Redefining hematopoietic progenitors cells and reaffirming lineage commitment by transcriptional atlas

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Abstract

Tracing the differentiation of the hematopoietic progenitor cells in human blood, particularly with the scarcity in adults have been challenging owing to the technical limitations and sensitivity. Often, the use of classic and traditional surface markers lacks the sensitivity and specificity to clearly define the hematopoietic progenitors. With the evolution of single cell sequencing approaches, including single cell RNA sequencing (scRNA seq) technologies, this have enable the opportunities to study the heterogeneity and hierarchy of HPCs. Here, scRNA-seq was used to capture, identify and define the characterization of HPCs in adults peripheral blood. In addition, HPCs lineage were reaffirmed by unique makers and key factor genes. Several key factors, including NFE2, LYL1, MYB, and RUNX1 of myeloid and RUNX2, HOXA9, and BCL11A of lymphoid progenitors, were identified as major switches that controlled hematopoietic lineage formation. The reaffirmed lineage commitment also revealed hematopoiesis was a continuum procedure controlled by the activation and suppression of transcription factors, with identified boundary. With these information, an elaborate and comprehensive transcriptional atlas of all types of hematopoietic progenitors was then constructed. Importantly, this newly created atlas can enable accurate definition, hierarchy, and biological insights of hematopoietic progenitors for facilitating new research development in cell engineering, regenerative medicine, and disease treatment. Moreover, this open a wide variety of possibilities for artificial precise control progenitor cells differentiation and targeted treatment of immune disease, providing hope for untreatable diseases.

Highlights

- Classic and traditional surface markers are often speciosity, inefficient and inaccurate for defining hematopoietic progenitor cells.
- Progenitors cells of multiple hematopoietic lineages were reaffirmed by unique makers and key genetic factors.
- Several key factors, including NFE2, LYL1, MYB, CDK4, ETV6, TESPA1, and

GATA2, and RUNX1 of myeloid progenitors and RUNX2, HOXA9, JUN/FOS, LST1, TCF4, DDIT4, HOPX, KLF10, HOXA3, TSC22D1, and BCL11A of lymphoid progenitors, were identified as major regulators that controlled hematopoietic lineage formation.

- Hematopoiesis is a continuum procedure controlled by the activation and suppression of transcription factors, with relative identified boundary.
- Transcriptional atlas of HPCs help to provide a well explanation of the immune reconstitution in adult and on its cytokines secretion and regulation.
- Application of this atlas can enable artificial precise control progenitor cell differentiation, in vitro accurate culture of each lineages cells, and targeted treatment of immune cell disease to be made possible for future development.

Introduction

Deciphering the hematopoiesis mechanism that underlies progenitor cells definition and fate decision are critical to understanding fundamental questions of hematopoietic lineage commitment. Toward the diversities of peripheral blood cells in the human body, it is like the multifarious stars existing in our galaxy. Although we know their existence, the exact characterization of these cell subsets remains a challenge.

Among the various cells types in peripheral blood (PB), hematopoietic stem cells (HSCs) are one of such rare cell subsets. These cells possess self-renewal capacity and can differentiate to all blood lineage cells. Such that, HSCs reside at the top of a hierarchy and differentiate to all blood lineage cells with tree-like pattern in classical hematopoietic hierarchical models [1]. Single-cell sequencing technique has been successfully implemented to explore molecular heterogeneity and hierarchy of HSCs [2-4]. The lineage commitment is affected by transcription factors (TFs) with a continuum fate decision procedure [5,6]. Though *in vitro* and *in vivo* studies have reaffirmed their critical role of transcription factors in lineage commitment, the function and regulatory mechanism of HSCs is still unclear [7]. In addition, the transcriptional atlas and the differentiation among subtypes of progenitors in adult peripheral blood remains to be elucidated.

The reason for the molecular mechanisms that controlled lineage commitment and progenitor behaviors remaining an enigma is due to various reasons. Firstly, the definition of hematopoietic progenitor cells (HPCs) is not standardized. Based on the classical and traditional definition, the characterization of HPCs is based on the presence of surface markers, such as CD34, CD38, FLT3 and KIT [5]. Similarly, the combination of surface markers have been proposed for the identification toward common lymphoid progenitors (CLP) and common myeloid progenitors (CMP). These markers include CD10 (MME) and CD38. Yet, owing to the rare and transient nature, the subtypes of CLP and CMP from HSCs have never been stringently identified and accurately captured. Secondly, the use of functional studies were also used for the characterization of HPCs. These include the selection of CD34 positive cell from bone marrow or cord blood. However, this approach can be affected by the type of culture methods and surface markers being used for the identification. Simply, the use of classical and traditional markers may be not insufficient, such as KIT, to differentiate mature subset cells. Thus, the use of combined surface markers for HPCs may cause biased and arbitrary.

In recent years, many studies have identified the lineage by using single cell sequencing. However, their is still several limitations of the transcriptional atlas being defined. The scRNA expression data of CD34 positive cells showed two well-defined subtypes of progenitor CLP and CMP being scattered and mixed distribution in T-SNE plots. This result implies that the transcriptional atlas for these two types of cells are not distinguishable by unsupervised dimensionality reduction and clustering. When compared with others mature cells, HPCs should exist as a relatively homogeneous progenitor population. The idea classification and defined subset of lineage cells should be separately distinguishable by unsupervised dimensionality reduction which carried the PCA key markers of genes as factors.

In addition, the hematopoietic hierarchical models are also inconsistent among the literature studies [1, 8, 9]. Most of the established lineage fate decision models were based on *in-vitro* / *in-vivo* functional studies or cell fate induces experiments [10, 11]. The use of cellular conditions can often lead to inaccurate interpretations of HPCs cell fate decision because cell lineages were induced by diverse experimental conditions and cannot be permissive to all possible branches of lineage commitment [10]. Similarly, single cell studies would have modified some aspects of classical hematopoiesis hierarchical model, as the hematopoietic progenitors are changing continuously among the progenitors differentiation process [5, 12]. In addition, progenitors cells were defined by classical markers and selected by surface markers [4, 5]. As each type of progenitors is very rare in whole blood, the captured cell numbers are often very low with less than one hundred cells [5]. This implies that some key progenitor subgroups may be missed. Thus, this would affect the reliability of results. As the classic definition of hematopoietic progenitors remains inefficient and inaccurate, this can cause the disorganization among the subgroups and without a reliable identification of the boundary among them [2]. Moreover, unsupervised dimensionality reduction and clustering of lineage cells demonstrated farraginous model [4]. The measurement of lineage definition, fate decision, and establishing the reliable lineage commitment between them are still a challenge [8, 13].

Herein, we reaffirmed the adult hematopoietic progenitors, and recovered the key transcript factors in lineages fate decision, and restructured the lineage commitment model of HPCs by single-cell sequencing. Our HPCs atlas defined the cellular biology of differentia stage progenitors among hematopoietic lineages generation. Our study can help to open new perspectives for the characterization, definition, and classification of HPCs.

Results

Single-cell identified two subtypes of progenitor cells in adults peripheral blood.

In order to enrich most of the subset of HPCs and later progenitors, such as CLP and CMP, lymphocyte and granulocyte were removed by surface marker panel (Lineage−) and progenitor cells were enriched by negative deletion (Fig. 1A). 10X genomics scRNA was conducted and totally 79,655 cells were captured after quality filters of mitochondrial RNA, low gene expression cells and erythrocyte cell. Unsupervised dimensionality reduction and clustering analysis showed 17 clusters by Seurat and UMAP (Fig. 1B). Total 3180 CD34 positive cells were identified in the dataset. Besides, samples from all donors were highly reproducible for cell composition. The HPCs marker of CD34 mainly expressed in two nearby clusters 6 and 14 with the co-expression of FLT3 and KIT (Fig. 1C).

To confirm whether the two clusters are subset of progenitor cells, the different expression of lymphocyte and granulocyte surface markers and known HSC related genes were compared (Fig. 1C). Both of the two clusters were found to express KIT and FLT3, but do not contained the expression of lymphocyte and granulocyte surface markers. Based on this, the two clusters can then be well distinguishable by three markers, defined with CD34+CD38+MME- and CD34+CD38-MME+ (Fig. 1C).

However, a portion of the cells can positively expressed CD38+ or MME in the two subgroups, respectively. KIT (CD117) and FLT3 were mainly expressed in one of the CD34+ clusters, with low positive percentages but also high expressed in the other clusters . This indicates that the progenitors of CD34+KIT and CD34+FLT3 belonging to two different clusters which may control the two differentiation directions.

We further analyzed the significantly identified genes among the two clusters with the other clusters. As found, we presented 16 progenitors related genes which expressed in more than 80% cells in the two clusters (Fig. 1C). Several HSCs key regulate genes were identified, such as high expression of *CDK6, SERPINB1, SPINT2, IMPDH2, TXN, SOX4, MYADM, HMGA1, CDK2AP1, CD82, TSC22D1, LYL1,* and middle expression of SPI1 [6], IFITM3, HMGB1, and PPIA. Based on the surface markers and the above hematopoietic stem cells functional key gens, we can confirm that the two identified clusters are hematopoietic progenitor cells. We also identified three new progenitor key genes of *PPIB, HMGA1, EGFL7*. It is very obviously that the gene of EGFL7 are very high and specific expressed in the two progenitors clusters, so that EGFL7 is a key functional gene in hematopoietic progenitor cells lineage differentiation and mature (Fig. 1D). The single cell sequencing identified two subgroups which are progenitor cells with category and functional heterogeneity.

The differentiation of transcript markers among progenitors

Our single cell expression atlas level showed the two clusters of myeloid progenitors (CD38+) and lymphoid progenitors (MME+) presented heterogeneity in these progenitors, suggesting that functional of the two-cluster subset are diversity. To determine the diversity, re-clustering of analysis was conducted and 11 clusters was generated (Fig 2A). Based on our finding, the subgroup of cells aggregation in two main directions. One is the cluster 0 called lymphoid progenitors, which showed the up-regulated expression of MME. The other is myeloid progenitors comprised with cluster 1, cluster 2, and cluster 5, with the gradual up-regulation of CD38 and KIT (Fig 2B). The three genes expression demonstrated contrary among the direction of myeloid progenitors and lymphoid progenitors. But the expression is not whole positive of the two direction cell types. This implies that CD38 and MME can be used as the markers distinguished myeloid progenitors and lymphoid progenitor differentiation directions, but not suitable to use in subtypes classification.

The efficient and accurately of classical hematopoietic progenitors markers which be used to distinguished CMP, GMP (Granulocyte-monocyte progenitors) and CLPs, were also re-analyzed. Our data demonstrated the expression of THY1 (CD90) is very rare (only 17 cells) in the two progenitor clusters which may cause as the progenitors were isolated from adult PBMC. To confirm whether the traditional classification markers THY1 and CD49f (ITGA6) expressed in progenitor, we analyzed four bone marrow single cell projects data of GSE120221, GSE117498, GSE145531 and GSE181989 (Fig. S1). Total 8296 CD34 positive cells were extracted and merged of the four projects data. The results showed positive expression cell numbers of THY1 is only 84 and CD49f is 824, and the distribution is inconsistent. This is similar with the other identified markers of FLT3, CD38 and KIT (Fig. S1). The different progenitor types of cells (CMP or CLP) were not distinguishable by unsupervised dimensionality reduction and clustering in bone marrow CD34+ cells. In addition, our data showed that the use of CD49f, THY1, FLT3 and KIT to classify or define the progenitor cells groups were very artificial and subjective. With the progenitors of CD34+FLT3+ cells defined a mixed population of progenitors, this finding may help to provide a well explanation that previously define or functional study of selected progenitors (such as CD34+FLT3+) presented multiple differential potential.

The essential regulate genes of HPCs lineages differentiation

To confirm the essential genes in HPCs status maintain and differentiation. We compared the expressions among three single cells data sheets, including bone marrow, PBMC and our CD34- cells subsets. The genes of SERPINB1, CDK6, HMGA1, IMPDH2, SPINT2, TXN, and EGFL7 were highly expressed in bone marrow and in our identified progenitor cells when compared with mature PBMC cells (Fig S2A). Those genes also high expressed in most of the subgroups of our

CD34+ cells. This indicates those genes are essential to regulating genes in HPCs status for maintenance and differentiation.

Classifications of the lineages differentiation

The transcriptomes among membrane marker-defined and transcriptomes-defined cells were thoroughly compared. Traditionally, membrane markers of progenitor CMP were defined with CD38+MME-FLT3+, while megakaryocytic–erythroid progenitors (MEP) were defined with CD38+MME-FLT3-. However, single cell clusters based on these markers were not distinguishable among the subgroups. As the expressions of CD38 and FLT3 were contradictory, this demonstrated FLT3 positive cells were negative of CD38 expression in most clusters (Fig 2B). With the CD38-FLT3+ subgroups progenitors were more original than the FLT3 negative cells, they are more likely to be CMPs. The expression is changed gradually and presented a continuum procedure. Although the expression tendency of FLT3+ was consistent with previously definition, it remained inefficient and inaccurate to classify the differentiation stages among the CMPs lineages. On the other hand, our result demonstrated clearly that CMPs lineages clusters can be distinguishable with the expression of KLF1, GATA1 and CSF3R (Fig 2B).

Our results showed KLF1 and GATA1 were uniquely expressed in the three clusters of 3, 8 and 9. This indicated that three clusters are likely to be either megakaryocyte progenitors (MkPs) or erythrocyte progenitors (ErP) of MEPs progenitors. The expression of KLF1 and GATA1 only presented in the late stage of CMPs directions, which indicated those two genes are the keys regulated genes in the differentiation from CMPs to MkPs or ErP. The other gene of GATA2 was widely expressed in most of the others clusters but mainly highly expressed in CMPs clusters. Interestingly, the expression of CSF3R disappeared in the MEPs or MkPs clusters (Fig 2B). During the lineage differentiation of CMPs, GATA2 play a very important role in all types of myeloid cell lines. The procedure of MEPs or MkPs differentiation was managed by up-regulated KLF1 and GATA1, and down-regulated with CSF3R. Hence, the cluster 5 was MEPs would act as the key progenitor to control the differentiation to MkPs or ErP progenitors.

As AVP was known to be uniquely expressed in HSC, our data showed both of AVP and CSF3R were mainly expressed in cluster 1 and 2 (Fig 2C and 2D). The high expression of CSF3R was consistent with G-CSF mobilization in peripheral blood HSC transplantation. Those clusters were the original multipotent clusters named as HPCs, which can prime to lineages of CLPs and CMPs. MPO was uniquely expressed in neutrophils in the border of cluster 1 (Fig S2B and S2C). This indicated that the granulocyte-monocyte progenitor branches were also present in the CMPs clusters. Our results showed the expression of IGHM, MME, and HOXA9 [19] was presented in cluster 0, which confirmed the cluster was lymphoid progenitor cells (Fig 2C). The expression level is increasing gradually of the transcription factors HOXA9 from cluster 1 to cluster 0 (Fig 2C).

Based on traditional surface markers, our data showed the classification of the progenitor subgroups or stages among lineage differentiation were inefficient and inaccurate. Importantly, this demonstrated that the expression and activation of transcription factors combined with unique marker genes would be more efficient for lineage definition of HPCs.

Differentiation and enrichment analysis

Differentiation and enrichment analysis was conducted among the clusters. As the expression changed gradually and presented in a continuum procedure, simple analysis on the different genes among clusters, especially when compared with nearby clusters, may not be efficient. To overcome this, we conducted the differentiation analysis among cluster 0 (CLPs) and cluster 3 (MEPs). Both of the two clusters are original clusters of CLPs or MEPs. The biology procedure analysis of Gene Ontology showed the high gene expression of CLPs were enriched in the regulation of leukocyte and T cell activation (Fig. 2E). While for CMPs, the gene expressions were highly enriched in mononuclear cell differentiation, erythrocyte differentiation and homeostasis (Fig. 2F). This was consistent with our previous results on CLPs and CMPs progenitors differentiation to two directions with strictly lineage affiliated by unsupervised dimensionality reduction and clustering.

Dynamics of progenitors cells subpopulations

It is clearly visible that CD38 and MME subgroups predominantly occupied the two directions of the progenitor differentiation, respectively. To confirm the trajectory change of the cells, trajectory analysis was conducted and three directions of progenitor cells differentiation were presented. Along the trajectory of right direction (State 1) which was specific expressed MME, only the cluster 0 and 4 were distributed (Fig. 3A and 3B). In contrast, the left direction (State 3) was composed by the CD38 positive expression clusters, such as cluster 1, 2, 3 and 5 (Fig. 3A and 3B) (Fig. S3). The expression tendency of MME and FLT3 was consistent with CLP differentiation, with contrary of KIT and CD38 which expressed with CMP lineages.

Heatmap analysis showed the expression tendency of selected marker genes in CMPs and CLPs. The CLPs branch highly expressed lymphoid marker genes of MME, IGHM and FLT3 (Fig. 3C). The CMPs branch was highly expressed with myeloid marker genes of GATA1, KLF1 and CD38. The expression of MME and CD38 were clearly inverse during the CLP and CMP lineages priming procedures (Fig. 3D). The last branch was highly expressed with NKG7, CD68 and FCGR3A (CD16), which are the markers of NK cells, monocyte and macrophages progenitor cells. This indicated lineage priming of these three types of cells may be distinct with CLPs and CMPs. In addition, the expression of transcription factors was distinguished among CLPs and CMPs directions. The expression of KLF1 and GATA1 only appeared in the later stage of myeloid progenitors named MEPs. This indicated that the HPCs lineages priming is likely controlled by key transcription factors. This may also indicated that the unperturbed adult hematopoiesis depends on replenishment from multiple potential progenitor.

Transcription factors promote the progenitor lineage priming with key receptors

To identify and characterize the committed cellular status, the critical co-expression of transcription factors for lineage fates were re-analyzed. Our study showed the CMP multipotent progenitors, which co-express with transcription factors GATA2, can differentiate into the lineage of myeloid and megakaryocyte cells (Fig. 2B). The expression of KLF1 was only present in the later stage of MEPs status. Furthermore, the expression of CSF3R was mainly showed in the original progenitors and almost disappeared in the later stage of MEPs progenitor. The cytokine receptors will co-express or co-silence with the relevant lineage key regulators, which indicated the progenitor lineages priming was multiple genes regulate procedure.

Likewise, the transcription factors regulation by SCENIC analysis showed the expression of transcription factors was completely distributed differently among CLP and CMP progenitors priming directions (Fig. 4A). The activation and expression of key regulators was much different among the priming stage of progenitors, such as GATA1 (Fig. 4B). As shown in our analysis, the transcription factors of BCL11A, YY1 and REL were the key regulators in CLP priming. And the KLF1, GATA1, and GATA2 were found to play a critical role during the lineage procedure of CMPs (Fig. 4C, Fig. S4).

To confirm which cluster was a megakaryocyte progenitor, the analysis on the expression of marker genes was performed. When compared with the key different genes and transcription factors among cluster 8 and cluster 9, the expression of KLF1 and GATA1 were gradually up-regulated in cluster 9 (Fig. 4A). The expression of KLF1 and GATA1 were up-regulated when the progenitor cells differentiate to megakaryocyte and erythrocyte lineages (Fig. S5). Our results also showed the expression of KIT, MS4A2 and TPSB2 were up-regulated or unique expression in cluster 8, indicating that the cluster was consisted of mast cell and basophil progenitors (Fig. S5).

G-CSF mobilization various type of progenitors in peripheral blood

Various types of progenitors were shown to exist in the PBMC of adults in the quiescent status. To examine whether any different among the mobilization and quiescent (unperturbed) progenitors, single cell sequencing was conducted in the

G-CSF mobilization peripheral blood progenitors. A total of 10450 CD34+ cells were enriched and sequenced, among which 16 clusters subgroups were showed after reclustering (Fig. 5A). Our results indicated that the total number of all types of progenitors were remarkable increased. All kinds of subtyped progenitors were shown to be existed in the quiescent status when compared before and after mobilization (Fig. 5B). This signifies that all types of progenitors were existed in the quiescent status of peripheral blood.

Since the progenitor was shown to gradually differentiate to mature stages in the peripheral blood, it would be expected fold changes of the more primitive progenitors to be higher than mature progenitors. Based on our analysis, the highest cell numbers and folder changed among the clusters was found to be in cluster 0 (Fig. 5C). The distribution and characterization of the featured markers were consistent with previously CD34 subgroups (Fig. 5D). Additionally, the AVP was mainly expressed in cluster 0 which was consistent with the analyzed results in the quiescent status of the progenitor cells. Hence, cluster 0 was the most original progenitor in the peripheral blood that can be identified as HPCs. In contrast, the expression of THY1 (CD49f) were only identified in very few cells. Gene expression analysis also demonstrated four genes, including *AVP*, *NPDC1*, *C1QTNF4*, and *JUN*, were high expressed in cluster 0 (Fig. 5F).

The progenitors were clustering into several directions, such as CLPs, CMPs and MEPs, which also consistent with the cluster distributions of quiescent status (Fig. 5A and Fig. 5D). One new direction of cluster 3 was presented in the all cells merged populations (Fig. 5E). The markers genes of that cluster were MPO and CSF3R. As myeloperoxidase (MPO) is the most abundant protein in human neutrophils and plays an important role in neutrophil antimicrobial responses [22, 23]. The other marker gene PRSS57 of neutrophils was also presented and up-regulated expression in the cluster [24]. So the cluster 3 was the progenitor of neutrophils, this was shown to be in a completely different direction with MEPs. Based on this analysis, it further showed NK cells progenitors and monocyte progenitor cells were not clustering nearby the other lineages (Fig. 5A).

In summary, there are three clusters which nearby the original progenitor of HPCs. It is clearly shown that during the HPCs priming and differentiation procedures, the HPCs priming into three progenitor cell types coinstantaneous and later maturing to diversity hematopoietic lineages. The lymphoid progenitors, myeloid progenitors, and megakaryocytic–erythroid progenitors were presented at the same time point and stage during the HPCs first step of priming process.

Re-definition of hematopoietic progenitor cells (HPCs)

As previously shown, cluster 0 was the most original progenitor in the peripheral blood that can be identified and defined as HPCs. All of the later mature progenitors are found to be developed from those original cells. Based on this rationale, we re-defined the three directions with common lymphoid progenitors (CLPs), GAPs (GATA genes controlled progenitors), and neutrophilic and monocyte progenitors (NMPs), which cannot be characterized by CD38, FLT3, and KIT (Fig. 6A). The differentiation of GAPs lineages was independently with the other two branches. The shape of each branch was controlled by the expression of key regulated genes which was shown to function like a switch. Through the transcription factors expression switches on-or-off and further gradually accumulated, generally synergistic with key receptor genes expression and signals activation, the original progenitor cells will be primed and differentiated to various progenitors.

During the myeloid progenitor differentiation procedure, the expression of GATA2 and CSF3R genes is the first branch being formed and presented (Fig. 6A and 6C). This was shown to be followed by the down-regulation of CSF3R genes and up-regulation of GATA2, which implies the progenitors were priming to oligo-potent progenitor GAPs. The expression of GATA1 and KLF1 were presented and gradually up-regulated at later stage. This subgroup was defined as GAPs mature stage named with megakaryocytic–erythroid progenitors (MEPs). With the differentiation of MEPs enhancement, the second branch was presented in the mature stage of MEPs. Toward the final stages of the MEPs, the expression of KIT, GATA1, and KLF1 controlled the differentiation of progenitor to megakaryocyte progenitors, erythrocyte progenitors

(Pro-ME), and mast cell or basophil progenitors (MBPs). In contrast to the other branch of myeloid progenitor differentiation process, the expression of GATA2 and CSF3R were restrained during differentiation trajectories when the progenitors were priming to neutrophilic and monocyte progenitors (NMPs, Fig 6A). In the lymphoid progenitor branch priming, critical transcription factor of HOXA9 and RUNX2 were identified and was shown to gradually up-regulated during the process of CLPs lineage differentiation (Fig. 6B). Similarly, the marker genes IGHM and MME of lymphocyte were also found to be up-regulated. This result provided strong evidence that the whole peripheral blood progenitors include a gradient of heterogeneity hematopoietic progenitors. The progenitor cells fate decisions and lineage commitment were mainly controlled by a combination of critical TFs and key receptor genes (Fig. 6). Also, the hierarchical organization of hematopoiesis was a continuum procedure, with key gene expressions presented and gradually being regulated. After the switch genes were activated and up-regulated, the progenitor cells would then differentiated into the next stages of priming. During this process, the decision of cell fate will be controlled and divided into different lineage branches. The fate deciding boundaries were then identified among the differentiation stages. The expression of GATA2 and CSF3R genes was shown to be the boundary for GAPs and NMPs. This finding is the first to validate that hematopoiesis was controlled by a switch-like model with identified relatively boundary, opening a new perspective on hematopoiesis (Fig. 6C).

Cell Atlas and characterization of all re-defined subtype progenitors cells

To clearly define the subgroups of progenitors hematopoietic, the characterization of all re-defined subtype progenitors cells were re-analyzed. Each of subgroups presents specific highly expression genes or negative express genes. These activated genes may enrich in some signal pathways which play a critical role in the lineages differentiation.

Pan markers of HPCs and unique makers of each subgroups

Our study showed the classical markers of CD49f and THY1 were not found in the multipotent HPCs progenitors. In contrast, ITGA4 (CD49d) was highly expressed in most of the progenitors, which is key molecular regulation of HSC homing [26]. Some reported HSC functional related marker genes was broadly expressed in most of the progenitor cells, such as HMGB1 [27], CD74 [28], PFN1 [29], TXN [30], ZFP36L2 [31], SERPINB1 [32], and HSP90AA1 [33] (Table 1 and Fig. 1B). These genes were shown to mediate the self-renewal, differentiation, mobilization, and maintenance of hematopoietic progenitor cells. In addition, two new genes of PPIA and EIF1 were identified. These genes were similarly to previous reported genes that pan-expressed in progenitors and highly expressed in matured subgroups of peripheral blood cells. A few progenitors' specific genes of CDK6[34], SOX4[35], CD82[36], EGFL7, IMPDH2[37], SPINK2[38] , TSC22D1[39] , FOXP1[40] were also identified in most progenitors. Among these specific genes, EGFL7 was identified as being pan-expressed in HPCs, CLPs and MEPs. As most of these genes were confirmed by functional validation, our data demonstrated that those high expression genes CD34 progenitor subsets were essential genes in progenitor status and differentiation.

Several key factors were identified, including NFE2, LYL1, MYB, RUNX1, CDK4, ETV6, TESPA1, and GATA2 of myeloid progenitors and RUNX2, HOXA9[6], JUN/FOS, LST1, TCF4, DDIT4, HOPX, KLF10, HOXA3, TSC22D1, and BCL11A of lymphoid progenitors (Fig. 6B). Interestingly, the two factors of RUNX2 and RUNX1 showed completely reverse expression tendency. Our data revealed RUNX2 monitored the differentiation of NMPs and GAPs, while RUNX1 controlled the differentiation of CLPs. Importantly, these key factors were shown to play reversely functional role during the differentiation of the HPCs. Through the up-regulated combination of NFE2, LYL1, MYB, RUNX1 , CDK4, ETV6, TESPA1, GATA2 and in restraining the expression of RUNX2, HOXA9, JUN/FOS, LST1, TCF4, DDIT4, HOPX, KLF10, HOXA3, TSC22D1, and BCL11A, the HPCs will diverse to differentiate to myeloid progenitors. Together with the activation of other receptors and unique transcript factors in each lineages progenitors, such as CSF3R for neutrophilic and GATA2 for GAPs, the whole hematopoietic lineage commitment was precisely controlled and gradually formed (Table 1, Fig. 6C). However, some unidentified regulators may still play a role during the process of hematopoietic lineage commitment. Thus, larger progenitor cell numbers and effective enrichment methods would be helpful to uncover those factors.

Our result also showed the selected specific marker genes and key transcript factors of each lineage progenitors (Table 1). Additionally, the negatively expressed genes were listed to distinguish each subgroup and clearly define the lineages progenitors (Table 1). It was identified that SLC40A1 was a unique new marker and key regulator of the whole lineages during GAPs differentiation. Our studies showed TAL1 only expressed in MEPs progenitors, which is the later stages of GAPs. LYL1 was up-regulated in the lineages of GAPs but decreased in the CLPs lineages. Our studies further confirmed that those two genes were critical regulators during myeloid lineages differentiation.

Pathways of each subgroups

Our current analyses results showed the JUN and JUND were enriched and highly expressed in most of the progenitors (Fig. 5 and 5F). This indicated that the JUN pathway may play a critical role in differentiation, mobilization, and maintenance of hematopoietic progenitor cells. Diversity pathways affect and control the differentiation of HPCs. The DEPTOR gene was found to be highly expressed in the clusters of MEPs. As DEPTOR was a negative regulator of the mTORC1 and mTORC2 signaling pathways [44]. That meaning the mTOR pathway should be suppressed in the MEPs mature and differentiation. The expression of NFKBIZ was very high expressed in the progenitors of monocyte and macrophages [45], that meaning the NFKB pathway was suppressed in the monocyte and macrophages mature and differentiation. The other key regulators and pathways of each subgroup will be shown in future.

Functional validation and primary culture

Previously studies already conducted many functional validations of HSCs key regulated genes. We hypothesis that our HPCs definitions and hematopoiesis model were right, that meanings the functional studies phenotype results would be consistent or can be explanation by our reaffirmed model. And furthermore, those conflicting and confusing results can be clearly interpretation by the reaffirmed hematopoiesis hierarchical organization model.

KLF1 and GATA1 were well-known play a key role in the erythroid and megakaryocytic differentiation [46, 47]. The expression of GATA1 progenitors generated mast cells, eosinophils, megakaryocytes and erythroid cells, and lacking expression can drive generated monocytes, neutrophils and lymphocytes [46]. It revealed that GATA1 was unique expressed in the later stages of MEPs and negatively expressed in the monocytes, neutrophils and lymphocytes progenitors (Fig. 5D). The result implies that the key transcript factor of GATA1 was very critical for the myeloid progenitor lineages differentiation. Our analysis results was found to be consistent with other functional studies results. Other evidence is transcript factor NFE2. It was reported that NFE2 acts as a critical regulator in hematopoietic cells. The knockout of NFE2 in mouse model displayed mild erythroid abnormalities and major defect in megakaryocyte biogenesis [48] [49]. Likewise, our results revealed NFE2 was up-regulated in the NMPs and MEPs lineage directions (Fig. 6B).

Based on findings from primary culture, M-CSF was shown to be critical for the survival of macrophage, which is consistent with our results that the receptor CSF1R of M-CSF only expression in monocyte progenitors and matured macrophages (data not shown). Our results showed stem cell growth factor (SCGF) was highly expression in NMP and GAP progenitors, especially in the whole lineages of MEPs that can explain SCGF burst promote erythroid activity.

Discussion

Taken together, we demonstrated the transcription atlas data of original status

HPCs in adult peripheral blood with large number of HPCs. Our study further reaffirmed and established a comprehensive model of hematopoiesis that can reconcile previous models and explain the conflict among functional studies. This helps to provide a detailed insight into lineage commitment from HPCs into almost branches of human peripheral blood progenitors.

Currently, there is without a standard consensus for the definition of all HPCs lineages. This is owing to the inconsistent classification with conflict functional outcomes among the current literatures [9, 13]. Our study succeed enriched the large numbers and almost whole subgroups of HPCs. This will be particularly attractive for large single cells studies to establish the comprehensive model of hematopoiesis with clearly HPCs definition and reliable lineage commitment. All of the hematopoietic progenitors were reaffirmed by unique makers and key factor genes, which can be well distinguishable. We are the first clearly definition all lineages of HPCs by unsupervised and inartificial clustering, which provide the unambiguous transcription atlas of each type of HPCs lineages. The unique markers genes of progenitor subgroups were highly consistent with previously characterization descriptions, such as KLF1 of MEPs, HPGDS of mast cell, and MPO of neutrophilic [22, 54, 55]. Our classification and definition of HPCs also help to explain the conflict and mechanism of *in vitro* and *in vivo* progenitor cell studies.

It is widely known that the HPCs would differentiate into common myeloid progenitors (CMPs) and common lymphoid progenitors (CLPs). Our data showed none of the MME positive cells co-expressed CD38, suggesting that classification of CLPs using solely CD38+ and MME+ was not sufficient. CMPs are defined with CD34+CD38+MME- which is consistent with classic definition, but our single cell expression atlas showed the CD34+CD38-MME+ progenitor cells is inconsistent with the traditional define of CLPs [4, 5]. Based on our study, we identified two subgroups of progenitors by single cell sequencing which are not consistent with classical definition, and showed specific classify surface or characterization markers genes, such as GATA1, MPO and KLF1. Likewise, we provided a clear evidence that the classical surface markers of CD38, KIT, THY1, CD49f and FLT3 were non-specific and inefficient to identify HSCs. This remind us the traditional lineage definition of HSC by above markers was imperfect and need to be improved. Evidently, the combination of transcriptional factors with unique marker genes would be more efficient for lineage definition of HPCs.

In brief, the unique markers were highly consistent with characterization of most progenitor subgroups. Secondly, the definition can very well explanation the conflict of functional studies. Thirdly, the expression of distinguished receptor of cytokines can perfectly explain the primary culture phenomenon of progenitor subgroups, such as CSF3R for neutrophilic and CSF1R for monocyte culture [56]. These three aspects strongly support the definition and classification of HPCs to be distinctly verifiable, reliable and reasonable. Moreover, our transcriptional atlas revealed the major role of each progenitor subgroups in hematopoiesis and the fate decision of downstream various hematopoietic cells.

It is well known that KLF1 and GATA1 are the keys regulate genes of erythrocyte or megakaryocyte differentiation [17, 18].Functional study have been shown that the upregulated transcriptional factor of GATA-1 can affect mast cells, eosinophils, megakaryocytes and erythroid cells generation, and downregulation to affect monocytes, neutrophils and lymphocytes generation [46]. Our finding reaffirmed the definition of GAPs lineages to show GATA1 to be solely expressed in the MEPs and up-regulated during maturation.Previously studies reported TAL1 and LYL1 are essential for maintenance of adult HSC function [41-43]. Since the induced pluripotent stem cells (iPSCs) was established through activation of four transcript factors, reprogram of pluripotent stem cells was widely investigated in various somatic cells [52]. GATA1 and TAL1 have been validated in the reprogram of megakaryocytes and erythroid cells [17, 53]. Although they finally induced mature erythroid cells based on those key transcript factors, but there are still many functional capacity limited during differentiation.

Through the expression changes of the three key transcription factors of KIT, KLF1 and GATA1, the later stage of MEPs can differentiate into two directions. The expression of transcriptional factors, both in original and mature stage of progenitors, revealed a basic split between myeloid-lymphocyte development and megakaryocyte-erythroid development. This provides a strong evidence that the transcription factors were the key regulators in each stages of the progenitor's differentiation. Generally, progenitor cells can be priming by key transcription factor activation, which derived progenitor differentiated to downstream specific mature lineages [20]. But previously study showed that transcriptional programs were not strictly lineage affiliated, and expression changes between HSCs and early progenitors occur independently of lineage choice [21]. That may cause by not reliable classification and definition of the subgroups and different stage of progenitor among lineage commitment, which based on the traditional cell surface markers in most of those investigations. We demonstrated the unique markers or transcriptional factors which control the progenitors differentiate to all kind of blood lineages in a continuum differentiation procedure. Our results strongly supported that in an ideally cell fate decision model, the activation of transcription factors and expression of progenitor lineage unique markers demonstrated a consistent lineage affiliated with relatively obvious subgroups boundary.

Our analysis on the transcriptional atlas revealed that the transcriptional factors activation and suppression play a critical role in the differentiation among lineage commitment. The bias during HPCs differentiation was gradually accumulated by activation of multiple lineage-specifying TFs, such as NFE2, LYL1, MYB, and RUNX1 of myeloid and RUNX2, HOXA9, and BCL11A of lymphoid progenitors. These TFs play critical role during the progenitor priming with dynamic entail quantitative expression and abundances change. By the multiple TFs activation and suppression, the downstream signal pathway would be activated. The stepwise controlled the differentiation of each branch during HPCs lineage commitment. During the process, individual progenitors would gradually acquire lineage biases along multiple directions during homeostasis. Our atlas results showed that key transcriptional factors priming HPCs for distinct downstream lineages, which finally generated and formatted the whole lineage commitment. The expression of key TFs could be considered as the relative reliable boundary among stages during progenitor mature. Through the control of TFs genes, the artificial precise committed progenitor cell differentiation can be made possible.

The underlying molecular mechanisms of G-CSF induces enhancement of hematopoietic remain unclear. Our results provide a new model of hematopoietic through scRNA of G-CSF mobilized progenitors lineages in peripheral blood. All types of the progenitors were remarkable increased, especially the HPCs. The CSF3R was mainly expressed in the original clusters (HPCs), which was the receptor of G-CSF [25]. G-CSF directly activates the downstream activation signals of HPCs through bind with CSF3R, which converted the quiescent status HPCs to activation progenitors. After the mobilization procedures, HPCs was priming and differentiation to all kinds of progenitors which can differentiate to mature myeloid and lymphocyte cells. In addition, SCGF, also known as CLEC11A, is a secreted glycoprotein that exhibits erythroid burst (BFU-E) promoting activity in the absence of SCF/c-kit Ligand [50, 51]. Furthermore, SCGF cooperates with GM-CSF, or G-CSF to enhance the growth of granulocyte-macrophage colonies (CFU-GM) [50, 51]. Because the CSF3R receptor of G-CSF was highly expression in progenitors of CMP, SCGF cooperates with G-CSF would activate and induced the progenitor differentiation to neutrophils and macrophages but reduce MEPs lineages generation. As key transcriptional factors and regulators controlled the whole hematopoietic lineage commitment formation, the use of primary cultures also showed factors of G-CSF and SCGF to induce the lineages formation. Both of these mechanisms would cooperate to play key roles during the lineage commitment formation. That reminds us two questions: First, how did the key transcript factors was activated during the lineage commitment procedure? Secondly, the receptors activated the signaling cascade and transcript factors, or the transcript factors were activated independently and factors enhance the commitment formation?

It was reported the HSC lineage commitment is a continuous process in recently single cell sequencing studies [5, 12]. But HSC heterogeneity and unclearly definition of HSC subgroups caused the mechanism of hierarchy formation still an enigma. The established hematopoietic lineage models were inconsistent and incompatible. This was due to the limitation of captured cell numbers and inefficient definition by identification using surface markers. Based on our clear definition and atlas of HPCs, it revealed that a gradient of hematopoietic progenitors existed during the lineage commitment and can be distinguished by the expression of unique TFs. Those progenitors gradually acquired lineage biases, divergence and prime to each branch. Our elaborate and comprehensive transcriptional atlas of HPCs can provide accurate definition, hierarchy, and biological insights of hematopoietic progenitors. As our study further explained how the diversified progenitor cells were primed, induced, and differentiated to the whole lineages of immune system cells, this enable the transcriptional atlas of HPCs to provide new perspective and well explanation about how the immune was reconstituted in adult. Recently, cytokines storm was reported in severe infection and coronavirus [57, 58]. Our atlas can clearly demonstrated the expression cytokines receptor in specific progenitor. By controlling the original stage and generation of cytokines, especially suppress the initial cytokines activation of the cascade activation, this could inhibit or reduce the effect of the follow-up cascade activation. Technically, this would be helpful in controlling the excessive activation by cytokines and preventing the occurrence of cytokines storm as a new therapeutic strategy. Our reaffirmed taxonomy will enhance more accurate prevention and treatment as well as immune monitoring in health and disease.

There were some limitations in our current study. Firstly, our study did not define the original progenitors with HSCs. Even though the characterization of these progenitors was most likely originated with stem cells, the markers of CD49f and THY1 were found to be negatively expressed. Our study showed two type of progenitor cells that are not clustering nearby the other lineages. One is the NK cells progenitors and the other is monocyte progenitors. Monocyte progenitors have more than two types of status progenitors, which located far away from the main cell populations. We speculated two possibilities to cause this phenomenon. One may be from the limitation of our enrichment method. This can cause the loss of major status progenitors from these two types of cells. This will have an effect to cause only the capture from the later stages. Secondly, the original source of NK cells progenitors and the monocyte progenitors were found to be independent and distinct with all of other progenitor cell types. As the whole atlas of our definition was established by unsupervised dimensionality reduction and clustering from statistical approach, the accuracy and efficiency should be considered. Artificial intelligence and deep learning would provide a more accurate, reliable, and distinguishable definition for each status and all progenitor subgroups during the whole procedure of hematopoiesis.

Importantly, our study helped to re-defined hematopoietic progenitor cells and reaffirming lineage commitment by transcriptional atlas. We are the first to clearly depict the transcriptional atlas and defined each subgroups of adult peripheral blood progenitor. Our results will enable the whole map of the transcriptional signal and pathway among lineage commitment. Moreover, we are also the first to identify the cell fate decision transcriptional factors in each branches among hematopoietic hierarchy formation. The precise control on the hematopoietic progenitor cells priming and differentiation can then be interfere by the key transcriptional factors and receptors signals, which we called Artificial Operation of HPCs.

In summary, our transcriptional atlas of HPCs established a comprehensive continuum and key regulators controlled model of the human adult peripheral blood hematopoiesis. Toward this transcriptional atlas, it provides accurate definition, hierarchy, and biological insights of hematopoiesis and lineage commitment of HPCs. Importantly, this can enable new research development in cell engineering, regenerative medicine, and disease treatment. Also, this can have critical insight and implications for the etiology and treatment of hematologic disorders. Moreover, this open a wide variety of possibilities for artificial precise control progenitor cells differentiation and targeted treatment of immune disease, providing hope for untreatable diseases.

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Author Contributions

Y.Y conceived the original idea, designed the study, and performed experiments on HPC's enrichment.Y.Y conducted the bioinformatics and statistical analysis of sequencing data.Y.Y wrote the manuscript and revised the manuscript. All authors approved the final draft of the manuscript.

Declaration of Interests

The authors have declared no competing financial interest.

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Data Availability Statement

The datasets used or analyzed during the current study are available from the corresponding author on reasonable request.

Methods

Sample collection and HPCs enrichment

Prior to blood collection, donor will received subcutaneous recombinant human granulocyte colony-stimulating factor with 300ug in each 12 hours for five days. After five days of continuous treatment, a total of 10mL of peripheral blood was collected in heparinized tubes from the stem cell donor, and kept at room temperature. PBMC and total white blood cells were obtained according to standard protocols. The protocol of peripheral blood mononuclear cell (PBMC) isolation was based on separation by density gradient centrifugation with Ficoll® (Cytiva, GE), a highly branched polysaccharide. Lineages (Lin−, CD3-CD19-CD56-) negative cells were removed by STEMCELL reagents (15272). Self-design method was applied to enrich the HPCs. Ethical approval was obtained from the ethics committee of the Shenzhen People's Hospital, as per the ethical standards recommended in the 1964 Declaration of Helsinki. Informed consent was obtained from the subjects in the study.

Single cell RNA preparation and sequencing

RNA-sequencing was also performed on human enriched HPCs (Lin−) purified from 10 donors, including one mobilization PBMC progenitor cells by G-CSF. The single cells suspension was loaded and captured by Chromium microfluidic chips and barcoded with a 10× Chromium Controller (10X Genomics). Barcoded single cells RNA was subsequently reverse-transcribed and sequencing libraries constructed with reagents from a Chromium Next GEM Single Cell 5' reagent kits v2 according to the manufacturer's instructions (10X Genomics). Sequencing was conducted by Illumina Nova 6000 (PE150).

Single-cell RNA-seq data processing

The raw sequencing reads of all samples were demultiplexed and aligned to the reference genome hg38 by Cellranger pipeline. All downstream single-cell unsupervised dimensionality reduction and clustering was conducted by Seurat [14]. Gene Ontology (GO) enrichment analysis of marker genes was implemented by the cluster Profiler R package. Trajectory analysis was conducted by Monocle2 [15]. Transcription factors regulation inference and gene regulatory networks analyses were performed using SCENIC [16].

Integrated analysis with online single cell dataset

The online scRNA data of GSE120221, GSE117498, GSE145531 and GSE181989 was downloaded from GSE database. Integrated analysis was performed by Seurat.

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List of Table and Legends

Table 1. The selected specific marker genes of all re-defined subgroups

Progenitor Cell Types	**Markers**	**Negative**
Pan-Progenitors	CD49d, EIF1,PPIA, HMGB1, CD74, PFN1, TXN, ZFP36L2, SERPINB1,HSP90AA1	
HPCs-Progenitors(C0)	CD34, CDK6, SOX4, CD82, EGFL7, IMPDH2, SPINK2, TSC22D1,SPI1(PU.1)	
CLP(C4,C13)	BCL11A, DNTT, RUNX2, MME, HOXA9, NKG7,CCR7	GATA2, SELL
NMPs(C3, neutrophilic,)	NFE2, MYB, LYL1, RUNX1, MPO, CSF3R, CLEC11A, MGST1	RUNX2, SLC40A1
GAPs (C1)	GATA2, NFE2, LYL1, MYB, RUNX1, SLC40A1	RUNX2, GATA1, KLF1
MEP(C2,C10,C14)	GATA2, NFE2, LYL1, MYB, GATA1,SLC40A1, CLEC11A, TAL1, KLF1, FCER1A, CPA3	SPINK2, CSF3R
C10(Mast cell or Basophil, MBPs)	MS4A2, TPSB2, HPGDS, HDC,KIT, LMO4, TPSAB1	
C14(Pro-ME)	HBD, TYMS, MCM7	
C5 (Pro-NK)	FCGR3A, GNLY, NKG7	
Monocytes and Macrophages(C6, C7)	FCGR3A, CD68, CST3, LYZ, SPI1, CEBPB, EGR1, CD86, PECAM1, CSF1R	

Figure 1. Two subtypes of progenitors were identified by unsupervised dimensionality reduction and clustering analysis of single cell data. A, Schematic

showing the progenitor cell enrichment and sequencing work flow. B,17 clusters were presented by UMAP anlaysis. The progenitors marker of CD34 mainly expressed in two nearby clusters 6 and 14. C, The heatmap results showed the different expression surface markers and known progenitors cell related markers in all clusters. Progenitor cells key regulate genes were identified, such as high expression of CDK6, SERPINB1, and SOX4. D, Violin plot presented the expression of HSCs traditional classification marker genes and known progenitor cell marker genes.

Figure 2. Transcriptome differentiation and characterization among re-clustered CD34 positive subgroups progenitor cells. A, UMAP analysis showed the CD34 positive subgroup cells aggregation in two mainly distinguished directions. B, Violin plots presented the expression unique marker genes, which demonstrated GATA2 expression of common myeloid progenitors (CMPs) and MME expression of common lymphoid progenitors (CLPs) characterization. C, The heatmap results showed the different expression marker genes confirmed the two distinguished directions belong different lineage cells of CLPs and CMPs. D, Feature plot of AVP and CSF3R expression showed cluster 1 was the most original progenitors in all of CD34 positive cells. E, The biology procedure analysis of Gene Ontology showed the high expression genes of CLPs were enriched in regulation of leukocyte and T cell activation. F, CMPs mainly high expression genes were enriched in mononuclear cell differentiation, erythrocyte differentiation and homeostasis.

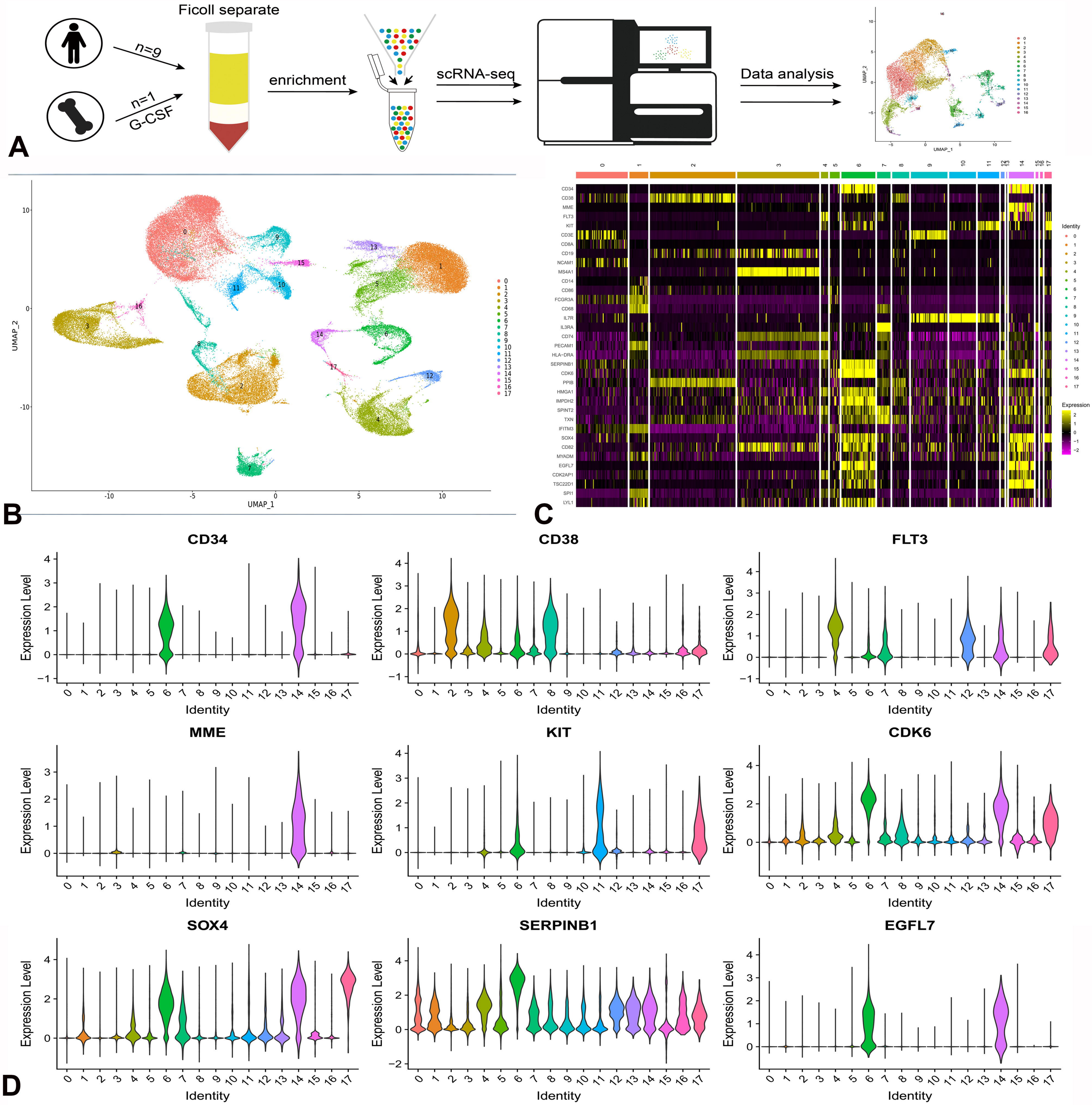
Figure 3. Dynamics of myeloid progenitor and lymphoid progenitor cells transcriptomes. A, trajectory analysis showed all clusters distributed in three branches. B, The expression tendency of unique markers genes was completely difference among the two lineages of myeloid and lymphoid progenitor. MME and FLT3 were up-regulated with CLP differentiation branches, with contrary of KIT and GATA2 up-regulated in CMP lineages. C, Heatmap analysis showed the expression tendency of selected marker genes in CMP and CLP. D, The expression of MME and CD38 were clearly inverse during the CLP and CMP lineages priming procedures.

Figure 4. Difference transcription factors promote the progenitor lineage priming. A, Heatmap results showed the different expression transcription factors among all progenitors subgroups. B, The activation and expression of key regulators GATA1 was obviously different among the priming stage of progenitors. C, Feature plots of the key transcription factors. BCL11A, YY1 and REL were the key regulators in CLP priming. KLF1, GATA1, and GATA2 were played a critical role in the lineage priming of CMPs.

Figure 5. The characterization of progenitors subtype in quiescent status and activated status. A, 16 clusters were presented of integrated CD34 positive progenitor cells by UMAP analysis. B, The cells distribution among the quiescent (PTE and GM01) and activate (GM02) status, which showed all kinds of progenitors were persisting existence in quiescent status of adult peripheral blood. C, The percentage changes column plot of CLP and CMP clusters. The cell numbers and folder were most obvious changed in the primitive cluster 0. D, Violin plot showed the feature markers distribution and characterization was consistent with quiescent status. E, Feature plots of unique marker genes. F, Dot plots presented the expression of unique marker genes and lineages specific genes.

Figure 6. New hematopoietic hierarchy of hematopoietic progenitor cells differentiation. A, T-SNE plots of re-defined clusters. B, Dot plots presented the expression of NFE2, LYL1, MYB, and RUNX1 of myeloid progenitors and RUNX2, HOXA9, and BCL11A of lymphoid progenitors. C, Reaffirmed lineage commitment model by scRNA transcriptional atlas. In the new hematopoietic hierarchy, the HPCs priming into three progenitor cell types coinstantaneous and later maturing to diversity hematopoietic lineages. The first progenitor cell type is lymphoid progenitors (CLPs) branches differentiated to T cell, B cell and NK cells. The second progenitor cell types GAPs (GATA genes controlled progenitors) branches differentiated to a middle stage of megakaryocytic–erythroid progenitors (MEPs) first,

which controlled by GATA2 and GATA1. In the later stages, MEPs differentiated to two directions controlled by KIT and KLF1. The KIT high expression branches will be derived to mast cell or basophil, eosinophil progenitors (MBPs). The other branch will differentiated to megakaryocytic and erythroid pro-progenitors (Pro-ME). The third progenitor cell type is neutrophilic and monocyte progenitors (NMPs), which differentiated to neutrophilic and monocyte. Dashed lines meanings haven’t strong directly evidence of the differentiation relationship which indicated partial lineage connections. The shaded area indicates the GATA2 and GATA1 expression domain.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Ficoll separate
n=9
n=1
G-CSF
enrichment
scRNA-seq
Data analysis
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UMAP_2
B
UMAP_1
UMAP_2
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
C
CD34
CD38
MME
FLT3
KIT
CD3E
CD8A
CD19
NCAM1
MS4A1
CD14
CD86
FCGR3A
CD68
IL7R
IL3RA
CD74
PECAM1
HLA-DRA
SERPINB1
CDK6
PPIB
HMGA1
IMPDH2
SPINT2
TXN
IFITM3
SOX4
CD82
MYADM
EGFL7
CDK2AP1
TSC22D1
SPI1
LYL1
Identity
Expression
D
CD34
CD38
FLT3
MME
KIT
CDK6
SOX4
SERPINB1
EGFL7
Expression Level
Identity

A
UMAP_1
UMAP_2
0
1
2
3
4
5
6
7
8
9
10
11
B
CD34
CD38
FLT3
MME
KIT
KLF1
CSF3R
GATA1
GATA2
Expression Level
Identity
C
Identity
Expression
AVP
CSF3R
KLF1
GATA1
GATA2
CD38
FLT3
KIT
IGHM
HOXA9
MME
CD68
GNLY
D
AVP
CSF3R
UMAP_1
UMAP_2
E
EnrichmentGO_BP_dot
positive regulation of lymphocyte activation
positive regulation of leukocyte activation
positive regulation of cell activation
positive regulation of T cell activation
positive regulation of leukocyte cell−cell adhesion
antigen processing and presentation
antigen processing and presentation of exogenous peptide antigen via MHC class II
antigen processing and presentation of peptide antigen via MHC class II
antigen processing and presentation of peptide or polysaccharide antigen via MHC class II
antigen processing and presentation of exogenous peptide antigen
antigen processing and presentation of exogenous antigen
antigen processing and presentation of peptide antigen
MHC class II protein complex assembly
peptide antigen assembly with MHC class II protein complex
peptide antigen assembly with MHC protein complex
MHC protein complex assembly
GeneRatio
0.075
0.100
0.125
0.150
0.175
Count
7.5
10.0
12.5
15.0
p.adjust
2.0e-08
1.5e-08
1.0e-08
5.0e-09
F
EnrichmentGO_BP_dot
carboxylic acid biosynthetic process
organic acid biosynthetic process
mononuclear cell differentiation
positive regulation of secretion by cell
positive regulation of secretion
response to oxygen levels
monocarboxylic acid biosynthetic process
multi−multicellular organism process
positive regulation of fibroblast proliferation
regulation of fibroblast proliferation
fibroblast proliferation
erythrocyte differentiation
erythrocyte homeostasis
nucleoside monophosphate metabolic process
definitive hemopoiesis
embryonic hemopoiesis
GeneRatio
0.06
0.08
0.10
0.12
Count
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6
7
8
p.adjust
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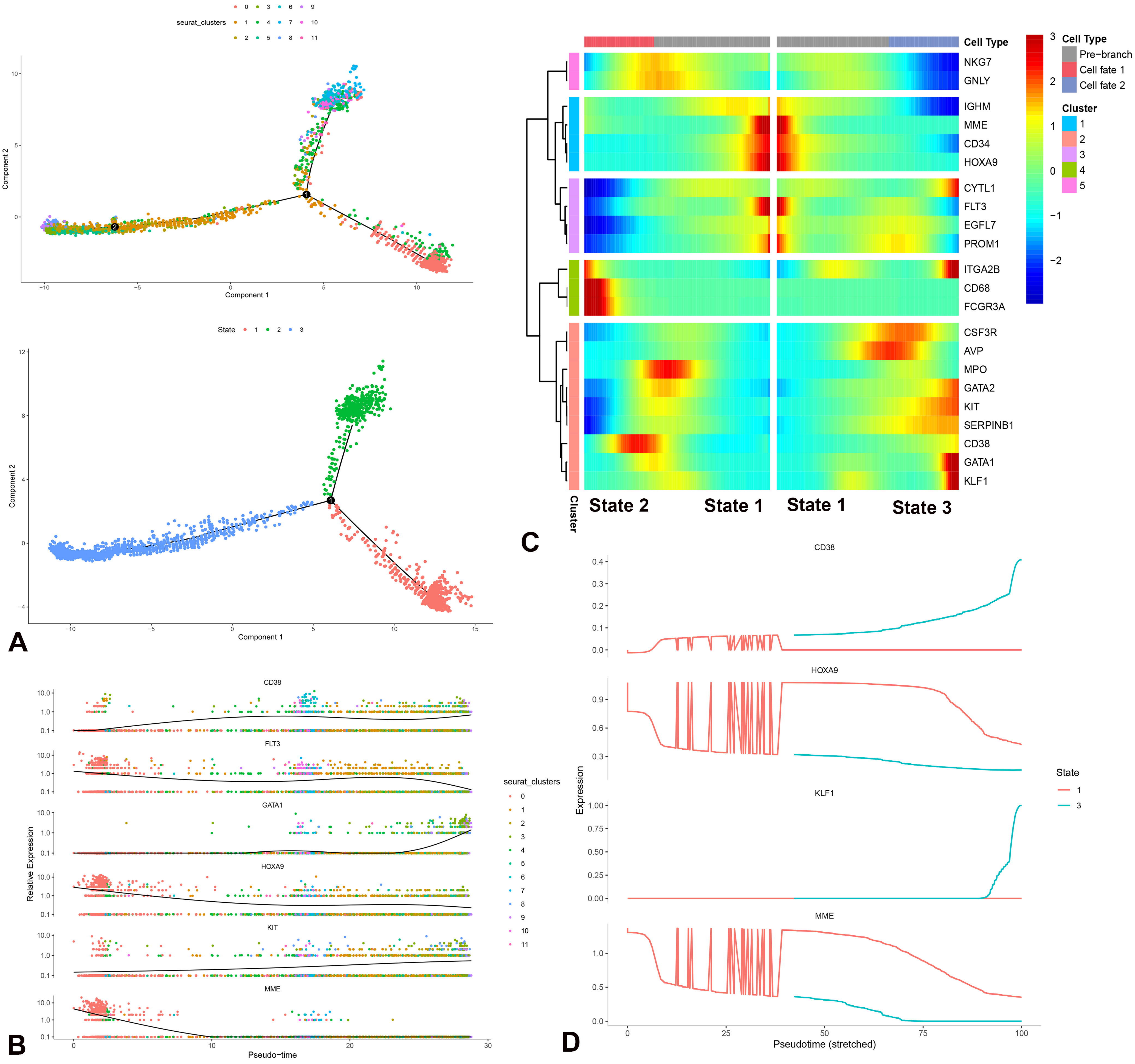
0.0100
0.0075
0.0050
0.0025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2
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4
5
6
7
8
9
10
11
Component 1
Component 2
State
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2
3
A
CD38
FLT3
GATA1
HOXA9
KIT
MME
Relative Expression
Pseudo−time
B
Cell Type
Pre−branch
Cell fate 1
Cell fate 2
Cluster
NKG7
GNLY
IGHM
MME
CD34
HOXA9
CYTL1
FLT3
EGFL7
PROM1
ITGA2B
CD68
FCGR3A
CSF3R
AVP
MPO
GATA2
KIT
SERPINB1
CD38
GATA1
KLF1
State 2
State 1
State 1
State 3
C
CD38
HOXA9
KLF1
MME
Expression
Pseudotime (stretched)
State
1
3
D

A
XBP1 (49g)
PRDM1_extended (82g)
IRF8 (50g)
IRF7 (55g)
IRF4 (15g)
CEBPD (22g)
SPI1 (194g)
MAFB_extended (86g)
FOSB_extended (27g)
FOS_extended (108g)
JUNB_extended (22g)
TCF7_extended (10g)
ETS1 (31g)
BCL11A_extended (15g)
BCLAF1_extended (36g)
STAT1 (61g)
IRF1 (57g)
YY1 (19g)
REL (23g)
EP300_extended (18g)
FOSL2 (17g)
KLF3 (45g)
JUN (12g)
JUND_extended (229g)
RAD21_extended (25g)
ZMIZ1_extended (53g)
KMT2A (29g)
FOXP1_extended (14g)
BRCA1_extended (18g)
MAZ_extended (30g)
KLF1_extended (51g)
MYC (32g)
STAT5A_extended (14g)
GATA1 (204g)
NFE2_extended (23g)
GATA2 (197g)
SOX4_extended (22g)
MYB (35g)
5
1
2
9
3
8
0
4
7
10
6
11
3
2
1
0
-1
-2
-3
B
GATA1 (204g)
Frequency
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50
100
150
0.00
0.02
0.04
0.06
0.08
0.10
AUC histogram
AUC
C
BCL11A_extended_15g
REL_23g
YY1_19g
KLF1_extended_51g
GATA1_204g
GATA2_197g
UMAP_1
UMAP_2
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-5
0
5
10
15
1.00
0.75
0.50
0.25
0.00

A
UMAP_1
UMAP_2
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
B
groups
UMAP_1
UMAP_2
GM01
GM02
PTE
C
Clusters
Percent
Samples
GM01
GM02
PTE
D
CD34
CD38
FLT3
MME
KIT
KLF1
CSF3R
GATA1
GATA2
Expression Level
Identity
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AVP
GATA1
IGHM
MPO
UMAP_1
UMAP_2
F
Features
Identity
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CD38
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MME
FLT3
JUN
C1QTNF4
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MPO
AVP
GATA2
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CSF3R
KLF1
HOXA9
IGHM
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75
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Average Expression
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1
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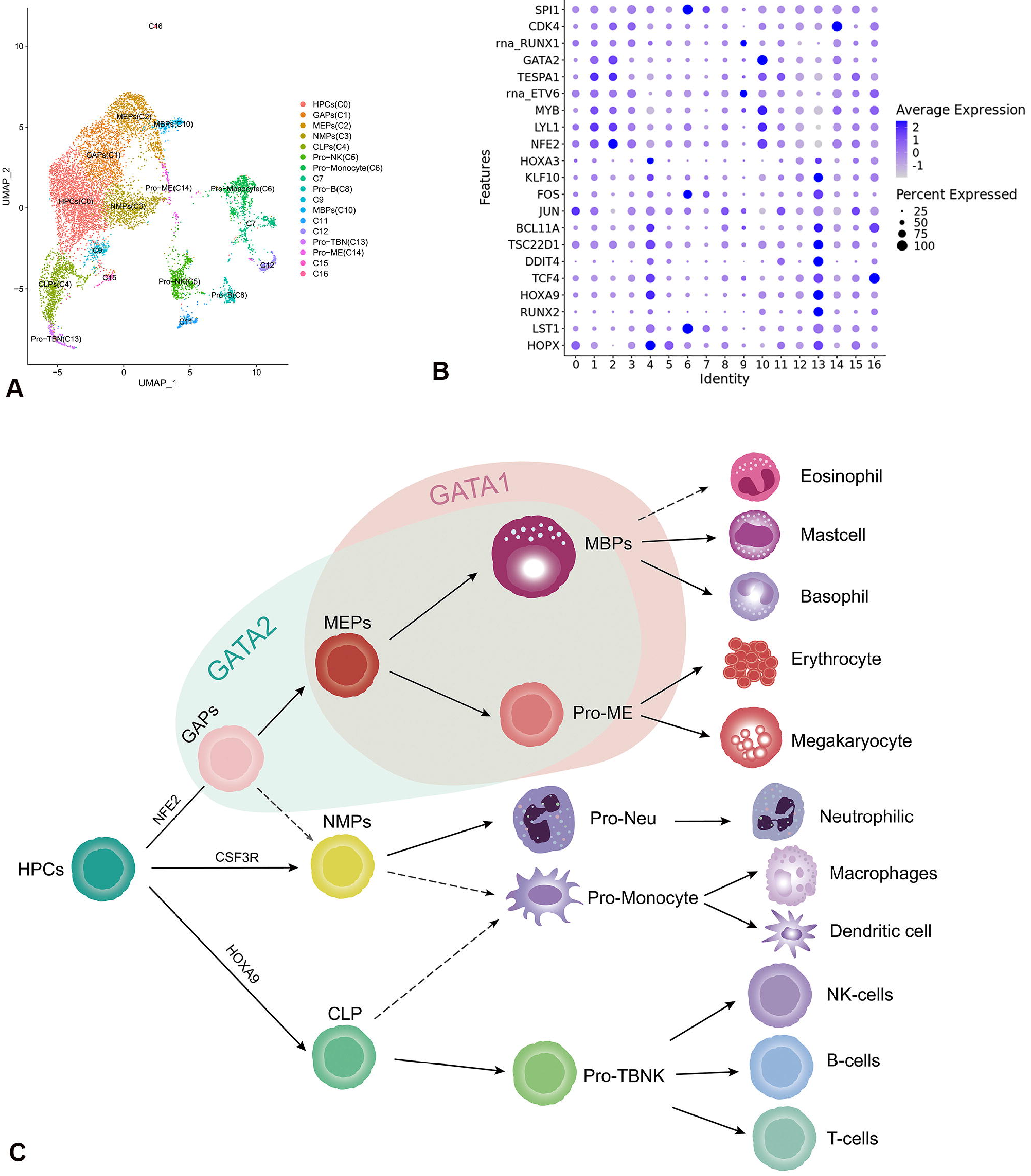
−1


A
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UMAP_2
C16
MEPs(C2)
MBPs(C10)
GAPs(C1)
Pro−ME(C14)
Pro−Monocyte(C6)
HPCs(C0)
NMPs(C3)
C7
C9
C12
CLPs(C4)
C15
Pro−NK(C5)
Pro−B(C8)
C11
Pro−TBN(C13)
HPCs(C0)
GAPs(C1)
MEPs(C2)
NMPs(C3)
CLPs(C4)
Pro−NK(C5)
Pro−Monocyte(C6)
C7
Pro−B(C8)
C9
MBPs(C10)
C11
C12
Pro−TBN(C13)
Pro−ME(C14)
C15
C16
B
Features
SPI1
CDK4
rna_RUNX1
GATA2
TESPA1
rna_ETV6
MYB
LYL1
NFE2
HOXA3
KLF10
FOS
JUN
BCL11A
TSC22D1
DDIT4
TCF4
HOXA9
RUNX2
LST1
HOPX
Identity
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
Average Expression
2
1
0
-1
Percent Expressed
25
50
75
100
C
GATA1
GATA2
MBPs
MEPs
Pro-ME
GAPs
NFE2
HPCs
CSF3R
NMPs
HOXA9
CLP
Pro-Neu
Pro-Monocyte
Pro-TBNK
Eosinophil
Mastcell
Basophil
Erythrocyte
Megakaryocyte
Neutrophilic
Macrophages
Dendritic cell
NK-cells
B-cells
T-cells