Excel Template for Identifying Mouse Myeloid Cell-Types in the Central Nervous System Based on Single-Cell RNA Sequencing Data

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Research

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Abstract

Background

The myeloid cells play a vital role in health and disease of central nervous system (CNS). However, how to clearly distinguish them is still a knotty problem. At present, single-cell RNA Sequencing (scRNA-Seq) technology can sequence thousands of cells at the single-cell level, and then divide the cells into different clusters according to the similarity of gene expression, but it is still difficult to further identity these cell clusters. Generally, there are some specific marker genes for cell-type identities. However, it is difficult to distinguish a variety of myeloid cells in the CNS, because these cells often have the same or cross gene markers, and some markers will change significantly in different pathological states. Therefore, establishing a simple and practical method to distinguish these cell populations is of great significance for the analysis of scRNA-Seq data.

Methods

Referring to CellMarker (http://biocc.hrbmu.edu.cn/CellMarker/), PanglaoDB (https://panglaodb.se/) and Mouse Cell Atlas (http://bis.zju.edu.cn/MCA/gallery.html), combining with the recent literatures, a simple Excel template was designed, in which a panel of gene makers corresponding to the myeloid cells were included. The 83 cell clusters from several recently reported single-cell data were used to verify the accuracy of this template.

Results

This template could easily distinguish myeloid cell-subtypes and non-myeloid cells. Comparing with literatures, the overall consistency rate was 93.98%. There was no statistically significant difference between the two groups (Bowker's test, $P > 0.05$). Kappa symmetric measures showed that the Kappa value = 0.642 ($P < 0.01$).

Conclusions

The cell identities of scRNA-Seq cluster data could be performed using our simple Excel formulae, a panel of gene markers and ideal cell clustering data are the basis for accurate identification of CNS myeloid cell-subtypes.

Background

The cell composition of central nervous system (CNS) includes not only neurons, oligodendrocytes and astrocytes, but also a variety of myeloid cells, such as microglia, monocytes, macrophages, dendritic cells and granulocytes [1]. These myeloid cells play a vital role in health and disease of CNS [2, 3]. Although there have been many studies on these cells, how to clearly distinguish them is still a difficult problem. The morphology, immunohistochemistry and flow cytometry are frequently used to identify these cells [4–6]. However, none of the above methods is perfect. They all have their own defects, such as poor objectivity, lack of specific markers, easy to produce cell recognition errors, etc.

Recently, single-cell RNA Sequencing (scRNA-Seq) technology can sequence thousands of cells at the single-cell level, and then divide the cells into different clusters according to the similarity of gene expression [7], but it is still difficult to further define these cell clusters because it is difficult for researchers to collect cell markers for interested cells [8]. At present, there are three main methods for cell-type identification based on single-cell transcriptome data: 1. Comparing the difference genes of a cluster with the marker genes of which cell-type in the database, and identify the cell-type in combination with their expressions. Common marker gene databases include CellMarker (http://biocc.hrbmu.edu.cn/CellMarker/) [8], PanglaoDB (https://panglaodb.se/) [9], Mouse Cell Atlas (http://bis.zju.edu.cn/MCA/gallery.html) [10], etc. In addition, we can also collect marker genes of certain cell-types in the literatures; 2. The expression profiles of genes in unknown cell clusters and known cell-types are used for similarity analysis. If the correlation was high, it would be identified as this kind of cells [11, 12]. For example, the R package (SingleR) can complete this analysis [13]; 3. Using the expression profiles of known cell-types to construct classifiers as the training sets, and the gene expression profiles of unknown cell clusters are input for classification and identification [11, 12]. For example, the R package (Garnett) can be used for this analysis [14]. Although, more and more automatic cell-type annotation tools have been developed, it is difficult to ensure that an automatic cell-type identification tool is suitable for all cell-types [15]. Therefore, researchers should select one of the defined results as a reference, and name the corresponding cell clusters in combination with manual annotation and relevant knowledge background. In any case, the specific marker genes are still the basis for defining cell cluster [11, 12]. Generally, the specific marker genes are selected according to the discipline background knowledges, literatures and databases. However, it is difficult to distinguish a variety of myeloid cells in the CNS, because these cells often have the same or cross gene markers, and some markers will change significantly in different pathological states [16]. For example, adgre1 (F4/80), the established marker for macrophages [17, 18], also expresses in monocytes, microglia and dendritic cells [19]. Gene expressions of microglial specific markers (P2RY12 and TMEM119) in microglia are often down regulated or even negative under the conditions of CNS injury, inflammation and degeneration [20–22]. Therefore, establishing a simple and practical method to distinguish these cell populations is of great significance for the analysis of scRNA-Seq data.

Methods

Excel template design for cell-type definition

Referring to CellMarker (http://biocc.hrbmu.edu.cn/CellMarker/) [8], PanglaoDB (https://panglaodb.se/) [9], Mouse Cell Atlas (http://bis.zju.edu.cn/MCA/gallery.html) [10], combining with the recent literatures [1, 3, 4, 6, 16, 18, 22-30], a simple excel template for cell definition was designed, in which a panel of gene makers corresponding to the myeloid cells, lymphocytes, common CNS cells, and proliferative cells were included (Fig. 1 and Table S1). Here, myeloid cells include monocytes (MNC), macrophages (MAC), microglia (MG), granulocytes (mainly neutrophils, NEUT), and dendritic cells (DC). In order to minimize the effects of lymphocytes on myeloid cell identifies, T, B, and NK cell specific gene markers were also listed in the table.

Excel template design for gene markers and expression extraction

To perform the cell identification of a cluster, we need four Excel tables: cell definition (Fig.1, Fig.2E and Table S1), cluster data (Fig2A and Table S1), avg_logFC extraction (Fig2B, D and Table S1), and gene extraction (Fig2C and Table S1). In cluster data table, column A is the genes in a cluster and column B is avg_logFC, which means average Log2 Fold Change, it is the ratio of the normalized mean gene counts in each cluster relative to all other clusters for comparison. In some literatures, the average value of gene expression is also used. In avg_logFC extraction table, the data in columns A and B should come from the corresponding columns of cluster data table, column C is extracted genes from column C of gene extraction table, column D is extracted values from column C using Excel command: VLOOKUP(Cn,A:B,2,0). In gene extraction table, the data in columns A is the gene markers from column B of cell definition table, column B is the genes from column A of avg_logFC extraction table, column C is extracted values from column A using Excel command: IF(COUNTIF(B:B,An)>0,An,"").

Cell-type identity workflow

The cell-type identity workflow included the follow steps (Fig2): 1. Copy lines A and B form cluster data table, and paste them to the corresponding columns A and B of avg_logFC extraction table, 2. Copy line A form avg_logFC extraction table, and paste it to the column B of gene extraction table, then we will get the extracted genes form gene markers (column A), 3. Copy column C form gene extraction table, and PasteSpecial it to the column C of avg_logFC extraction table, then we will get the extracted values in column D, 4. Copy column D form avg_logFC extraction table, and PasteSpecial it to any blank column we like in the cell definition table, 5. In cell definition table, we can perform cell identities by comparing the extracted values (upregulated and downregulated genes are shown as red and green, respectively) to the cell-types (column A) and gene markers (column B).

Data

The sources of gene expression data used in this paper are shown in Table 1 [10, 31-33]. The data in each literature are displayed in the form of Excel (Fig. 2A).

Consistency test of cell-type identity methods

In order to test the consistency of our cell identity method with the literatures, the identification results were divided into three grades: excellent, satisfactory and poor, based on Table 2. Bowker's test and Kappa symmetric measures were used to test the difference and consistency of the paired data between the two groups, respectively. For Bowker's test, $P < 0.05$ was considered to be a statistically significant difference. For Kappa symmetric measures, Kappa $\geq$ 0.75 indicates good consistency, 0.4 $\leq$ kappa < 0.75 indicates general consistency, and kappa < 0.4 indicates poor consistency. The data were analyzed using SPSS software v.26 (SPSS Inc., Chicago, IL, USA).

Results

Descriptive comparison of our method with the literatures in CNS myeloid cells

Using our cell-type identification method, we identified CNS myeloid cells in the four data reported in the literatures (Table 1).

In the Supplementary Table 3 of Ximerakis, et al. [31], they listed the most discriminating genes per cell-type. From this table, we chose monocytes (MNC), macrophages (MAC), microglia (MG), neutrophils (NEUT), dendritic cells (DC), neuronal-restricted precursors (NRP), immature neurons (ImmN), mature neurons (mNEUR), astrocyte-restricted precursors (ARP), astrocytes (AST), oligodendrocyte precursor cells (OPC), oligodendrocytes (OL), ependymocytes (EPC), and hypendymal cells (HypEPC) as "gold standard" to test our method. As shown in Fig. 3, Table 3 and Table S2, among the 14 cell clusters being compared, we identified MNC as MNC (mixed with a few NEUT and DC), and NRP as proliferative cells. The other 12 cell clusters were completely consistent.

The 15 clusters of adult mouse brain from the Table S3 of Han, et al. [10] were also identified, the results were shown in Table 4 and Table S3. We found that among the 15 cell clusters being compared, pan-GABAergic and Schwann cell were not within the scope of our evaluation, the reported cluster 4 (Macrophage_*Klf2* high) was mixed with a few MG, the other 12 cell clusters were completely consistent.

The $CD11b^{+}CD45^{+}CD3^{-}B220^{-}Ly6G^{-}$ cells isolated using fluorescence-activated cell sorting (FACS) from adult mouse brain parenchyma, choroid plexus, leptomeninges, and perivascular space (embj2021108605-sup-0008-datasetev1) by Sankowski et al. [32] were also compared. As shown in Table 5 and Table S4, we found that among the 17 cell clusters compared, there 14 were completely consistent. The non-consistent clusters included stromal cells (cluster 15) which was not within the scope of our evaluation, the reported cluster 6 (CNS-associated macrophages, CAMs) which expressed MG specific markers, and cluster 9 (CAMs) which the typical genes of MAC were not elevated.

Of course, our cell identification process was not smooth sailing. When we analyzed another data (Table S2 of Mimouna et al.) [33], we encounter thorny problems. In this report, Louvain graph-based community clustering was used to divide the cells into different clusters, and PanglaoDB was used to identify putative cell and/or activation state for each individual Louvain cluster. We still identified the cell-types using our method based the author's data. As shown in Table 6 and Table S5, although the cell-type identification was basically consistent, in both reported and our results, the cell-types in each of the nine clusters were mixed, which indicates that the cell clustering in this data is not ideal.

Descriptive comparison of our method with the literatures in peripheral blood and bone marrow myeloid cells

In order to test whether our method was suitable for the identification of non-CNS myeloid cells, the 21 peripheral blood cell clusters and 17 bone marrow cell clusters of adult mice from the Table S3 of Han, et al. [10] were also identified.

The peripheral blood results were shown in Table 7 and Table S6. We found that among the 21 cell clusters being compared, cluster 14 (Erythroblast_*Car2* high), cluster 20 (B cell_*Igha* high), and cluster 21 (Erythroblast_*Hba-a2* high) were not within the scope of our evaluation, the reported cluster 18 (Macrophage_*Pf4* high) was mixed with a few NEUT, the other 17 cell clusters were completely consistent. The bone marrow results were shown in Table 8 and Table S7. We found that among the 17 cell clusters being compared, cluster 3 (Neutrophil progenitor), cluster 8 (Hematopoietic stem progenitor cell), cluster 9 (Erythroblast), and cluster 15 (Mast cell) were not within the scope of our evaluation, the other 14 cell clusters were completely consistent.

Statistical comparison of our method with the literatures

According to the grading evaluation method in Table 2, we graded the results of all data analysis (Table 3-8). Excluding those clusters (N/A) that are not within the scope of our analysis, we obtained a total of 83 valid cases. As shown in Fig. 4, the excellent, satisfactory and poor results in literatures were 74, 3 and 6, respectively, and they were 77, 1, and 5 in our results. The overall consistency rate was 93.98% (78/83). The Bowker's test showed that there was no statistically significant difference between the two groups ($P > 0.05$). Kappa symmetric measures showed that the Kappa value = 0.642 ($P < 0.01$), indicated general consistency.

Discussion

For the last few decades, although advanced techniques, such as flow cytometry, can be used to identify CNS myeloid cell-subtypes, it is still difficult to be very accurate due to the lack of absolutely specific markers and the instability of marker expression under different pathophysiological conditions [16]. Although, scRNA-Seq is a promising new technology to solve this problem (Cembrowski, 2019), for ordinary researchers, various programming language analysis packages for scRNA-Seq data are really not an easy task, and for bioinformatics experts, they do not necessarily know the specific markers for CNS myeloid cell-subtype identifies. Therefore, building a bridge to connect the knowledge gap between ordinary researchers and bioinformatics experts is the key to solve this problem.

In this report, a simple excel template was designed, in which a panel of gene makers corresponding to the myeloid cells, lymphocytes, common CNS cells, and proliferative cells were included. For users, as long as the gene expression data of cell clusters are obtained, the clusters can be named directly using this excel template. It should be emphasized that this template is mainly suitable for determining the major categories of myeloid cells. If researchers need to further distinguish the subtypes of certain cells, it is necessary to add corresponding gene markers. Therefore, this Excel template is open, and researchers can modify or add new genes based on their need. In addition, in the selection of gene markers, we consider not only their relative specificity, but also the crossover and commonality of different cells. Therefore, in the Excel template, we defined the positive gene marker as "P", negative as "N", and if the marker could be positive or negative, we defined it "P/N" (Fig. 1 and Table S1). For example, Ptprc (the gene of CD45) was the common marker of myeloid cells and lymphocytes [34–36]. Therefore, we used it as a common marker of myeloid cells and lymphocytes to distinguish CNS non-myeloid cells (such as astrocytes, oligodendrocytes, neurons, etc.). In addition, in theory, the protein molecule CD45 expressed by Ptprc gene is positive in many leukocytes, but in the process of collecting gene markers and drawing the Excel template, we found that Ptprc gene is not expressed in every cell cluster, so we defined it as P/N. In addition to Ptprc, there are many similar examples. We will not list them one by one. Please see Fig. 1 and Table S1 for details. For a certain cell, although there are some relatively specific gene markers, we do not use a single or a small number of markers to identify it. We use a panel of gene markers to comprehensively evaluate it and then define it. This can effectively distinguish the cell-types with similar or cross gene expression and ensure the accuracy of cell cluster identification. In this Excel template, there are 73 gene markers (excluding non-myeloid CNS cells) in each panel can be used to distinguish myeloid cell-subtypes and lymphocytes (Fig. 1 and Table S1). For example, MNC could express Ptprc (P/N), Cd14 (P/N), Itgam (P/N), Itgax (P/N), Csf3r (P/N), Adgre1(P/N), Ly6c1 (P/N), S100a4 (P/N), Cd68 (P), Ly86 (P/N), Ctsb (P/N), Ccr2 (P/N), Ly6c2 (P), Plac8 (P), Pf4 (P/N), Lyz1 (P), Hmox1 (P/N), F13a1(P), Lyst (P/N), Prtn3 (P/N), Elane (P/N), and Pilra (P/N). Although, several molecules (Cd68, Ly6c2, Plac8 and Lyz1) are positive (P) in MNC, they are also expressed in other cells. Therefore, there is no absolute specific marker of MNC in this template. Nevertheless, we can still determine its cell type using comparative analysis. The typical examples can be found in table S4 (C8 and 11). For those cell-types with their own specific gene markers, it is easy to identify cell clusters using comparative analysis. Typical examples are Ms4a7, Lyve1, Cbr2, Mrc1 and CD163 for MAC; Hexb, Olfml3, Sparc, Tgfbr1, P2ry12 and Tmem119 for MG; Ltf, Ly6g, Mmp8, Camp, Ngp, Fcnb, Cebpe, Retnlg, S100a8, S100a9, Lcn2, G0s2, Wfdc21 for NEUT. Of course, due to the limitations of knowledge background and research level, this Excel template still has some defects. For example, for DC, the expressions of H2-Ab1, H2-Eb1, H2-Aa, Cd74 and Cd209a should be positive, but these markers can also be expressed in MAC and B cells, especially B cells do not belong to myeloid cells, which is easy to cause misjudgment. Therefore, in this template, we also added B cell markers to facilitate distinguish B cells from DC.

In order to verify the accuracy of this Excel template, the 83 cell clusters from several recently reported single-cell data were used (Table 1). The results showed that comparing with literatures, the overall consistency rate was 93.98%. The Bowker's test showed that there was no statistically significant

difference between the two groups (P >0.05). Kappa symmetric measures showed that the Kappa value = 0.642 (P < 0.01). These indicate that our method is general consistency with the literatures. Next, we will analyze the possible causes of inconsistency.

Comparing with the report of Ximerakis, et al. [31], only one cluster is inconsistent (Table 3). Our results showed that there were a few NEUT and DC mixed with their MNC. The possible reason is that they take Plac8 as a specific marker of MNC. In fact, Plac8 is also expressed in NEUT and DC [10]. Comparing with the cell-type identifies in adult brain of Han, et al. [10], the cluster 4 is inconsistent (Table 4). The reason may be that the reported cluster 4 was mixed with a few MG, because we can find the typical microglia markers (Hexb, Olfml3, Sparc, Tgfbr1, P2ry12 and Tmem119) in Table S3. Comparing with the report of Sankowski, et al.[32], the clusters 6 and 9 are inconsistent (Table 5). Both clusters were identified as CAMs, however, the expression of typical genes of MACs (Mrc1, Cd163, Lyve1, Pf4, Ms4a7, Stab1, and Cbr2) were not elevated in both clusters. In contrast, MG specific markers (Hexb, Olfml3, and Sparc) were significantly elevated in cluster 6, while the other genes in cluster 9 were not within the scope of our evaluation. Comparing with the cell-type identifies in peripheral blood and bone marrow of Han, et al. [10], excepting cluster 18 of peripheral blood was mixed with a few NEUT, the others were completely consistent. These indicate that our Excel template is also very effective for the analysis of non-CNS myeloid cells.

From the above analysis, we can deduce that the appropriate gene markers and ideal scRNA-Seq data clustering are key factors for the accuracy of cell definition. We can understand the importance of cell clustering through the following example. When we analyzed another data (Table S2 of Mimouna et al.) [33], both the reported and our results were not ideal. Analyzing the reasons, we find that their data clustering methods are different from the other literatures mentioned above. The cell clustering method in this literature is Louvain graph-based community clustering, which may be the reason why clustering is not ideal. Although, our Excel template still can be used to identify the cell-types based on the author's data, the cell-types in each of the nine clusters were mixed (Table 6). Therefore, the data used in this Excel template should be processed through the standard scRNA-Seq analysis process, including quality control, standardization, data correction, feature selection and data dimensionality reduction, finally the cells were divided into different clusters according to the similarity of gene expression.

Conclusions

In conclusion, the cell identities of the scRNA-Seq data could be performed using our simple Excel formulae, a panel of gene markers must be compared to obtain accurate analysis of CNS myeloid cell-subtypes. For data with better cell clustering, this template could effectively distinguish myeloid cell-subtypes, various lymphocytes and other CNS cells. For data with poor clustering, this template could also identify various cell-types, but it would need to be further subdivided.

Abbreviations

ARP: Astrocyte-restricted precursors

AST: Astrocytes

CAMs: CNS-associated macrophages

CNS: central nervous system

DC: Dendritic cells

EPC: Ependymocytes

FACS: fluorescence-activated cell sorting

HypEPC: Hypendymal cells

ImmN: Immature neurons

MAC: Macrophages

MG: Microglia

MNC: Monocytes

mNEUR: mature neurons

NEUT: Neutrophils

NRP: Neuronal-restricted precursors

OL: Oligodendrocytes

OPC: Oligodendrocyte precursor cells

scRNA-Seq: single-cell RNA Sequencing

Declarations

Ethical Approval and Consent to participate

Not applicable

Consent for publication

Not applicable.

Availability of supporting data

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

Competing interests

The authors declare that they have no competing interests.

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Author contributions

HZL and JGH participated in study design, data interpretation and writing. XYL, JLL and SQD participated in literature search, data collection, data analysis tables and figures. All authors read and approved the final manuscript.

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Author disclosure statement

The authors declare no competing financial interest.

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Tables

Table 1 The sources of gene expression data used in this paper

Data	Mice	Tissue	single cell	scRNA-seq	Clustering	Cluster annotation
Ximerakis, et al. Nat Neurosci. 2019; 22(10):1696-1708. Table S3	C57BL/6J mice (male, 2-3 months of age, and 21-22 months of age).	8 young and 8 old brains	Dissociated brain	Chromium Single Cell 3' Chip (10x Genomics), the sequencing was performed on NextSeq 500 instrument (Illumina)	Seurat package (v.2.3) in R (v.3.3.4)	Using multiple cell-type-specific/enriched marker genes that have been previously described in the literature (Plac8 for MNC).
Han, et al. Cell. 2018; 172(5):1091-1107.e17. Table S4	Wild-type C57BL/6J mice (SPF, female, 6-10 week-old).	Brain, blood and bone marrow	Brain was dissociated using accutase; bone marrow was treated red blood cell lysis buffer; blood was treated red blood cell lysis buffer or Ficoll separation	Microwell-seq, the 3' ends of the transcripts are then enriched during library generation using PCR and sequenced using the Illumina Hiseq platform	Seurat was used for dimension reduction, clustering and differential gene expression analysis.	Single cell MCA (scMCA) analysis built by authors (Fig 7a)
Sankowski, et al. EMBO J. 2021; e108605. embj2021108605-sup-0008-datasetev1	SPF and GF C57BL/6J mice (mixed sex, 6-10 weeks old)	The brain parenchyma, choroid plexus, leptomeninges, and perivascular space (20 mice per group).	Parenchyma and perivascular space cells were isolated using Percoll gradient. The choroid plexuses and leptomeninges were treated by mechanical dissociation through a 70 micron cell strainer. Viable $CD11b^{+}CD45^{+}CD3^{-}B220^{-}Ly6G^{-}$cells were FACS-isolated.	High-throughput scRNA-seq using the high-sensitivity method mCEL-Seq2, the sequencing was performed on Illumina HiSeq 3000 sequencing system (pair-end multiplexing run) at a depth of 130,000–200,000 reads per cell.	Seurat version 3	Generating maps for the myeloid cell populations based on published signature genes (Jordao et al, 2019). Fig 1B
J Exp Med. 2021;218(1):e20192386. Table S2	C57BL/6 mice (mixed sex, 6–10 weeks old)	EAE mouse spinal cord	CNS-infiltrating cells were isolated using Percoll density gradient. $F4/80^{+}CD11b^{+}CD45^{+}$ cells were sorted using FACS.	Chromium Single Cell 3' Chip (10x Genomics), The sequencing was performed on the Illumina NovaSeq system using a 28-8-98 paired-end cycle.	R version 4.0.1 software (R Core Team, 2019), fastMNN implementation, Louvain graph–based community clustering.	Cluster-specific markers were searched using the Wilcoxon rank-sum test. An automated cell type assignment was performed with singleR using training sets derived from the Immunological Genome Project database. PanglaoDB was used to identify putative cell and/or activation state for each individual Louvain cluster. The cell type and cell activation state transitions were identified by performing trajectory

	analysis with slingshot.

Table 2 The grade evaluation criterion of cell identity

Consistency	Accuracy	Grade
Consistent	Both completely accurate	Both excellent (A)
	Both partially accurate	Both satisfactory (B)
	Neither is accurate	Both poor (C)
Non-consistent	One is completely accurate	Excellent (A)
	One is partially accurate	Satisfactory (B)
	One is not accurate	Poor (C)

Table 3 Comparison of the cell-type identifies with Ximerakis, et al.

Clusters	Reported cell types	Our cell types	Consistency	Reason
MNC	MNC	MNC (mixed with a few NEUT and DC)	Part	Plac8 is also expressed in NEUT and DC
MAC	MAC	MAC	Yes	
MG	MG	MG	Yes	
NEUT	NEUT	NEUT	Yes	
DC	DC	DC	Yes	
NRP	NRP	Proliferative cells	N/A	not within the scope of our evaluation
ImmN	ImmN	neuron	Yes	
mNEUR	mNEUR	neuron	Yes	
ARP	ARP	AST	Yes	
AST	AST	AST	Yes	
OPC	OPC	OPC	Yes	
OL	OL	OL	Yes	
EPC	EPC	Ependymal	Yes	
HypEPC	HypEPC	Ependymal	Yes	

Abbreviations:

ARP: Astrocyte-restricted precursors

AST: Astrocytes

DC: Dendritic cells

EPC: Ependymocytes (a kind of ependymal cells)

HypEPC: Hypendymal cells (a kind of ependymal cells)

ImmN: Immature neurons

MAC: Macrophages

MG: Microglia

MNC: Monocytes

mNEUR: mature neurons

NEUT: Neutrophils

NRP: Neuronal-restricted precursors

OL: Oligodendrocytes

OPC : Oligodendrocyte precursor cells

Table 4 Comparison of the cell-type identifies in adult brain with Han, et al.

Clusters	Reported cell types	Our cell types	Consistency	Reason
1	Myelinating oligodendrocyte	OL	Yes	
2	Microglia	MG	Yes	
3	Astrocyte_*Mfe8* high	AST	Yes	
4	Macrophage_*Klf2* high	MAC/MG	Part	The reported cluster 4 was mixed with a few MG
5	Astrocyte_*Atp1b2* high	AST	Yes	
6	Oligodendrocyte precursor cell	OPC	Yes	
7	Neuron	Neuron	Yes	
8	Macrophage_*Lyz2* high	MAC	Yes	
9	Astroglial cell (Bergman glia)	AST	Yes	
10	Pan-GABAergic	Proliferative cells	N/A	not within the scope of our evaluation
11	Astrocyte_*Pla2g7* high	AST	Yes	
12	Schwann cell	Unkonw	N/A	not within the scope of our evaluation
13	Granulocyte_*Il33* high	NEUT	Yes	
14	Hypothalamic ependymal cell	Ependymal cells	Yes	
15	Granulocyte_*Ngp* high	NEUT	Yes	

Abbreviations:

AST: Astrocytes

DC: Dendritic cells

MAC: Macrophages

MG: Microglia

MNC: Monocytes

NEUT: Neutrophils

OL: Oligodendrocytes

OPC : Oligodendrocyte precursor cells

Table 5 Comparison of the cell-type identifies with Sankowski, et al.

Clusters	Reported cell types	Our cell types	Consistency	Reason
C0	MG	MG	Yes	
C1	CAMs	MAC	Yes	
C2	MG	MG	Yes	
C3	CAMs	MAC	Yes	
C4	CAMs	MAC	Yes	
C5	MG	MG	Yes	
C6	CAMs	MG	No	The expression of typical genes of MAC including Mrc1, Cd163, Lyve1, Pf4, Ms4a7, Stab1, and Cbr2 were not elevated. In contrast, MG specific markers Hexb, Olfml3 and Sparc were significantly elevated.
C7	CAMs	MAC	Yes	
C8	$Ly6c^{low}$ monocytes	MNC	Yes	
C9	CAMs	Unknow	N/A	The expression of typical genes of MAC including Mrc1, Cd163, Lyve1, Pf4, Ms4a7, Stab1, and Cbr2 were not elevated. The other genes were not within the scope of our evaluation.
C10	MG	MG	Yes	
C11	$Ly6c^{hi}$ monocytes	MNC	Yes	
C12	DCs	DC	Yes	
C13	CAMs	MAC	Yes	
C14	Prolif. Cells	Prolif. Cells	Yes	
C15	Stromal cells	Unknow	N/A	not within the scope of our evaluation
C16	Lymphocytes	NK	Yes	

Abbreviations:

CAMs: central nervous system (CNS)-associated macrophages

DC: Dendritic cells

MAC: Macrophages

MG: Microglia

MNC: Monocytes

NEUT: Neutrophils

NK: Natural killer cells

Table 6 Comparison of the cell-type identifies with Mimouna et al.

Clusters	Reported cell types	Our cell types	Consistency	Reason
C1	MAC/MG/others	MAC/MG/others	Yes	Cell clustering was not ideal.
C2	MAC/MG/NEUT	MAC/MG/NEUT	Yes	
C3	MNC/MAC/MG	MAC/MG/NEUT	Part	
C4	MAC/MG/NEUT	MAC/MG/NEUT	Yes	
C5	MNC/MAC	MAC/MG/NEUT	Part	
C6	NEUT	MAC/MG/NEUT	Part	
C7	MAC/MG/others	MAC/MG/NEUT	Yes	
C8	T/others	MAC/MG/NEUT	Part	
C9	MNC/MAC	MAC/MG/NEUT	Part	

Abbreviations:

MAC: Macrophages

MG: Microglia

MNC: Monocytes

NEUT: Neutrophils

Table 7 Comparison of the cell-type identifies in peripheral blood with Han, et al.

Clusters	Reported cell types	Our cell types	Consistency	Reason
1	T cell_*Trbc2* high	T	Yes	
2	B cell_*Ly6d* high	B	Yes	
3	Macrophage_*S100a4* high	MAC	Yes	
4	Neutrophil_*Retnlg* high	NEUT	Yes	
5	Neutrophil_*Ltf* high	NEUT	Yes	
6	Neutrophil_*Camp* high	NEUT	Yes	
7	Neutrophil_*Il1b* high	NEUT	Yes	
8	NK cell_*Gzma* high	NK	Yes	
9	Macrophage_*Ace* high	MAC	Yes	
10	Monocyte_*Elane* high	MNC	Yes	
11	B cell_*Vpreb3* high	B	Yes	
12	Monocyte_*F13a1* high	MNC	Yes	
13	T cell_*Gm14303* high	T	Yes	
14	Erythroblast_*Car2* high	Proliferative cells	N/A	not within the scope of our evaluation
15	B cell_*Rps27rt* high	B	Yes	
16	Dendritic cell_*Siglech* high	DC	Yes	
17	Basophil_*Prss34* high	Unknow	N/A	
18	Macrophage_*Pf4* high	MAC/NEUT	Part	The reported cluster 18 was mixed with a few NEUT
19	B cell_*Igha* high	Unknow	N/A	not within the scope of our evaluation
20	Macrophage_*Flt-ps1* high	MAC	Yes	
21	Erythroblast_*Hba-a2* high	Unknow	N/A	not within the scope of our evaluation

Abbreviations:

B: B cells

DC: Dendritic cells

MAC: Macrophages

MG: Microglia

MNC: Monocytes

NEUT: Neutrophils

NK: NK cells

T: T cells

Table 8 Comparison of the cell-type identifies in bone marrow with Han, et al.

Clusters	Reported cell types	Our cell types	Consistency	Reason
1	Neutrophil_*Cebpe* high	NEUT	Yes	
2	Neutrophil_*Mmp8* high	NEUT	Yes	
3	Neutrophil progenitor	MNC/MAC/NEUT	N/A	not within the scope of our evaluation
4	Monocyte_*Prtn3* high	MNC	Yes	
5	Macrophage_*Ms4a6c* high	MAC	Yes	
6	Neutrophil_*Ngp* high	NEUT	Yes	
7	Pre-pro B cell	B	Yes	
8	Hematopoietic stem progenitor cell	Unknow	N/A	not within the scope of our evaluation
9	Erythroblast	Proliferative unknow cell	N/A	not within the scope of our evaluation
10	Neutrophil_*Fcnb* high	NEUT	Yes	
11	B cell_*Igkc* high	B	Yes	
12	Macrophage_*S100a4* high	MAC	Yes	
13	T cell_*Ms4a4b* high	T	Yes	
14	Dendritic cell_*Siglech* high	DC	Yes	
15	Mast cell	Unknow	N/A	not within the scope of our evaluation
16	Dendritic cell_*H2-Eb1* high	DC	Yes	
17	Monocyte_*Mif* high	MNC	Yes	

Abbreviations:

B: B cells

DC: Dendritic cells

MAC: Macrophages

MG: Microglia

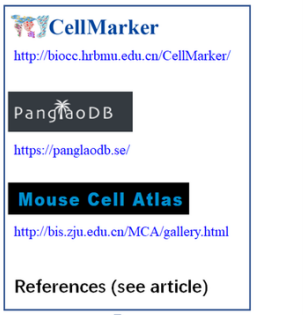
MNC: Monocytes

NEUT: Neutrophils

NK: NK cells

T: T cells

Figures



Cell types	gene markers	MNC	MAC	MG	NEUT	DC	T	B	NK	NK/T
ALL	Ptprc	P/N	P/N	P/N	P/N	P/N	P/N	P/N	P/N	P/N
MNC/MAC/MG/ NEUT/DC	Cd14	P/N	P/N	P/N	P/N	P/N	N	N	N	N
	Itgam	P/N	P/N	P/N	P/N	P/N	N	N	N	N
	Itgax	P/N	P/N	P/N	P/N	P/N	N	N	N	N
No DC/T/B/NK	Csf3r	P/N	P/N	P/N	P/N	N	N	N	N	N
No NEUT/T/B/NK	Adgre1	P/N	P/N	P/N	N	P/N	N	N	N	N
No MG/T/B/NK	Ly6c1	P/N	P/N	N	P/N	P/N	N	N	N	N
	S100a4	P/N	P/N	N	P/N	P/N	N	N	N	N
MNC/MAC/MG	Cd68	P	P	P/N	N	N	N	N	N	N
	Ly86	P/N	P/N	P/N	N	N	N	N	N	N
	Ctsb	P/N	P	P/N	N	N	N	N	N	N
MNC/MAC/DC	Ccr2	P/N	P/N	N	N	P/N	N	N	N	N
MNC/MAC/NEUT	Ly6c2	P	P/N	N	P	N	N	N	N	N
MNC/NEUT/DC	Plac8	P	N	N	P/N	P/N	N	N	N	N
MAC/MG/DC	Aif1	N	P/N	P	N	P/N	N	N	N	N
MNC/MAC	Pf4	P/N	P	N	N	N	N	N	N	N
	Lyz1	P	P	N	N	N	N	N	N	N
	Hmox1	P/N	P/N	N	N	N	N	N	N	N
	F13a1	P	P/N	N	N	N	N	N	N	N
MNC/NEUT	Lyst	P/N	N	N	P/N	N	N	N	N	N
	Prtn3	P/N	N	N	P/N	N	N	N	N	N
	Elane	P/N	N	N	P/N	N	N	N	N	N
	Pilra	P/N	N	N	P/N	N	N	N	N	N
MAC/MG	C1qa	N	P/N	P	N	N	N	N	N	N
	C1qb	N	P/N	P	N	N	N	N	N	N
	C1qc	N	P/N	P	N	N	N	N	N	N
	Csf1r	N	P	P	N	N	N	N	N	N
	Cx3cr1	N	P/N	P	N	N	N	N	N	N
	Fcgr1	N	P/N	P/N	N	N	N	N	N	N
	Trem2	N	P/N	P/N	N	N	N	N	N	N
	Hpgds	N	P/N	P/N	N	N	N	N	N	N
	Gpr34	N	P/N	P	N	N	N	N	N	N
	Lpcat2	N	P/N	P	N	N	N	N	N	N
	Ctsd	N	P	P/N	N	N	N	N	N	N
	Stab1	N	P	P/N	N	N	N	N	N	N
MAC/DC	H2-Ab1	N	P/N	N	N	P	N	P/N	N	N
	H2-Eb1	N	P/N	N	N	P	N	P/N	N	N
	H2-Aa	N	P/N	N	N	P	N	P/N	N	N
	Cd74	N	P/N	N	N	P	N	P/N	N	N
	Cd209a	N	P/N	N	N	P	N	P/N	N	N
MG/DC	Siglech	N	N	P/N	N	P/N	N	N	N	N
MAC	Ms4a7	N	P	N	N	N	N	N	N	N
	Lyve1	N	P	N	N	N	N	N	N	N
	Cbr2	N	P/N	N	N	N	N	N	N	N
	Mrc1	N	P/N	N	N	N	N	N	N	N
	CD163	N	P/N	N	N	N	N	N	N	N
MG	Hexb	N	N	P	N	N	N	N	N	N
	Olfml3	N	N	P	N	N	N	N	N	N
	Sparc	N	N	P	N	N	N	N	N	N
	Tgfbr1	N	N	P/N	N	N	N	N	N	N
	P2ry12	N	N	P/N	N	N	N	N	N	N
	Tmem119	N	N	P/N	N	N	N	N	N	N
NEUT	Ltf	N	N	N	P/N	N	N	N	N	N
	Ly6g	N	N	N	P	N	N	N	N	N
	Mmp8	N	N	N	P/N	N	N	N	N	N
	Camp	N	N	N	P/N	N	N	N	N	N
	Ngp	N	N	N	P/N	N	N	N	N	N
	Fcnb	N	N	N	P/N	N	N	N	N	N
	Cebpe	N	N	N	P/N	N	N	N	N	N
	Retnlg	N	N	N	P/N	N	N	N	N	N
	S100a8	N	N	N	P	N	N	N	N	N
	S100a9	N	N	N	P	N	N	N	N	N
	Lcn2	N	N	N	P	N	N	N	N	N
	G0s2	N	N	N	P	N	N	N	N	N
	Wfdc21	N	N	N	P	N	N	N	N	N
T	Cd3d	N	N	N	N	N	P/N	N	N	P/N
	Cd3e	N	N	N	N	N	P/N	N	N	P/N
	Cd3g	N	N	N	N	N	P/N	N	N	P/N
B	Ms4a1	N	N	N	N	N	N	P/N	N	N
	CD79a	N	N	N	N	N	N	P/N	N	N
	CD79b	N	N	N	N	N	N	P/N	N	N
NK	Gzma	N	N	N	N	N	N	N	P/N	P/N
	Ncr1	N	N	N	N	N	N	N	P/N	P/N
	Klrb1c	N	N	N	N	N	N	N	P/N	P/N

Figure 1

Excel template design for cell-type definition

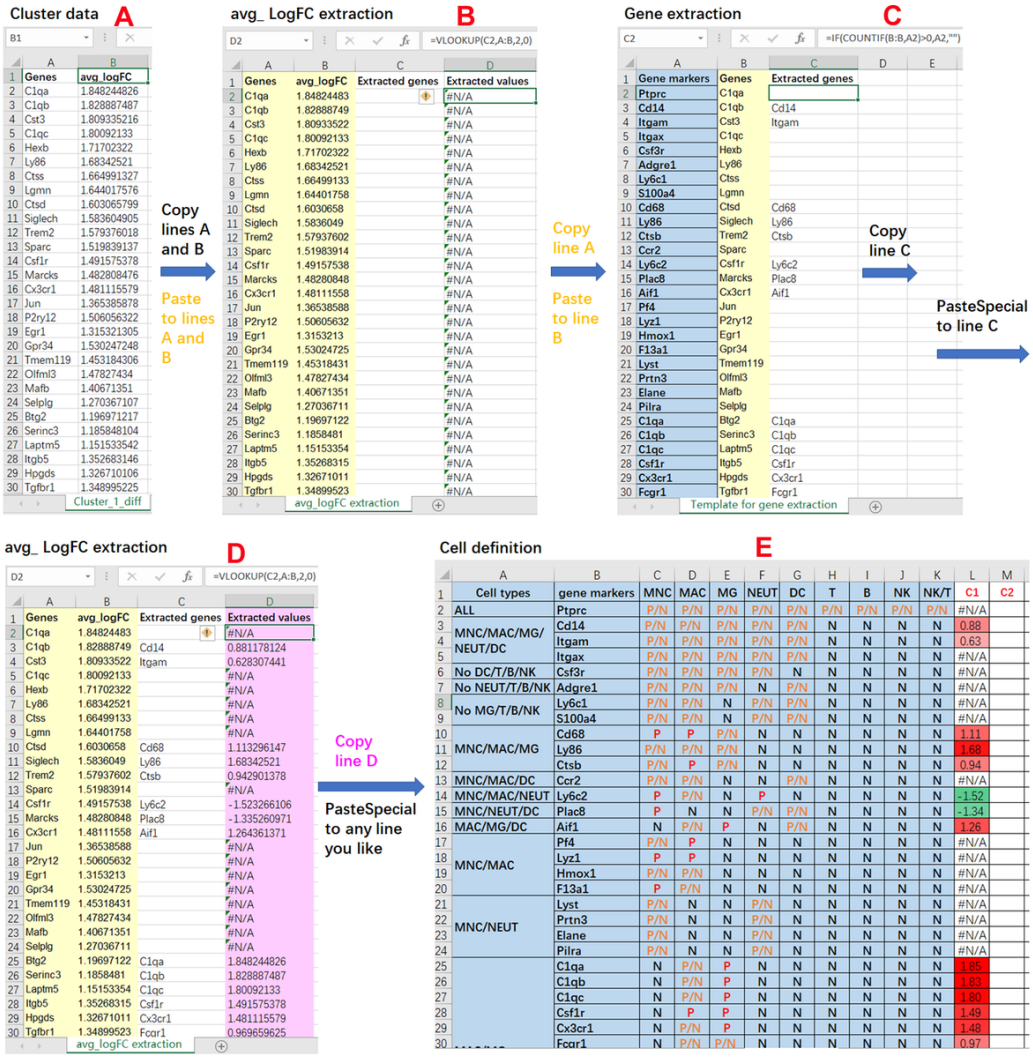


Figure 2

Excel template design for gene markers and expression extraction, and cell-type identity workflow

Cell types	gene markers	MNC	MAC	MG	NEUT	DC	T	B	NK	NK/T	MNC	MAC	MG	NEUT	DC	NRP	mNEUR	ARP	AST	OPC	OL
ALL	Ptprc	P/N	P/N	P/N	P/N	P/N	P/N	P/N	P/N	P/N	1.71	0.82	0.73	0.96	1.05	N/A	#N/A	N/A	N/A	N/A	N/A
MNC/MAC/MG/NEUT/DC	Cd14	P/N	P/N	P/N	P/N	P/N	N	N	N	N	N/A	2.30	1.56	N/A	N/A	N/A	#N/A	N/A	N/A	N/A	-0.65
	Itgam	P/N	P/N	P/N	P/N	P/N	N	N	N	N	N/A	0.30	1.08	1.14	0.42	N/A	#N/A	N/A	N/A	N/A	N/A
	Itgax	P/N	P/N	P/N	P/N	P/N	N	N	N	N	N/A	N/A	N/A	N/A	N/A	N/A	#N/A	N/A	N/A	N/A	N/A
No DC/T/B/NK	Csf3r	P/N	P/N	P/N	P/N	N	N	N	N	N	N/A	N/A	0.97	1.41	N/A	N/A	#N/A	N/A	N/A	N/A	N/A
No NEUT/T/B/NK	Adgre1	P/N	P/N	P/N	N	P/N	N	N	N	N	0.56	1.26	1.11	N/A	N/A	N/A	#N/A	N/A	N/A	N/A	N/A
No MG/T/B/NK	Ly6c1	P/N	P/N	N	P/N	P/N	N	N	N	N	N/A	N/A	-1.62	N/A	N/A	N/A	-1.68	N/A	-1.74	N/A	-1.89
	S100a4	P/N	P/N	N	P/N	P/N	N	N	N	N	2.32	N/A	N/A	N/A	1.88	N/A	#N/A	0.30	N/A	N/A	N/A
MNC/MAC/MG	Cd68	P	P	P/N	N	N	N	N	N	N	0.76	1.60	2.27	-0.76	0.36	-0.75	-0.80	-0.75	-0.86	-0.77	-0.97
	Ly86	P/N	P/N	P/N	N	N	N	N	N	N	N/A	0.89	2.83	-1.06	N/A	-1.04	-1.14	-1.02	-1.19	-1.07	-1.32
	Ctsb	P/N	P	P/N	N	N	N	N	N	N	0.39	1.52	1.56	-1.44	-0.66	-1.27	-0.52	-0.33	-0.34	N/A	-0.47
MNC/MAC/DC	Ccr2	P/N	P/N	N	N	P/N	N	N	N	N	1.89	0.85	N/A	N/A	1.59	N/A	#N/A	N/A	N/A	N/A	N/A
MNC/MAC/NEUT	Ly6c2	P	P/N	N	P	N	N	N	N	N	2.47	N/A	N/A	2.02	N/A	N/A	#N/A	N/A	N/A	N/A	N/A
MNC/NEUT/DC	Plac8	P	N	N	P/N	P/N	N	N	N	N	3.70	N/A	N/A	0.95	1.55	N/A	#N/A	N/A	N/A	N/A	N/A
MAC/MG/DC	Aif1	N	P/N	P	N	P/N	N	N	N	N	N/A	1.48	2.59	-0.97	0.37	-0.86	-1.03	-0.85	-1.06	-0.97	-1.19
MNC/MAC	Pf4	P/N	P	N	N	N	N	N	N	N	N/A	3.67	N/A	N/A	N/A	N/A	#N/A	N/A	N/A	N/A	N/A
	Lyz1	P	P	N	N	N	N	N	N	N	0.62	0.29	N/A	0.37	N/A	N/A	#N/A	N/A	N/A	N/A	N/A
	Hmox1	P/N	P/N	N	N	N	N	N	N	N	0.72	0.85	0.28	N/A	N/A	N/A	#N/A	N/A	N/A	N/A	N/A
	F13a1	P	P/N	N	N	N	N	N	N	N	1.20	1.99	N/A	N/A	N/A	N/A	#N/A	N/A	N/A	N/A	N/A
MNC/NEUT	Lyst	P/N	N	N	P/N	N	N	N	N	N	N/A	N/A	N/A	N/A	N/A	N/A	#N/A	N/A	N/A	N/A	N/A
	Prtn3	P/N	N	N	P/N	N	N	N	N	N	N/A	N/A	N/A	N/A	N/A	N/A	#N/A	N/A	N/A	N/A	N/A
	Elane	P/N	N	N	P/N	N	N	N	N	N	N/A	N/A	N/A	N/A	N/A	N/A	#N/A	N/A	N/A	N/A	N/A
	Pilra	P/N	N	N	P/N	N	N	N	N	N	0.98	N/A	0.34	1.52	N/A	N/A	#N/A	N/A	N/A	N/A	N/A
MAC/MG	C1qa	N	P/N	P	N	N	N	N	N	N	-2.59	1.77	4.12	-2.49	-1.29	-2.20	-2.71	-2.43	-2.77	-2.63	-2.97
	C1qb	N	P/N	P	N	N	N	N	N	N	-2.29	1.73	4.03	-2.45	-0.82	-2.27	-2.55	-2.27	-2.61	-2.43	-2.80
	C1qc	N	P/N	P	N	N	N	N	N	N	-2.18	1.77	3.93	-2.42	-1.45	-2.02	-2.42	-2.22	-2.50	-2.32	-2.69
	Csf1r	N	P	P	N	N	N	N	N	N	N/A	1.29	3.03	-1.27	-0.52	-1.12	-1.34	-1.21	-1.39	-1.27	-1.52
	Cx3cr1	N	P/N	P	N	N	N	N	N	N	N/A	0.46	2.85	-1.06	-0.78	-1.02	-1.12	-1.00	-1.17	-1.06	-1.30
	Fcgr1	N	P/N	P/N	N	N	N	N	N	N	N/A	0.96	1.55	N/A	N/A	N/A	#N/A	N/A	N/A	N/A	N/A
	Trem2	N	P/N	P/N	N	N	N	N	N	N	-1.10	0.60	3.40	-1.42	-1.34	-1.51	-1.57	-1.41	-1.63	-1.49	-1.77
	Hpgds	N	P/N	P/N	N	N	N	N	N	N	N/A	1.00	1.78	N/A	N/A	N/A	#N/A	N/A	-0.54	N/A	-0.63
	Gpr34	N	P/N	P	N	N	N	N	N	N	-1.09	N/A	3.04	-1.16	-1.06	-1.13	-1.24	-1.14	-1.29	-1.17	-1.42
	Lpcat2	N	P/N	P	N	N	N	N	N	N	-0.37	N/A	2.25	-0.45	-0.71	-0.80	-0.90	-0.81	-0.95	N/A	-0.81
	Ctsd	N	P	P/N	N	N	N	N	N	N	-1.46	0.40	3.38	-1.48	-2.36	-1.95	-1.98	-1.29	-1.18	-1.46	-1.89
	Stab1	N	P	P/N	N	N	N	N	N	N	N/A	1.94	0.72	N/A	N/A	N/A	#N/A	N/A	N/A	N/A	N/A
MAC/DC	H2-Ab1	N	P/N	N	N	P	N	P/N	N	N	1.79	3.48	N/A	N/A	4.49	N/A	#N/A	N/A	N/A	N/A	N/A
	H2-Eb1	N	P/N	N	N	P	N	P/N	N	N	1.31	3.38	N/A	N/A	4.24	N/A	#N/A	N/A	N/A	N/A	N/A
	H2-Aa	N	P/N	N	N	P	N	P/N	N	N	1.72	3.49	N/A	N/A	4.42	N/A	#N/A	N/A	N/A	N/A	N/A
	Cd74	N	P/N	N	N	P	N	P/N	N	N	2.53	4.11	N/A	N/A	4.99	N/A	#N/A	N/A	N/A	N/A	N/A
	Cd209a	N	P/N	N	N	P	N	P/N	N	N	N/A	1.50	N/A	N/A	2.85	N/A	#N/A	N/A	N/A	N/A	N/A
MG/DC	Siglech	N	N	P/N	N	P/N	N	N	N	N	N/A	N/A	2.53	N/A	N/A	N/A	-0.88	N/A	-0.92	-0.83	-1.03
MAC	Ms4a7	N	P	N	N	N	N	N	N	N	N/A	2.28	N/A	N/A	N/A	N/A	#N/A	N/A	N/A	N/A	N/A
	Lyve1	N	P	N	N	N	N	N	N	N	N/A	1.43	N/A	N/A	N/A	N/A	#N/A	N/A	N/A	N/A	N/A
	Cbr2	N	P/N	N	N	N	N	N	N	N	N/A	1.81	N/A	N/A	N/A	N/A	#N/A	N/A	N/A	N/A	N/A
	Mrc1	N	P/N	N	N	N	N	N	N	N	N/A	2.35	N/A	N/A	0.29	N/A	#N/A	N/A	N/A	N/A	N/A
	CD163	N	P/N	N	N	N	N	N	N	N	N/A	1.08	N/A	N/A	N/A	N/A	#N/A	N/A	N/A	N/A	N/A
MG	Hexb	N	N	P	N	N	N	N	N	N	-1.85	-0.53	4.35	-2.30	-1.40	-2.35	-2.50	-2.23	-2.29	-2.20	-2.65
	Olfml3	N	N	P	N	N	N	N	N	N	-1.15	-0.59	2.98	-1.14	-1.14	-1.02	-1.21	-1.08	-1.25	-1.04	-1.39
	Sparc	N	N	P	N	N	N	N	N	N	-1.81	-1.49	1.75	-1.62	-1.59	-1.27	-1.76	N/A	N/A	-1.17	-1.55
	Tgfbr1	N	N	P/N	N	N	N	N	N	N	N/A	N/A	1.94	-0.47	-0.26	-0.42	-0.62	-0.54	-0.59	-0.36	-0.68
	P2ry12	N	N	P/N	N	N	N	N	N	N	-1.38	-0.72	3.48	-1.51	-1.31	-1.40	-1.60	-1.33	-1.58	-1.52	-1.78
	Tmem119	N	N	P/N	N	N	N	N	N	N	-1.19	-0.76	3.08	-1.19	-1.14	-1.17	-1.27	-1.16	-1.33	-1.22	-1.46
NEUT	Ltf	N	N	N	P/N	N	N	N	N	N	N/A	N/A	N/A	2.96	N/A	N/A	#N/A	N/A	N/A	N/A	N/A
	Ly6g	N	N	N	P	N	N	N	N	N	N/A	N/A	N/A	1.88	N/A	N/A	#N/A	N/A	N/A	N/A	N/A
	Mmp8	N	N	N	P/N	N	N	N	N	N	0.38	N/A	N/A	2.76	N/A	N/A	#N/A	N/A	N/A	N/A	N/A
	Camp	N	N	N	P/N	N	N	N	N	N	N/A	N/A	N/A	5.20	N/A	N/A	#N/A	N/A	N/A	N/A	N/A
	Ngp	N	N	N	P/N	N	N	N	N	N	N/A	N/A	N/A	5.19	N/A	N/A	#N/A	N/A	N/A	N/A	N/A
	Fcnb	N	N	N	P/N	N	N	N	N	N	N/A	N/A	N/A	0.60	N/A	N/A	#N/A	N/A	N/A	N/A	N/A
	Cebpe	N	N	N	P/N	N	N	N	N	N	N/A	N/A	N/A	1.08	N/A	N/A	#N/A	N/A	N/A	N/A	N/A
	Retnlg	N	N	N	P/N	N	N	N	N	N	N/A	N/A	N/A	6.01	N/A	N/A	#N/A	N/A	N/A	N/A	N/A
	S100a8	N	N	N	P	N	N	N	N	N	N/A	N/A	N/A	7.53	N/A	N/A	#N/A	N/A	N/A	N/A	N/A
	S100a9	N	N	N	P	N	N	N	N	N	N/A	N/A	N/A	7.18	N/A	N/A	#N/A	N/A	N/A	N/A	N/A
	Lcn2	N	N	N	P	N	N	N	N	N	N/A	N/A	N/A	4.00	N/A	N/A	#N/A	N/A	N/A	N/A	N/A
	G0s2	N	N	N	P	N	N	N	N	N	N/A	-0.55	-0.59	3.10	-0.46	-0.36	-0.49	-0.27	N/A	1.33	-0.31
	Wfdc21	N	N	N	P	N	N	N	N	N	N/A	N/A	N/A	4.12	N/A	N/A	#N/A	N/A	N/A	N/A	N/A

Figure 3

Representative results of cell type identification

Literatures * Our Crosstabulation					
		Our			Total
		A	B	C	
Literatures	A	73	1	0	74
	B	3	0	0	3
	C	1	0	5	6
Total		77	1	5	83

A: excellent
B: satisfactory
C: poor

Bowker's test			
	Value	Degree of freedom	Approximate significance (2-sided)
Bowker's test	2.000	2	.368
N of Valid Cases	83		

Symmetric Measures					
		Value	Asymptotic Standardized Error[a]	Approximate T[b]	Approximate Significance
Measure of Agreement	Kappa	.642	.146	7.200	.000
N of Valid Cases		83			

a. Not assuming the null hypothesis.
b. Using the asymptotic standardized error assuming the null hypothesis.

Figure 4

Bowker's test and Kappa symmetric measures of literatures and our results

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- TableS1Exceltemplatedesign.xlsx
- TableS2TheidentifyresultsofXimerakisetal..xlsx
- TableS3IdentifyofbrainfromHanetal..xlsx
- TableS4TheidentifyresultsofSankowskietal..xlsx
- TableS5TheidentifyresultsofMimounaetal..xlsx
- TableS6IdentifyofperipheralbloodfromHanetal..xlsx
- TableS7IdentifyofbonemarrowfromHanetal..xlsx