A Novel Murine Model of Differentiation-mediated Cytomegalovirus Reactivation from Latently Infected Bone Marrow Hematopoietic Cells

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SUPPORTING DATA

Table S1. Antibody panels for Immunophenotyping of M-CSF or GM-CSF differentiated BMCs

Fluorophores	Panel1 Myeloid and maturation markers	Panel2 Macrophages	Panel3 Dendritic cells(DCs)
FITC	CD40		CD24
PerCP-Cy5.5	MHCII	MHCII	MHCII
V500	FVD eFluor® 506	FVD eFluor® 506	FVD eFluor® 506
E450/BV421	CD115	CD135	mPDCA1
APC	CD86	CD11c	
AF700	CD45	CD45	CD45
APC-Cy7	CD11b	CD11b	
PE	CD64	CD115	
PE-CF594 (PE TxRed)	CD80		Lymph Dump
PE-Cy7	CD11c	MerK	CD11c
BUV395	F4/80		CD11b

Table S2. Antibody panels for bone marrow cells sorting

Fluorophores	Panel1, Lymphocytes	Panels, CD11b positive cells	Panel3 Stem cells and progenitor cells
BUV395	LD Blue	LD Blue	LD Blue
BV421	CD3e	CD115	CD135
BV510			CD127
FITC	CD3+CD19+CD49b	CD3+CD19+CD49b	CD3+CD19+CD49b
PerCP5.5	CD335 (NKp46)	Ly6G	Lin (Ter119, CD11c, CD3e, CD11b, Ly6G, CD335, CD19, MHCII)
PE		CD11c	CD45
PE TxRed	CD19 or B220	Siglec F	CD117
PC7		F4/80	CD244
APC		mPDCA1	CD150
A700	CD11b	CD11b	
AC7		Ly6C	CD48

Table S3. Antibodies used for Immunophenotyping of differentiated BMCs.

Antigen and Fluorophores	Clone	Vendor and catalog #	Volume/100ul
Panel1, Myeloid and maturation markers			
CD40-FITC		Biolegend, 124607	
I-A/I-E-PerCP-Cy5.5	M5/114.15.2	BD Biosciences, 562363	0.5
CD115-BV421	AFS98	Biolegend, 135513	2ul
CD86-APC	GL1	BD Biosciences, 561964	0.2ul
CD45- AlexaFluor700	30-F11	BD Biosciences, 560510	0.3ul
CD11b-APC-Cy7	M1/70	BD Biosciences, 557657	0.15ul
CD64-PE	X54-5/7.1	BD Biosciences, 558455	0.3ul
CD80-PE-CF594	16-10A1	BD Biosciences, 562504	0.5ul
CD11c-PE-Cy7	HL3	BD Biosciences, 558079	0.2ul
F4/80-BUV395	T45-2342	BD Biosciences, 565614	0.3ul
Panel2, Macrophages			
MHCII-PerCP-Cy5.5	M5/114.15.2	BD Biosciences, 562363	0.5
CD135-BV421	A2F10.1	BD Biosciences, 562898	2ul
CD11c-APC	N418	eBioscience, 17-0114-82	0.5ul
CD45- AlexaFluor700	30-F11	BD Biosciences, 560510	0.3ul
CD11b-APC-Cy7	M1/70	BD Biosciences, 557657	0.2ul
CD115-PE	T38-320	BD Biosciences, 565249	0.5ul
MerTK-PE-Cy7	DS5MMER	ThermoFisher, 25-5751	0.3ul
Lymph Dump for Panel3			
NK1.1-PE-CF594	PK136	BD Biosciences, 562864	0.3ul
CD3e-PE-CF594	145-2C11	BD Biosciences, 562332	0.6ul
CD19-PE-CF594	ID3	BD Biosciences, 562291	0.6ul
Ly6G-PE-CF594	1A8	BD Biosciences, 562700	0.3ul
SiglecF-PE-CF594	E50-2440	BD Biosciences, 562757	0.15ul
Panel3, Dendritic cells			
CD24-FITC	M1/69	BD Biosciences, 561777	2ul
MHCII-PerCP-Cy5.5	M5/114.15.2	BD Biosciences, 562363	0.5
mPDCA1-APC	JF05-1C2.4.1	Miltenyi BioTech, 130-102-829	1ul
Lymph Dump (See above)			1.95ul
CD11c-PE-Cy7	N418	ThermoFIsher, 25-0114-82	0.3ul
CD11b-BUV395	M1/70	BD Bioscience, 563553	0.3ul

Table S4. List of antibodies used in BMC sorting.

Antibody	Clone	Vendor and Catalog #	Volume/ 100ul
Panel1 for Lymphocytes (FITC positive cells)			
CD3e-BV421	145-2C11	BD Biosciences, 562600	0.5ul
CD19-PE-CF594	ID3	BD Biosciences, 562291	0.6ul
CD335(NKp46)-PerCP	29A1.4	eBioscience, 46-3351	0.5ul
CD11b-AlexaFluor700	M1/70	eBioscience, 56-0112-80	0.2ul
Panel2 for CD11b positive cells			
CD115-BV421	AFS98	Biolegend, 135513	2ul
Ly6G-PerCP-Cy5.5	IA8-Ly6G	eBioscience, 46-9668-82	0.5ul
CD11c-PE	N418	eBioscience, 12-0114-81	0.5ul
SiglecF-PE-CF594	E50-2440	BD Biosciences, 562757	0.2ul
F4/80-PE-Cy7	BM8	Biolegend, 123114	0.3ul
CD11b-AlexaFluor700	M1/70	eBioscience, 56-0112-80	0.2ul
Ly6C-APC-Cy7	AL-21	BD Biosciences, 560596	0.5ul
mPDCA1-APC	JF05-1C2.4.1	Miltenyi Biotec, 130-102-829	1ul
Panel3 for HSPCs (FITC and CD11b double negative cells)			
CD135-BV421	A2F10.1	BD Biosciences, 562898	2ul
CD127-BV510	A7R34	Biolegend, 135033	1.2ul
CD45-PE	30-F11	Biolegend, 103106	0.5ul
CD117-PE-CF594	2B8	BD Biosciences, 562417	0.5ul
CD244.2-PE-Cy7	M2B4(B6)458.1	Biolegend, 133512	1.2ul
CD150-APC	TC15-12	Biolegend, 115909	0.6ul
CD48-APC-Cy7	HM48	Biolegend, 103432	1ul
CD11b-AlexaFluor700	M1/70	eBioscience, 56-0112-80	0.2ul
Lineage markers-PerCP-Cy5.5	See below		4.0ul
Lineage Markers Cocktail			
TER119- PerCP-Cy5.5	TER-119	eBioscience, 45-5921	0.5ul
CD11b-PerCP-CY5.5	M1/70	eBioscience, 45-0112-82	0.5ul
CD11c-PerCP-Cy5.5	N418	eBioscience, 45-0114	0.5ul
CD3e- PerCP-Cy5.5	145-2C11	eBioscience, 45-0031	0.5ul
Ly6G- PerCP-Cy5.5	RB6-8C5	eBioscience, 45-5931	0.5ul
CD335-PerCP-eFluor710	29A1.4	eBioscience, 46-3351	0.5ul
CD19-PerCP-Cy5.5	eBioD3	eBioscience, 45-0193	0.5ul

I-A/I-E-PerCP-Cy5.5	M5/114.14.2	BD Biosciences, 562363	0.5ul

Supplemental Figure 1

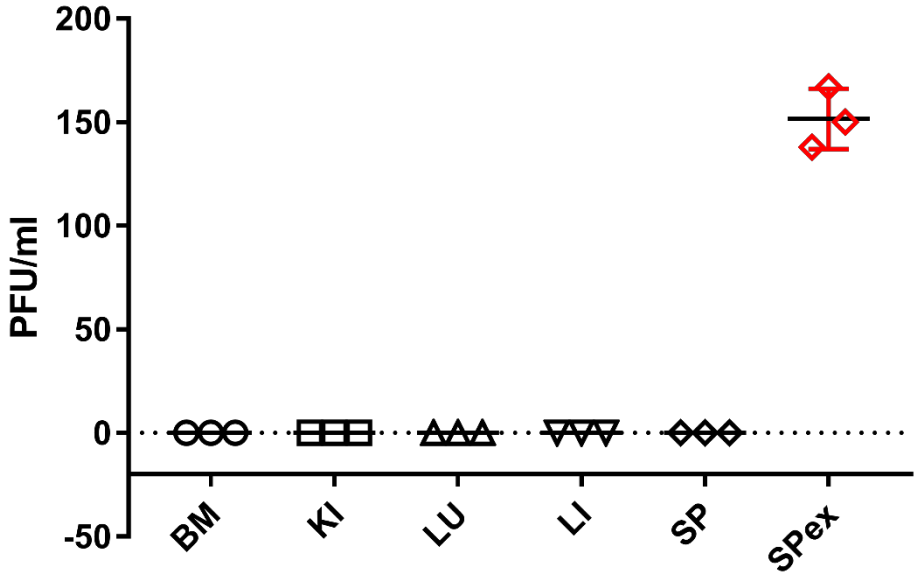

Figure S1. Latency is established in BALB/c mice 3 months post infection (p.i.)

Plaque assays were performed on mouse embryonic fibroblasts (MEFs) monolayer with the tissue lysate from the organs that were harvested from BALB/c mice 3months p.i. with Smith virus, and supernatant of explanted spleens from the same batch of mice. The data are presented as mean ± SD of PFU per ml of homogenate or supernatant. N=3. BM, Bone marrow cells; KI, Kidneys; LU, lungs; LI, livers, SP, spleens; SPex, spleen tissues harvested from BALB/c 3 months p.i. were explanted (cultured) for 18 days.

Supplemental figure 2

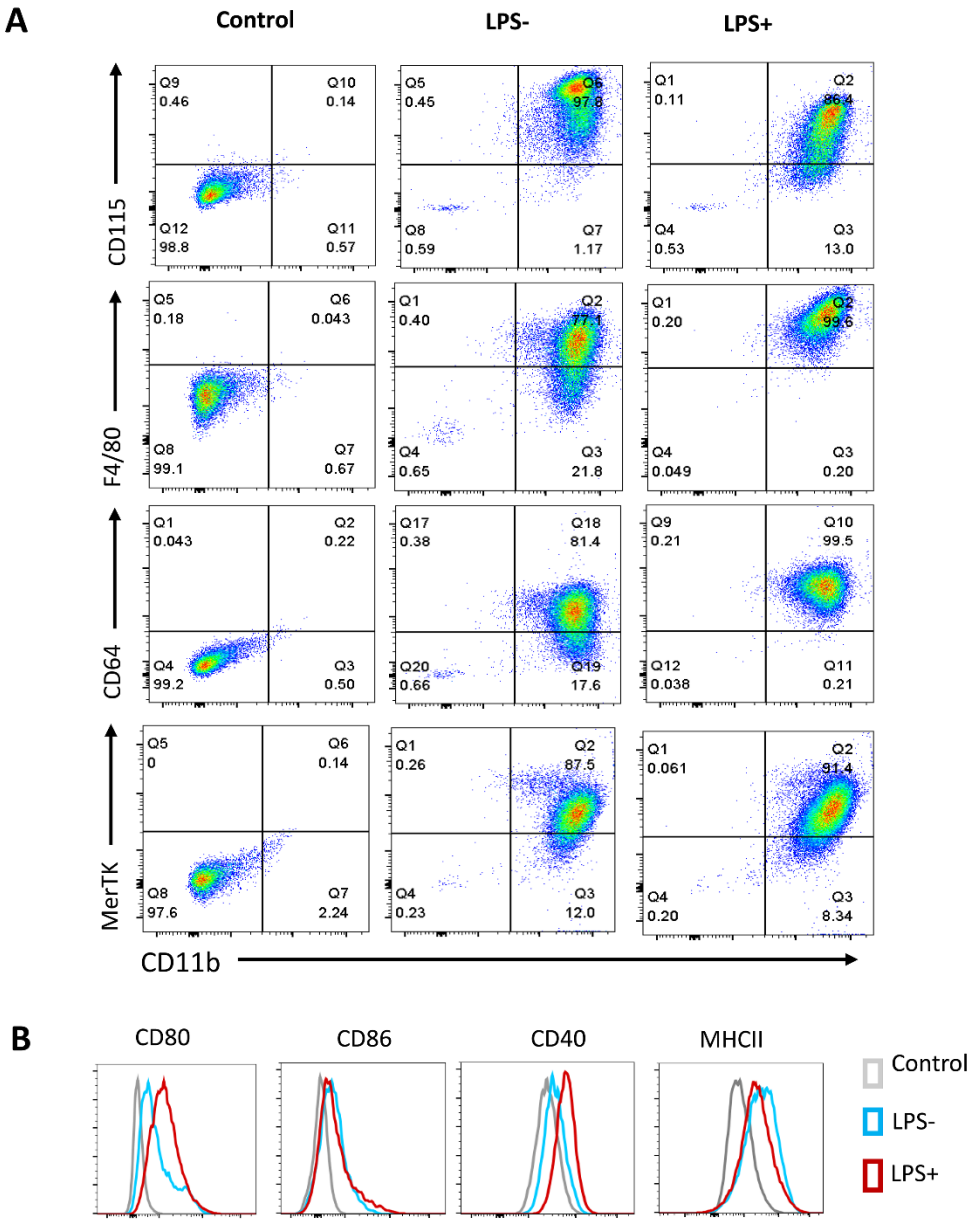

Figure S2. Immunophenotype of M-CSF differentiated BMCs.

(A). Phenotypes of representative M-CSF BMC culture in Day10 with or without LPS added in day9. The dot graphs show the surface marker expression on CD45+CD11b+ live cells.

(B). Maturation marker expression upon LPS treatment. The histograms show the indicated maturation marker expression in CD45+CD11b+F4/80+ cells.

Supplemental figure 3

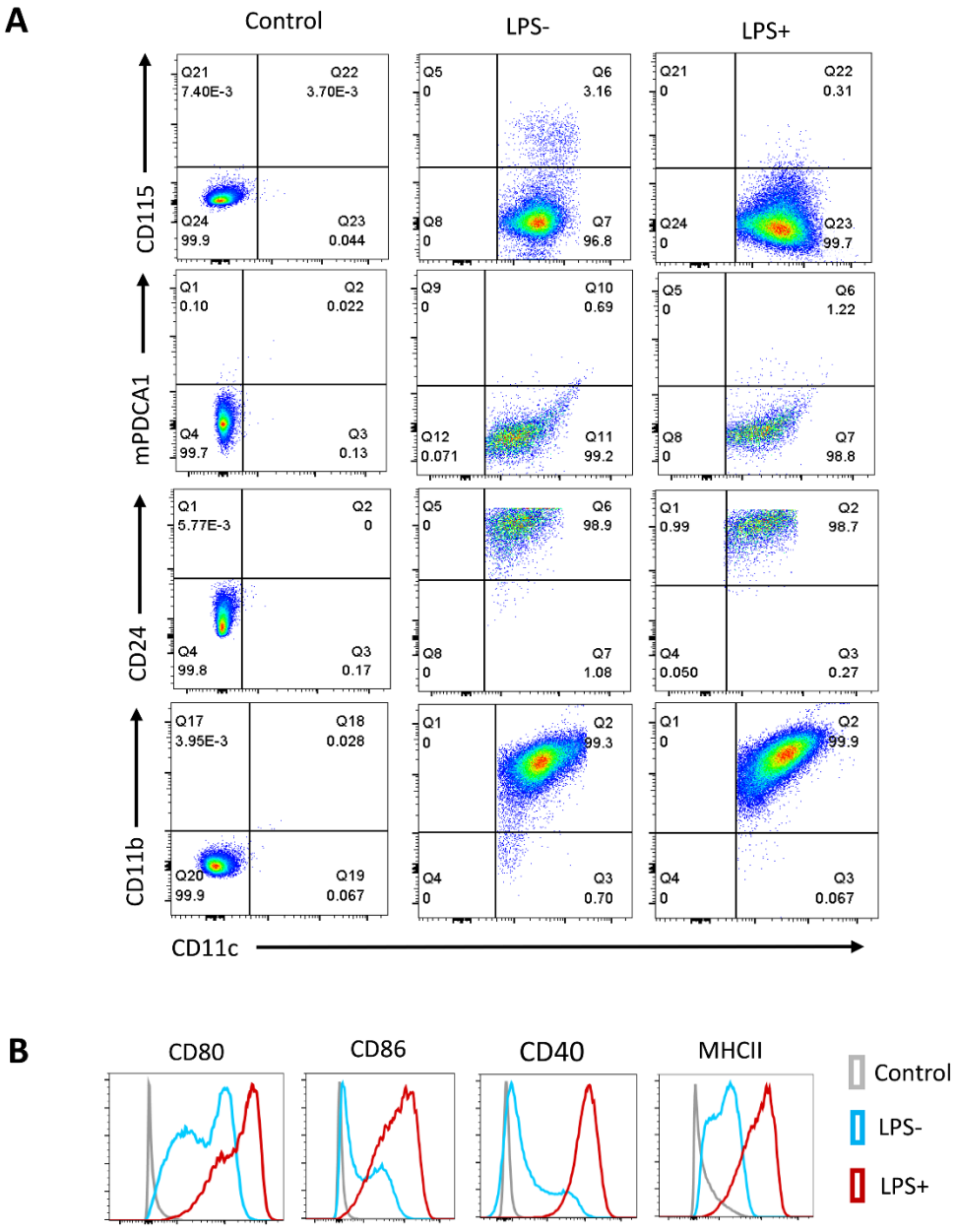

Figure S3. Immunophenotype of GM-CSF/IL-4 differentiated BMCs.

(A). Phenotype of representative GM-CSF/IL-4 BMC culture at day10 with or without LPS added at day9. The dot graphs show the indicated surface marker expression in CD45+ Lymph Dump-CD11c+ live cells.

(B). Maturation marker expression following LPS treatment. The histograms show the indicated maturation marker expression in CD45+ Lymph Dump-CD11c+ cells.

Supplemental figure 4

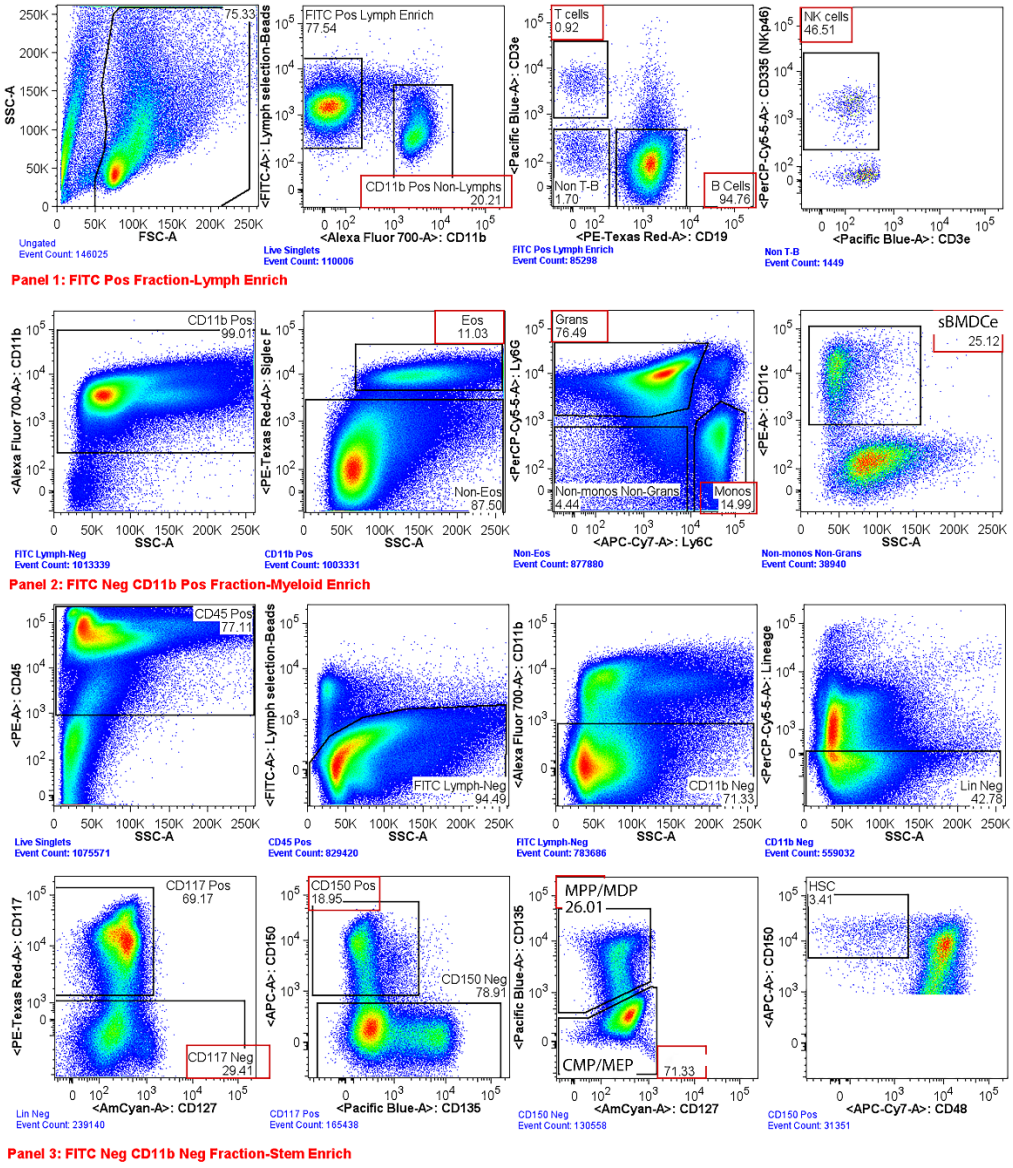

Figure S4. Gating strategy FITC/CD11b selected BMCs.

In **Panel 1**(top), three population of FITC+ CD11b- lymphoid cells were sorted and collected including T-cells (CD3e+CD19-), B-cells (CD3e- CD19+), and NK cells (CD3e- CD19- CD335+). In **Panel 2** (middle), four populations of FITC- CD11b+ cells were sorted and collected, including Eos (SiglecF+), Grans (SiglecF- Ly6G+), Monos (SiglecF- Ly6C+), and sBMDCe (SiglecF- Ly6G- Ly6C- CD11c+). In **Panel3** (bottom), three populations of FITC- CD11b- CD45+ Lin- CD117+ cells were sorted and collected, including HSC (CD150+CD135-CD48-), MPP/MDP/ (CD150- CD127- CD135+), and CMP/MEP (CD150- CD127- CD135-). Abbreviation: Eos: Eosinophil; Grans: granulocytes; Monos: Monocytes; sBMDCe: sorted bone-marrow dendritic cell-enriched fraction; HSC: hematopoietic stem cells; CMP: common myeloid progenitor cells; MDP: macrophage-DC progenitor; MEP: megakaryocytic-erythroid progenitors: MPP: multipotent progenitor cell.

Supplemental figure 5

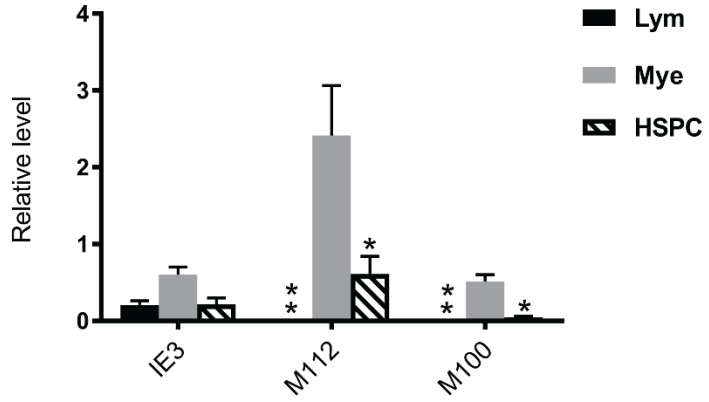

Figure S5. MCMV RNA expression in different populations of BMCs 5 days p.i.

BMCs from 5 BALB/c mice at 5 days p.i. with Smith virus were pooled and subjected to FITC bead separation, and CD11b positive bead separation successively resulting into three populations: FITC positive (lymphoid cells), FITC negative CD11b positive (Myeloid cells), and FITC negative CD11b negative (HSPCs). RNA extracted from these three populations of cells, was subjected to RT-qPCR with primers and probes specific to viral gene IE3, M112, and M100 respectively. Results are presented as the Mean ± SD after normalization against cellular gene Eef2 RNA expression. N=4 for all populations of cells. Multiple individual student t tests were performed to determine the difference of viral gene expression in different populations of cells. *P≤ 0.05; **P≤ 0.01. Lym: lymphoid cells; Mye: Myeloid cells; HSPC: hematopoietic stem cells and progenitor cells.