diverse ways of gene expression regulation during terminal erythropoiesis

The A and B compartments in Hi-C are generally related to active and inactive areas in chromatin, respectively. Chromatin accessibility is also positively associated with genes’ expression and activity. To test whether these are applicable during terminal erythropoiesis, we cross-analyzed datasets in Hi-C, ATAT-seq, and RNA-seq. We identified 3,787 genes switched from compartment B to A, which represents increased gene activity. When compared to the RNA-seq data, 121 genes switched from B to A showed increased expression, whereas 612 genes with significantly increased expression did not show compartment change in PCA analysis (Fold change>4, p<0.01). Among these 121 genes, we identified 25 genes with increased chromatin accessibility in ATAC-seq (Figure 5A). Notably, most of the genes in these datasets do not overlap, which indicates that gene expression is regulated in diverse ways during terminal erythropoiesis.

GO analysis shows that these 25 genes are closely related to terminal erythropoiesis (Figure 5B). We reviewed literature and listed related studies on these genes in Table 1. Within these 25 genes, Kel, Rhag and Abcg2 are well known red blood cell markers. Genes like Arl4a, Hpf1, Snca, and Trim10 have been reported to be involved in terminal erythropoiesis. The roles of other genes in erythropoiesis remain unclear.

Figure 4. Gene expression changes during terminal erythropoiesis
(A) Volcano plot of RNA-seq data comparing cells at 30 h to those at 0 h.
(B) Heatmap analysis of RNA-seq data between cells at 30 and 0 h groups in triplicate. The most significantly differentially expressed genes are listed on the right.
DISCUSSION

In this study, we report for the first time the genomewide chromatin 3D architectural changes during murine terminal erythroid differentiation. Our study reveals decreased long-range chromosome interactions and TADs during terminal erythropoiesis. Similar to the decreased TADs in the late-stage murine terminal erythroblasts, it was reported that chicken mature erythrocytes lack typical TADs found in mammals and chicken embryonic fibroblasts. A more recent study comparing erythrocytes 3D genome organization across various vertebrates further showed lack of TADs in vertebrate erythrocytes, including late-stage erythroblasts in mouse. Because chicken mature erythrocytes also undergo nuclear condensation despite retaining their nuclei, these findings suggest that the terminal erythropoiesis is evolutionally conserved among vertebrates. It is possible that immature chicken erythroblasts in the bone marrow could have typical TADs as those found in the murine erythroblasts and gradually decrease. It would be interesting to explore from the perspective of comparative biology.

Besides compartment switching, we also found increased trans interactions in BB and AB compartments during development, suggesting increased inter-chromosomal interactions. Overall, our study reveals a dynamic process during chromatin condensation in terminal erythropoiesis. In this process, a small portion of genome DNA

Figure 5. Cross analyses of Hi-C, ATAC-seq, and RNA-seq.
(A) Green: genes switched from compartment B to A in Hi-C; Pink: genes with increased peaks in ATAC-seq; Blue: genes with increased expression in RNA-seq. Representative genome browser view of genes with increased expression and chromatin accessibility is on the right.
(B) Gene Ontology analysis of the 25 overlapped genes in three datasets.
| OFFICIAL GENE SYMBOL | Gene Name | Alias | Function | Reference |
|----------------------|-----------|-------|----------|-----------|
| 1110034G24Rik        | RIKEN cDNA 1110034G24 gene(1110034G24Rik) | Shld1 | ATP-binding cassette, sub-family G (WHITE), member 2(ABCG2) | Jonker et al.16 |
| ABCG2                | ABC15, ABCB, BCRP, BCRP1, BMDP, CD338, CDw338, EST157481, GOUT1, MRX, MXR, MXR1, UAQL1, MXR-1 | ABCG2 null mice show similar phenotype as erythropoietic protoporphyria | Jonker et al.16 |
| Arl4a                | ADP-ribosylation factor-like 4A(Arl4a) | Knockdown of arl4a in Zebrafish induces reduced definitive hematopoiesis by interfering with Notch Receptor maturation | Guo et al.17 |
| Cdc42ep3             | CDC42 effector protein (Rho GTPase binding) 3(Cdc42ep3) | BORG2, CEP3, UB1 | Knockdown of Cpeb4 causes inhibition of terminal erythropoiesis | Hu et al.18 |
| Cpeb4                | cytoplasmic polyadenylation element binding protein 4(Cpeb4) | KIAA1673 | Modulation of PARP2, which sustains erythropoiesis | Farres et al.19 |
| Gda                  | guanine deaminase(Gda) | GAH, Cytoplasmic PSD-95 Interactor, PS1-Nedasin, EC 3.5.4.3 | Modulation of PARP2, which sustains erythropoiesis | Farres et al.19 |
| Ghitm                | growth hormone inducible transmembrane protein(Ghitm) | TMBIM5, DERP2, HSPC282, PTD010, Myo21 | Modulation of PARP2, which sustains erythropoiesis | Kurgina et al.20 |
| Hace1                | HECT domain and ankyrin repeat containing, E3 ubiquitin protein ligase 1(Hace1) | KIAA1320, EC 2.3.2.26, EC 6.3.2, SPPRS | Modulation of PARP2, which sustains erythropoiesis | Kurgina et al.20 |
| Hpf1                 | histone PARylation factor 1(Hpf1) | C4orf27, FLJ20534, Chromosome 4 Open Reading Frame 27, UPF0609 Protein C4orf27 | Modulation of PARP2, which sustains erythropoiesis | Farres et al.19 |
| Ifit1b1l1            | interferon induced protein with tetratricopeptide repeats 1B like 1(Ifit1B1l1) | IFNA1, G10P1, IFI56, GARG-16, IFI-S6K | Modulation of PARP2, which sustains erythropoiesis | Farres et al.19 |
| Kel                  | Kell blood group(Ke) | CD238, ECE3 | Endopeptidase that cleaves big endothelin-3 | Lee et al.21 |
| Lrrc1                | leucine rich repeat containing 1(Lrc1) | LANO, DJ523E19.1 | Endopeptidase that cleaves big endothelin-3 | Lee et al.21 |
| Mllt3                | myeloid/lymphoid or mixed-lineage leukemia; translocated to, 3(Mllt3) | YEATS3, AF9 | Regulate Erythrocyte/Megakaryocyte fate decision | Pina et al.22 |
| Ncoa7                | nuclear receptor coactivator 7(Ncoa7) | ERAP140, DJ187J11.3, TLDC4 | Regulate Erythrocyte/Megakaryocyte fate decision | Pina et al.22 |
| Pkhd1l1              | polycystic kidney and hepatic disease 1-like 1(Pkhd1l1) | Fibrocystin-L | Regulate Erythrocyte/Megakaryocyte fate decision | Pina et al.22 |
| Pla2g16              | phospholipase A2, group XVII(Pla2g16) | HREV107-3, HREV107, AdPLA, PLAAT-3 | Regulate Erythrocyte/Megakaryocyte fate decision | Pina et al.22 |
| Rhag                 | Rhesus blood group-associated A glycoprotein(Rhag) | SLC42A1, CD241, RH50A | Regulate Erythrocyte/Megakaryocyte fate decision | Pina et al.22 |

(Continued on next page)
switches between active and inactive states. Chromatin penetrates the compartmental boundary and builds new interactions, which is featured by decreased intra-chromosomal and increased inter-chromosomal interactions (Figure 6).

Another key feature in terminal erythropoiesis is nuclear opening that is mediated by partial histone release and facilitates nuclear condensation.\textsuperscript{9,30,31} This process occurs more frequently during the polychromatic to orthochromatic stage. We previously reported that caspases play critical roles in this process by cleaving lamin B to generate transient nuclear openings for histone release. Histone cytoplasmic release during terminal erythropoiesis was also reported to be mediated by exportin 7, a highly erythroid-specific exportin.\textsuperscript{11} Moreover, a recent report showed that loss of \textit{Wdr}, a gene essential for lamin B degradation, could induce anemia in zebrafish and disrupt erythroid nuclear condensation in mouse.\textsuperscript{12} Together, these studies demonstrate that histone release is essential in nuclear condensation and enucleation. It would be interesting to investigate whether inhibition or downregulation of these proteins would affect chromatin 3D structural changes. In this respect, erythroid specific and inducible knockout models will be essential to reveal the detailed mechanisms.

| OFFICIAL GENE SYMBOL | Gene Name Alias | Function | Reference |
|----------------------|-----------------|----------|-----------|
| Scai                 | suppressor of cancer cell invasion(Scai) | NET40, FLJ36664, C9orf1126 | Renella et al.\textsuperscript{25} |
| Sec61g               | SEC61, gamma subunit(Sec61g) | S5S1 | |
| Smc2os               | structural maintenance of chromosomes 2, opposite strand(Smc2os) | | |
| Sncα                 | synuclein, alpha(Sncα) | NACP, PARK1, PD1, PARK4 | Regulate oxidative stress |
| Trim10               | tripartite motif-containing 10(Trim10) | RFB30, HERF1, RNF9 | regulating splicing of exon 16 in 4.1R and required for terminal erythropoiesis |
| Tspan8               | tetraspanin 8(Tspan8) | CO-029, TM4SF3 | Zhao et al.\textsuperscript{27} |
| Zfand6               | zinc finger, AN1-type domain 6(Zfand6) | AWP1, ZFAND5B, ZA20D3 | |
| Zfp760               | zinc finger protein 760(Zfp760) | | |

Table 1. Continued

**Figure 6. Schematic overview of chromatin 3D structural changes during terminal erythropoiesis**

Blue and red strings represent independent chromosomes. Blue and red dots represent intra-chromosomal interactions. Purple dots represent inter-chromosomal interactions. During differentiation, chromosomal interactions within the same chromosome decrease, whereas the interactions between chromosomes increase.
Limitations of the study
The current study used cultured mouse fetal liver cells. The culture artifact could be a potential concern. In addition, the rapid differentiation process of mouse fetal liver cells within 48 h makes it difficult to dissect the chromatin reconstruction at each stage of terminal erythropoiesis.

STAR+METHODS
Detailed methods are provided in the online version of this paper and include the following:

- **KEY RESOURCES TABLE**
- **RESOURCE AVAILABILITY**
  - Lead contact
  - Materials availability
  - Data and code availability
- **EXPERIMENTAL MODEL AND SUBJECT DETAILS**
- **METHOD DETAILS**
  - Mice
  - Cell culture
  - Cell surface marker staining
  - Total RNA extraction
  - Hi-C data analysis
  - RNA-seq analysis
  - ATAC-seq analysis
- **QUANTIFICATION AND STATISTICAL ANALYSIS**

SUPPLEMENTAL INFORMATION
Supplemental information can be found online at https://doi.org/10.1016/j.isci.2022.105554.

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AUTHOR CONTRIBUTIONS
H.B., Y.H., J.W., A.M., D.H., Y.L., H.B., X.H., K.R., and E.L. performed the experiments and interpreted data. Y.H. performed the Hi-C experiment. J.W., Z.X., and D.W. analyzed the Hi-C data. H.B., Y.L., F.Y., and P.J. designed the experiments, interpreted data, and edited the manuscript. H.B. and P.J. wrote the manuscript.

DECLARATION OF INTERESTS
The authors declare no competing interests.

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STAR METHODS

KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| Antibodies          |        |            |
| APC Rat Anti-Mouse TER-119 | BD | Cat# 557909, RRID:AB_398635 |
| FITC Rat Anti-Mouse CD71 | BD | Cat# 567260, RRID:AB_2916522 |
| Deposited data      |        |            |
| Hi-C-seq data for erythrocyte differentiation | This paper | GSE184974 |
| ATAC-seq and RNA-seq data for erythrocyte differentiation | This paper | GSE185470 |
| Experimental models: Organisms/strains |        |            |
| C57BL/6j mice | Jax Lab | strain #: 000,664 |
| Software and algorithms | | |
| runHiC python package | Wang⁵² | https://pypi.org/project/runHiC/ |
| cooltools | Venev et al.⁵³ | https://github.com/open2c/cooltools |
| cworld matrix2insulation and insulation2tads | NA | https://github.com/dekkerlab/cworld-dekker |
| juicebox | NA | https://github.com/aidenlab/Juicebox |
| MACS2 | Zhang et al.³⁴ | NA |
| seqMINER | Ye et al.³⁵ | NA |
| bedtools | Quinlan et al.³⁶ | NA |
| TopHat2 | Kim et al.³⁷ | NA |

RESOURCE AVAILABILITY

Lead contact
Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Peng Ji (peng-ji@fsm.northwestern.edu).

Materials availability
This study did not generate new unique reagents.

Data and code availability
- All data were deposited at NCBI GEO database. The accession number for Hi-C is GEO: GSE184974. The accession number for ATAC-seq and RNA-seq data is GEO: GSE185470.
- This paper does not report original code.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

E13.5 fetal liver cells were acquired by breeding male and female C57BL/6J mice purchased from the Jackson laboratory. The mice were maintained at animal facility in center for comparative medicine, Feinberg School of Medicine at Northwestern University. After 1 night of breeding (day 1), female mice were separated and observed for 2 weeks. At day 14 after breeding, the pregnant female mice were sacrificed, and fetal livers were then retrieved. The fetal livers were then homogenized by repeated pipetting and filtered through 40 µm cell strainer to obtain single cells. The filtered cells were incubated with biotin-antimouse Ter119 antibody for 15 min and washed with PBS. After washing, the cells were resuspended and Ter119 positive cells were pulled down with streptavidin magnetic beads. The remained Ter119 negative cells were then used for the following experiments.
The Ter119 negative cells were either processed for sequencing directly or cultured in erythropoietin (EPO)-containing medium (15% FBS, 1% BSA, 10µg/ml Insulin, 200µg/ml Holo-transferrin, 10⁻⁴M β-Mercaptoethanol, 1% Penicillin-Streptomycin, 2mM Glutamine, 2U/ml EPO in IMDM) for up to 48 h. The cells were harvested at 30 h for the follow up studies. ¹⁵,²⁶

**METHOD DETAILS**

**Mice**

C57BL/6J mice were purchased from the Jackson laboratory (strain #: 000,664). Both male and females were used. All animal studies were performed in accordance with the Guidelines for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committees at Northwestern University.

**Cell culture**

Purification of mouse fetal liver erythroblast precursors and in vitro culture were performed as previously described. ³¹ Briefly, fetal liver erythroid precursors were isolated from the E13.5 fetal liver by depletion of Ter119 positive cells using a biotinylated anti-Ter119 antibody. The Ter119 negative cells were then incubated in EPO-containing medium (15% FBS, 1% BSA, 10µg/ml Insulin, 200µg/ml Holo-transferrin, 10⁻⁴M β-Mercaptoethanol, 1% Penicillin-Streptomycin, 2mM Glutamine, 2U/ml EPO in IMDM) for differentiation and enucleation for up to 48 h. The cells were harvested at 30 h for further analysis.

**Cell surface marker staining**

Cells were washed in PBS and resuspended in 100 µL PBS with a concentration of 1 million cells/ml. 0.5 µg APC-Ter119 antibody (BD, Cat# 557909) and FITC-CD71(BD, Cat# 567260) were added to the cell suspension. In turn, Hoechst 33,342 was added to the cell suspension to a final concentration of 20 µg/mL. The cells were incubated in dark under room temperature for 10 min and washed with PBS. The washed cells were then resuspended with PBS containing 1 µg/mL propidium iodide to exclude dead cells. The resuspended cells will then be analyzed by flow cytometry within 1 h.

**Total RNA extraction**

Cells were harvested and washed for 3 times with PBS. Qiagen RNeasy kit was used to extract total RNA. Briefly, cells were lysed and homogenized in lysis buffer. Ethanol was then added to the lysate followed by centrifugation through silica membrane. After 2 times wash, RNAs were eluted by elution buffer and stocked for further analysis.

**Hi-C data analysis**

The adapters of the raw reads were trimmed and mapped against the mm10 reference genome using the runHiC python package (https://pypi.org/project/runHiC/). The contact matrix was binned at multiple resolutions: 5, 10, 25, 40, 50, 100, 250, 500kb, 1, 2.5, 5 and 10Mb using the run-cool2multirescool script from the 4DN consortium. The ICE normalization was performed for each resolution. For A/B compartment analysis, we first identified A/B compartment at 100kb resolution using the cooltools (https://github.com/open2c/cooltools) call-compartment function. To quantify the A and B compartment interactions, we used the cooltools compute-saddle function. The cworld matrix2insulation and insulation2tads (https://github.com/dekkerlab/cworld-dekker) were used for insulations scores calculation and TAD identification. We also generated the multi-resolution.hic files using the juicer tools for the convenience of example Hi-C matrices visualization in juicebox (https://github.com/aidenlab/Juicebox). The accession number for Hi-C is GSE184974.

**RNA-seq analysis**

RNA-seq reads were aligned to the mouse genome (NCBI37/mm10) using TopHat2. ³⁷ Expression of each gene was calculated from the resulting alignment bam file by HTSeq-count. ³⁷ Differentially expressed genes were determined using R package DESeq²⁰ with 2-fold change or 4-fold change as cutoff. Gene ontology analysis for changed genes was performed using DAVID. ³⁷
**ATAC-seq analysis**

Paired-end ATAC-seq reads were aligned to the mouse genome (NCBI37/mm10) using Bowtie2\(^2\) with option “--very-sensitive-local”. Mitochondrial reads were excluded from downstream analysis. Peak calling was performed on each individual sample by MACS2\(^3\) with parameter “-BAMPE”. Peaks from different ATAC-seq samples were merged using bedtools.\(^4\) Merged peaks were used as genome coordinate for K-means clustering which was performed using seqMINER.\(^5\) 10 million randomly sampled mapped reads from each sample were used as input. Signals were calculated in a 1 kb region (±500 bp) surrounding the center of the peak with 20 nt bin. Peaks assignment to the genes and subsequent ontology analysis were done with GREAT.\(^6\) The accession number for ATAC-seq and RNA-seq is GSE185470.

**QUANTIFICATION AND STATISTICAL ANALYSIS**

Results are expressed as mean ± SEM unless otherwise indicated. Statistical comparisons between two groups were performed with a two-tailed unpaired Student’s t test using GraphPad Prism version 6.0 software. p <0.05 was considered to be significant.