SUPPLEMENTARY DATA

Systematic Study of Immune Cell Diversity in ischemic postconditioning Using High-Dimensional Single-Cell Analysis with Mass Cytometry

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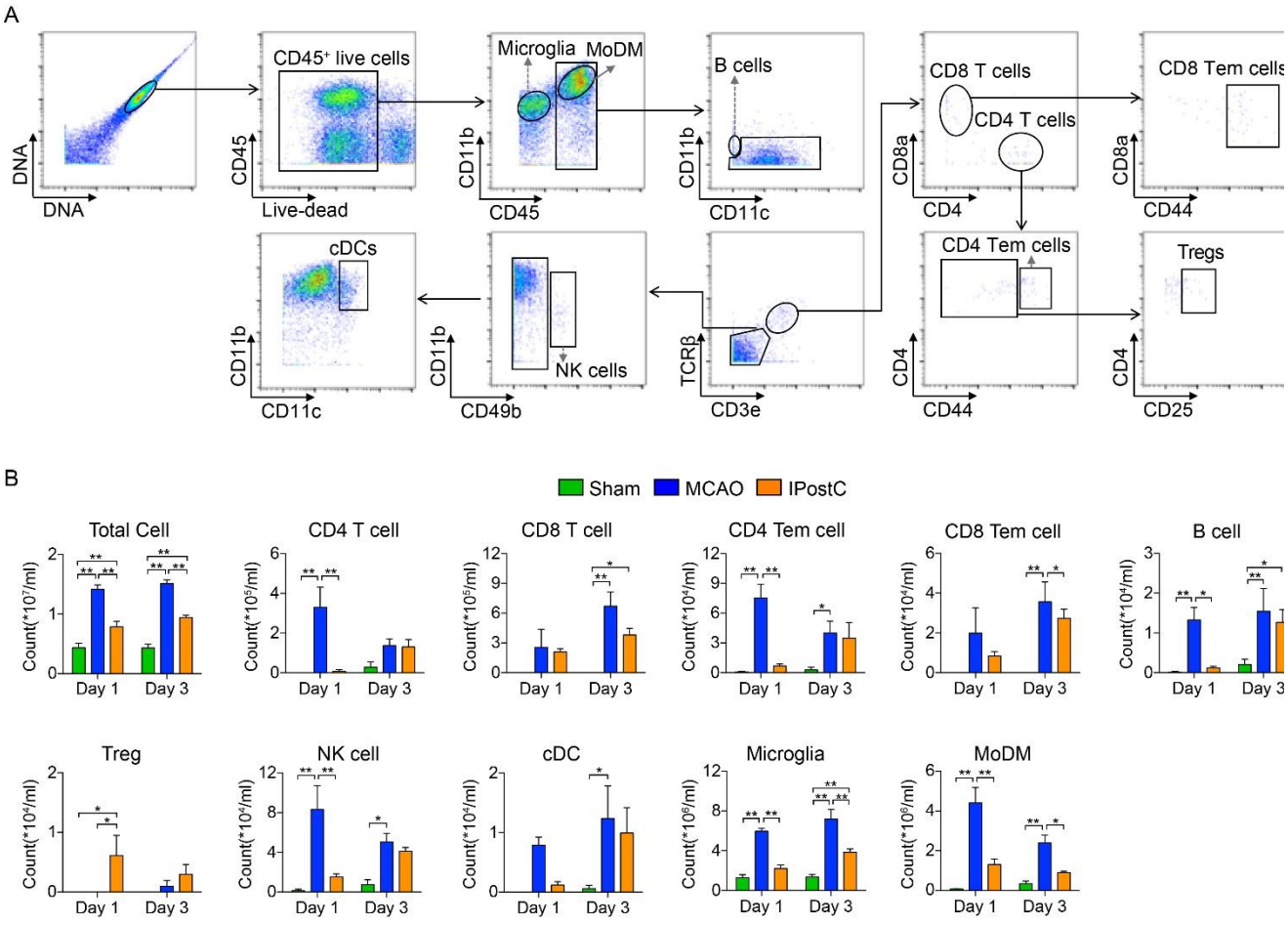

Supplementary Figure 1. Bar graphs show the percentage of cell populations identified in the viSNE clustering at day 1 after stroke. Data are presented as Mean ± SD, and dots on the bars represent individual samples. MoDMs, monocyte-derived macrophages; CD4 Tem/ CD8 Tem cell, effector memory CD4 and CD8 T cells. Treg, regulatory T cells; cDC, conventional dendritic cells; NK cells, natural killer cells. n=5/group. *, **, P<0.05, 0.01, respectively, between the two indicated groups.

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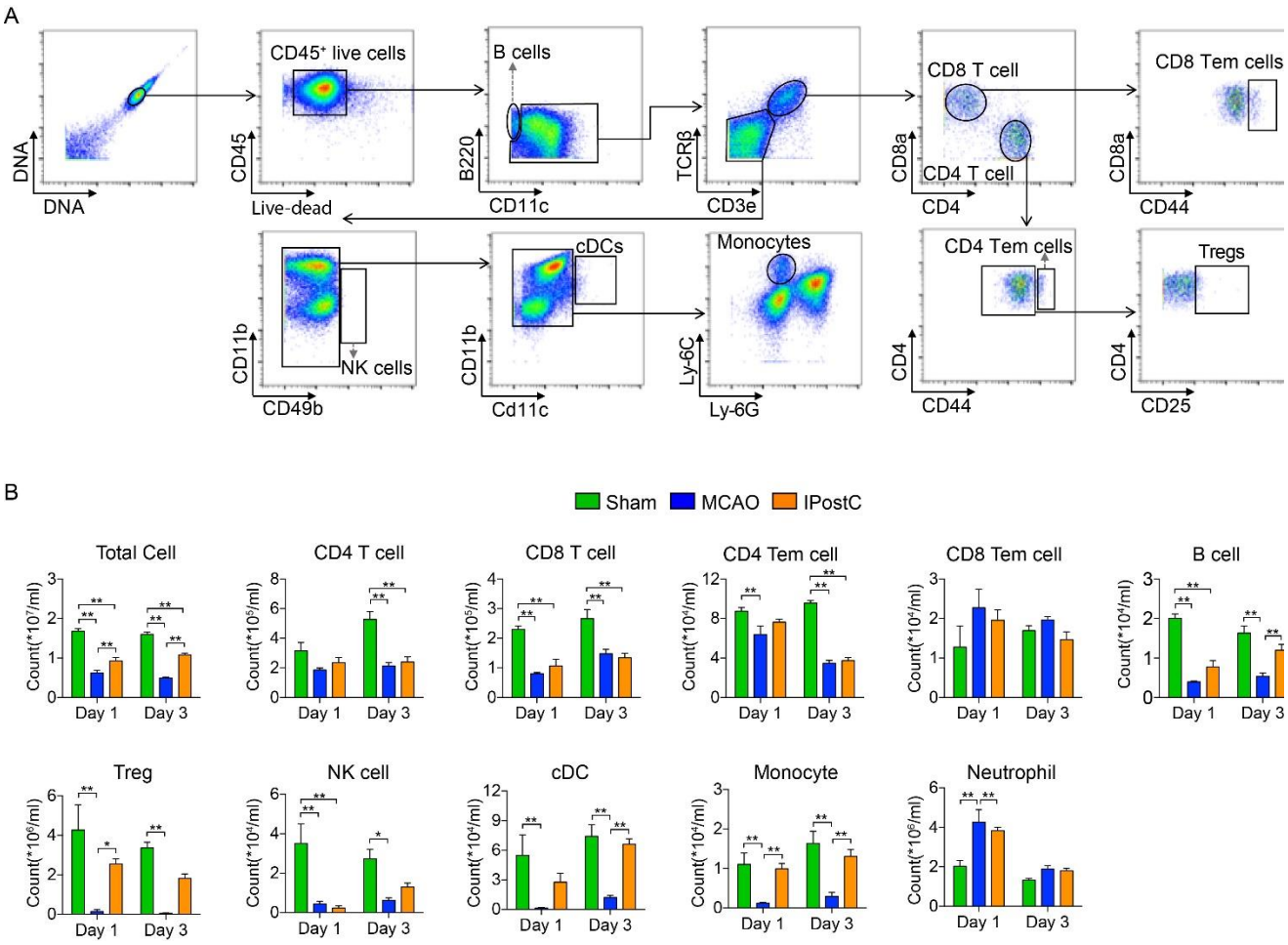

Supplementary Figure 2. Analysis of inflammatory cells in the ischemic brain. (A) Manual gating strategies for diverse immune cell types in samples of the ipsilateral ischemic hemisphere 3 days after stroke. (B) Quantitative data of the total number of immune cells and their subtypes were identified from manual gating strategy.

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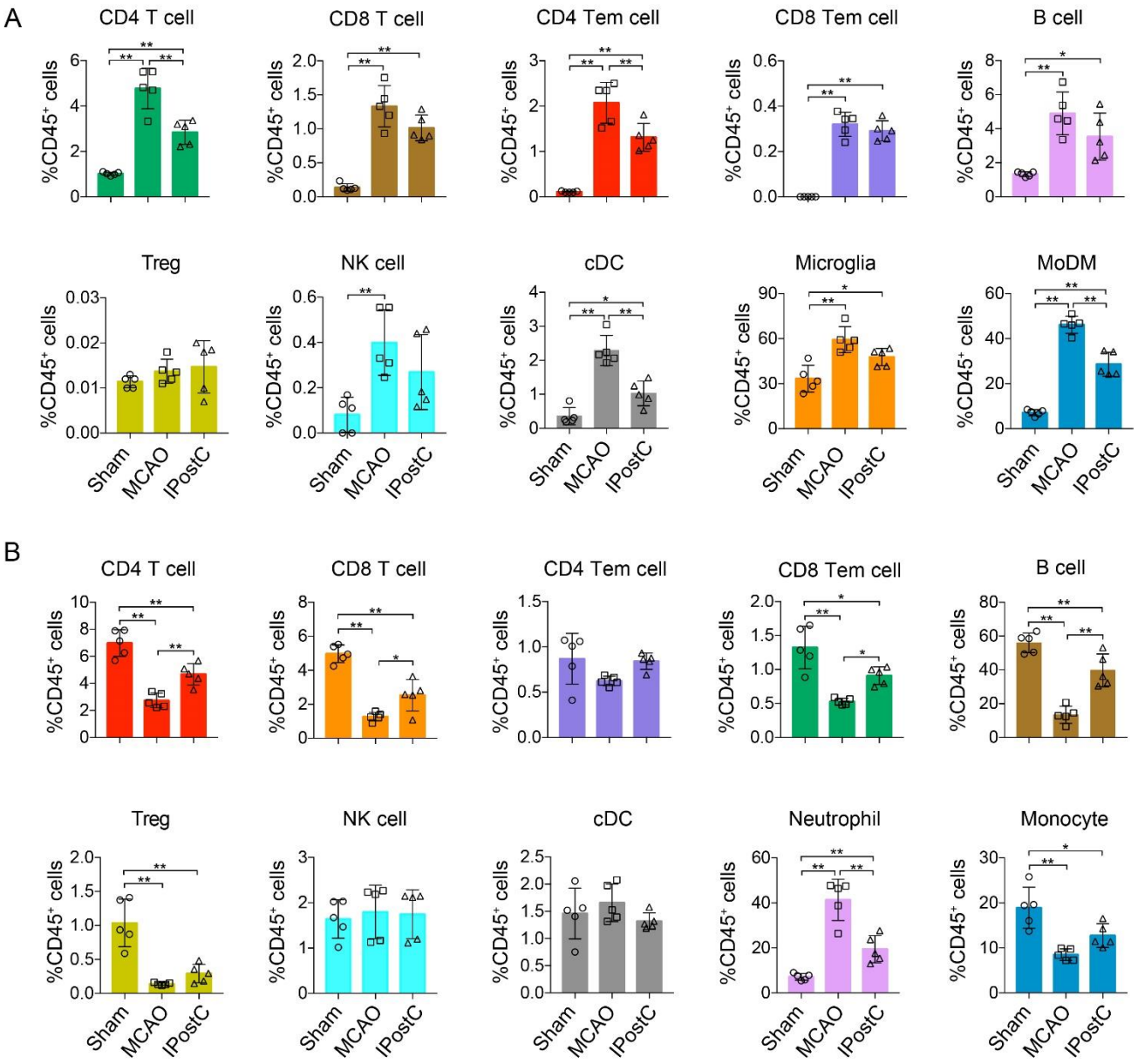

Supplementary Figure 3. Analysis of peripheral blood leukocytes after stroke. (A) Manual gating strategies for immune cell subsets in peripheral blood samples collected 3 days after stroke. (B) Quantitative data of the total number of immune cells and their subtypes were identified from manual gating strategy.

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Supplementary Table 1. The panel of Cytof antibody.

CyTOF antibody panel. The full metal-conjugated antibody panel. Used for CyTOF experiments. Amount of each antibody is referred to one sample with $3*10^6$ cells, at a 100μl final staining volume.

Metal isotope	Antibody	Host	Amount(μl)	Clone	Source	Cat.#
141Pr	Ly6G	Mouse	0.5	1A8	Fluidigm	3141005B
142Nd	CD11c	Mouse	0.5	N418	Fluidigm	3142003B
143Nd	CD357	Mouse	0.5	DTA1	Fluidigm	3143019B
145Nd	CD45RB	Mouse	0.5	C363.16A	Fluidigm	3145012B
147Sm	CD45	Mouse	0.5	30-F11	Fluidigm	3147003B
148Nd	CD11b	Mouse	0.5	M1/70	DVS Science	3148003B
150Nd	CD44	Human/Mouse	0.5	IM7	DVS Science	3150018B
151Eu	CD25	Mouse	0.5	3C7	Fluidigm	3151007B
154Sm	CD48	Mouse	0.5	HM48-1	DVS Science	3154004B
159Tb	F4/80	Mouse	0.5	BM8	DVS Science	3159009B
162Dy	Ly6C	Mouse	0.5	HK1.4	Fluidigm	3162014B
163Dy	CD54	Mouse	0.5	YN1/1.7.4	Fluidigm	3163020B
165Ho	CD3e	Mouse	0.5	145-2C11	DVS Science	3165020B
168Er	CD8a	Mouse	0.5	53-6.7	Fluidigm	3168003B
169Tm	TCRβ	Mouse	0.5	H57-597	Fluidigm	3169002B
170Er	CD49b	Mouse	0.5	HMa2	DVS Science	3170008B
172Yb	CD4	Mouse	0.5	RM4-5	Fluidigm	3172003B
176Yb	CD45R/B220	Human/Mouse	0.5	RA3-682	DVS Science	3176002B
144Nd	pPLCg2	Mouse	0.5	K86-689.37	DVS Science	3144015A
171Yb	pERK1/2	Mouse	0.5	D13,14,4E	Fluidigm	3171010A
146Nd	pEGFR	Mouse	0.5	D7A5	Fluidigm	3146007A
156Gd	p-p38	Mouse	0.5	T180/Y182	Fluidigm	3156002A
153Eu	pStat1	Mouse	0.5	4a	Fluidigm	3153005A
174Yb	pStat4	Mouse	0.5	38	DVS Science	3174005A
158Gd	pStat3	Mouse	0.5	4	Fluidigm	3158005A
152Sm	pAKT	Mouse	0.5	D9E	Fluidigm	3152005A
149Sm	p4E-BP1	Mouse	0.5	23684	Fluidigm	3149005A
175Lu	pS6	Mouse	0.5	S235/S236	DVS Science	3175009A
155Gd	IRF-4	Human/Mouse	0.5	3E4	Fluidigm	3155014B
173Yb	IRF-5	Mouse	0.5	10T1	Fluidigm	Not applicable
161Dy	iNOS	Mouse	0.5	CXNFT	Fluidigm	3161011B
166Er	Arg-1	Mouse	0.5	Polyclonal	Fluidigm	3166023B
160Gd	Tbet	Human/Mouse	0.5	4B10	Fluidigm	3160010B
167Er	Gata3	Human/Mouse	0.5	TWAJ	Fluidigm	3167007A
164Dy	IkBa	Mouse	0.5	L35A5	Fluidigm	3164004A

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Supplementary Table 2. Identified immune cell types and their identifying markers.

Ischemic brain		**Peripheral blood**	
Cell Types	**Defined Markers**	**Cell Types**	**Defined Markers**
Microglia	$CD45^{low}CD11b^{+}$	Monocytes	$CD45^{+}Ly6C^{+}Ly6G^{-}$
Monocyte derived macrophages (MoDM)	$CD45^{high}CD11b^{+}$	B cells	$CD45^{+}B220^{+}$
B cells	$CD45^{high}B220^{+}$	CD4 T cells	$CD45^{+}TCR\beta^{+}CD3^{+}CD4^{+}$
CD4 T cells	$CD45^{high}CD3^{+}CD4^{+}$	CD8 T cells	$CD45^{+}TCR\beta^{+}CD3^{+}CD8^{+}$
CD8 T cells	$CD45^{high}CD3^{+}CD8^{+}$	CD4 Tem cells	$CD45^{+}TCR\beta^{+}CD3^{+}CD4^{+}CD44^{+}$
CD4 Tem cells	$CD45^{high}CD3^{+}CD4^{+}CD44^{+}$	CD8 Tem cells	$CD45^{+}TCR\beta^{+}CD3^{+}CD8^{+}CD44^{+}$
CD8 Tem cells	$CD45^{high}CD3^{+}CD8^{+}CD44^{+}$	Tregs	$CD45^{+}TCR\beta^{+}CD3^{+}CD4^{+}CD25^{+}$
Tregs	$CD45^{high}CD3^{+}CD4^{+}CD25^{+}$	NK cells	$CD45^{+}CD3^{-}CD49b^{+}$
NK cells	$CD45^{high}CD3^{-}CD49b^{+}$	cDC	$CD11b^{+}CD11c^{+}$
cDC	$CD11b^{+}CD11c^{+}$		

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Supplementary Table 3. Changed endogenous functional immune features in different cell types.

Ischemic Brain				
CD4 T cell	**CD8 T cell**	**CD4 Tem cell**	**B cell**	**cDC**
p-P38 p-4E-BP1 p-EGFR p-AKT IκBα	p-P38 p-ERK1/2	p-PLCg2	p-EGFR	p-4E-BP1 p-EGFR IκBα Arg-1 IRF4
Microglia	**MoDM**			
p-4E-BP1 p-P38 p-ERK1/2 IκBα iNOS	p-4E-BP1 p-P38 p-EGFR p-PLCg2 Arg-1 IκBα			
Peripheral Blood				
CD4 T cell	**CD8 T cell**	**CD4 Tem cell**	**CD8 Tem cell**	**B cell**
p-P38 p-STAT1 p-STAT3 IRF4 iNOS	p-EGFR p-S6 iNOS	p-P38 p-STAT1 p-STAT3 p-STAT4 iNOS IRF4	p-P38 p-STAT3 p-STAT4 p-ERK1/2 p-EGFR p-S6 IRF4	p-P38 p-S6 iNOS
Treg	**NK cell**	**cDC**	**Monocyte**	
p-P38 p-ERK1/2 p-PLCg2 p-STAT4	p-P38 p-EGFR p-STAT1 p-STAT4 p-S6	p-P38 IκBα	p-P38 p-PLCg2 Arg-1 IRF4	

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Supplementary Table 4. Changed cell types in different endogenous functional immune features.

Ischemic Brain	
p-4E-BP1	CD4 T cell, CD8 T cell, cDC, Microglia, Monocyte
p-P38	CD4 T cell, Microglia, Monocyte
p-EGFR	CD4 T cell, B cell, cDC, Monocyte
p-PLCg2	CD4 Tem cell, Monocyte
p-ERK1/2	CD8 T cell, Microglia
p-AKT	CD4 T cell
IκBα	CD4 T cell, cDC, Microglia, Monocyte
iNOS	Microglia
Arg-1	cDC, Monocyte
IRF4	cDC
Peripheral Blood	
p-P38	CD4 T cell, CD4 Tem cell, CD8 Tem cell, B cell, Treg, NK cell, cDC, Monocyte
p-STAT3	CD4 T cell, CD4 Tem cell
p-STAT4	CD4 Tem cell, CD8 Tem cell, Treg, NK cell
p-PLCg2	Treg
p-ERK1/2	Treg
IκBα	cDC
p-S6	CD8 T cell, CD8 Tem cell, B cell, NK cell
p-ERK1/2	CD8 Tem cell
p-PLCg2	Monocyte
p-EGFR	CD8 T cell, CD8 Tem cell, NK cell
p-STAT1	CD4 T cell, CD4 Tem cell, NK cell
iNOS	CD4 T cell, CD8 T cell, CD4 Tem cell, CD8 Tem cell, B cell
Arg-1	Monocyte
IRF4	CD4 T cell, CD4 Tem cell, CD8 Tem cell, Monocyte