OMTM, Volume *30*

Supplemental information

iPSC-derived mesenchymal stem cells attenuate cerebral ischemia-reperfusion injury by inhibiting inflammatory signaling and oxidative stress

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Supplemental Materials

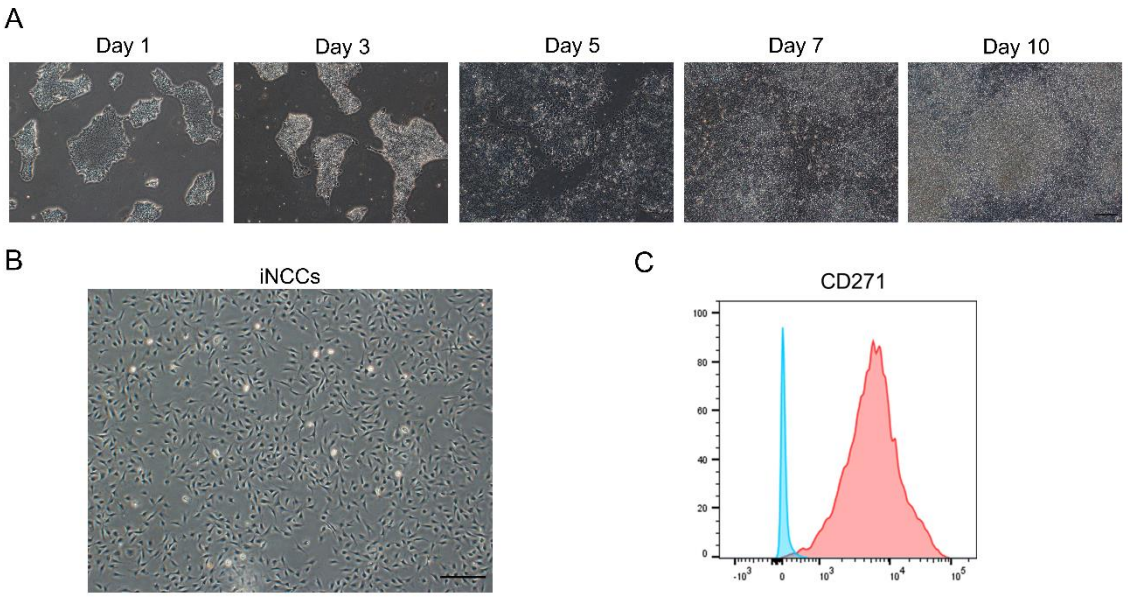


Figure S1. Morphological changes in the induction of iPSCs to iNCCs and characterization of iNCCs

(**A**) Morphological changes of iPSCs during iNCC differentiation (Days 1, 3, 5, 7, and 10.) Scale bar = 200 μm. (**B**) Cell morphology of iNCCs. Scale bar = 200 μm. (**C**) Surface marker analyses of iNCCs using flow cytometry. The blue histogram represents the isotype control and the red overlay represents each antigen. NCC positive marker CD271 was measured.

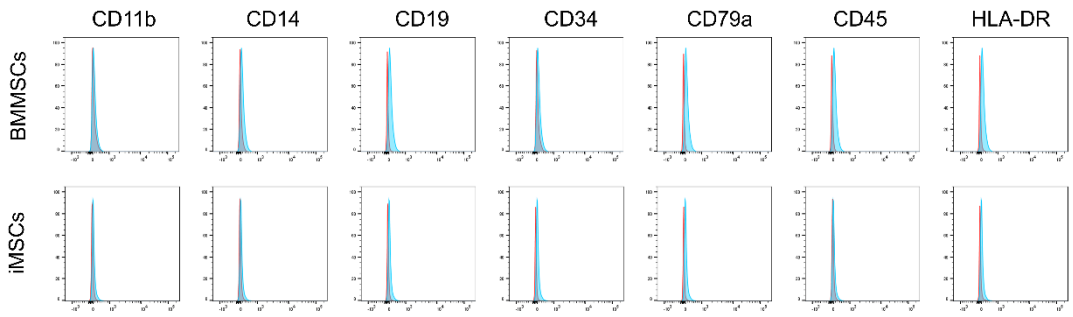


Figure S2. Surface marker analyses of BMMSCs and iMSCs

Surface marker analyses of BMMSCs (top) and iMSCs (bottom) using flow cytometry. The blue histogram represents the isotype control and the red overlay represents each antigen. MSC negative markers CD45, CD34, CD19, CD14, CD11b, CD79a, and HLA-DR were measured.

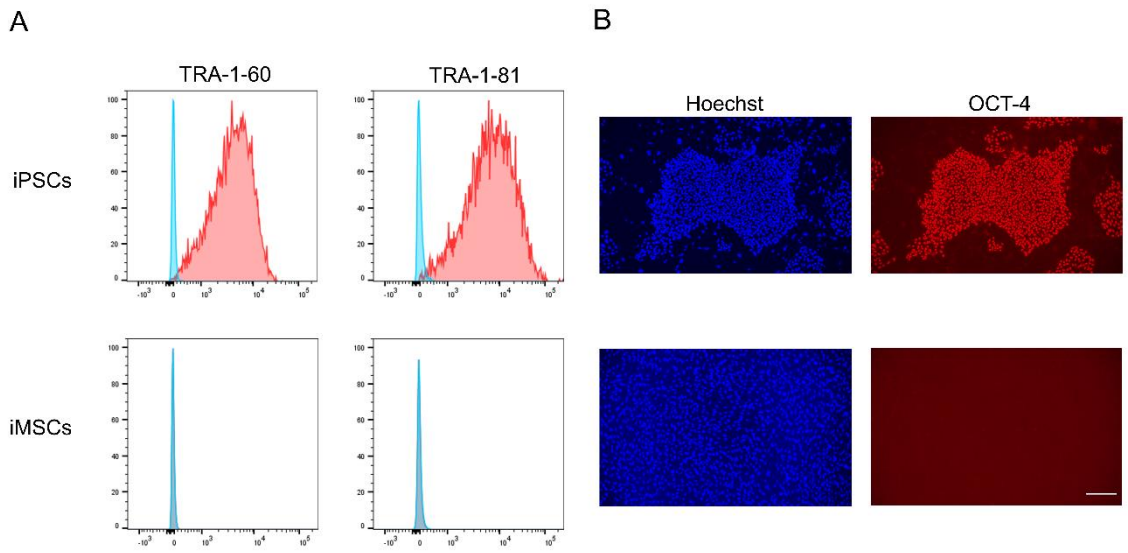


Figure S3. Surface and intracellular marker analyses of iPSCs and iMSCs

(**A**) Surface marker analysis of the pluripotency markers TRA-1-60 and TRA-1-81 in iPSCs and iMSCs using flow cytometry. The expression of TRA-1-60 (left) and TRA-1-81 (right) in iPSCs (top) and iMSCs (bottom) is shown. (**B**) Oct4 expression was confirmed by immunostaining. Hoechst (left) and Oct4 staining (right) of iPSCs (top) and iMSCs (bottom) are shown. Scale bar = 200 μm.

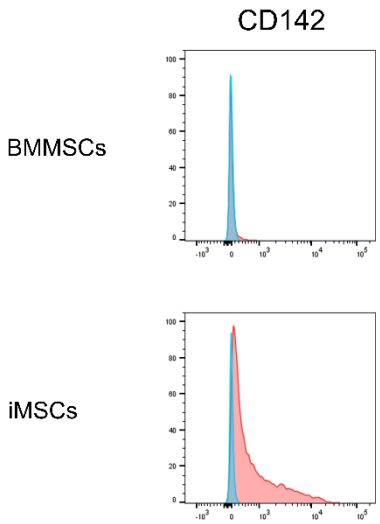
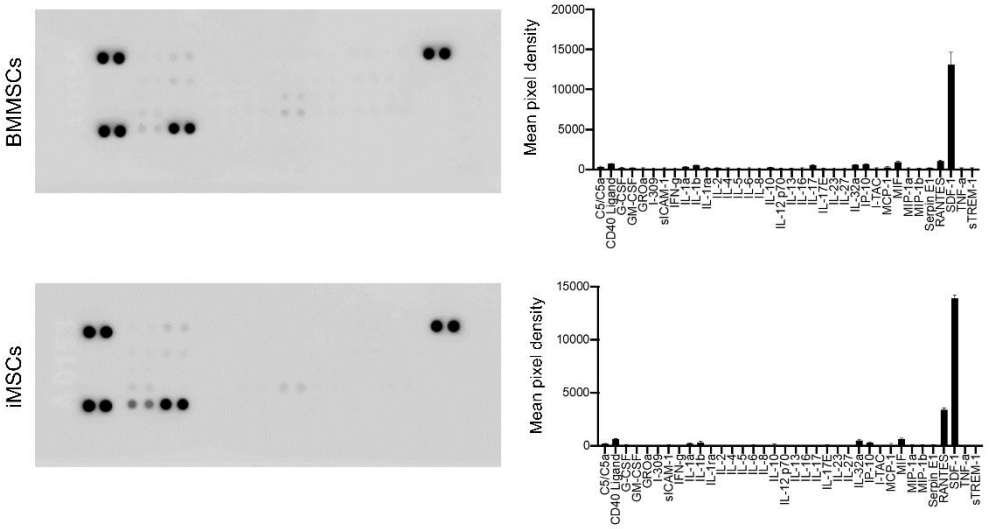


Figure S4. Evaluation of CD142 expression in BMMSCs and iMSCs

Surface marker analysis of BMMSCs and iMSCs using flow cytometry. The blue histogram represents the isotype control and the red overlay represents each antigen. Surface marker analysis of CD142 in BMMSCs (top) and iMSCs (bottom).

A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GM-CSF
GRO
GRO a/b/g
I-309
sICAM-1
IFN-g
IL-1a
IL-1b
IL-1ra
IL-2
IL-4
IL-5
IL-6
IL-8
IL-10
IL-12 p70
IL-13
IL-15
IL-17
IL-17E
IL-23
IL-27
IL-32a
IP-10
I-TAC
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MIF
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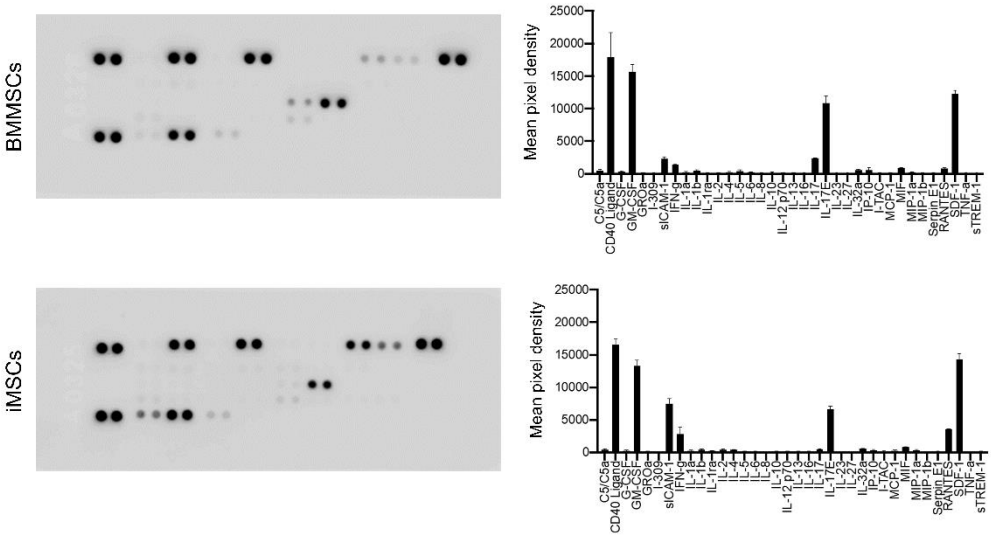
Serpin E1
RANTES
SDF-1
TNF-a
sTREM-1

B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iMSCs
Mean pixel density
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GRO a/b/g
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IFN-g
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C

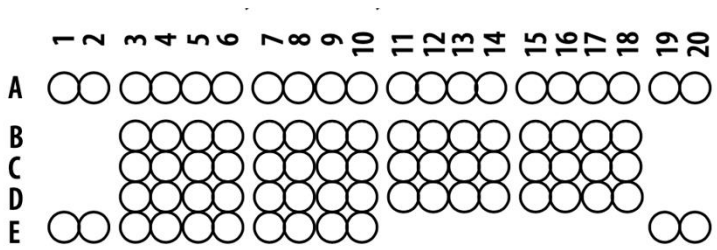
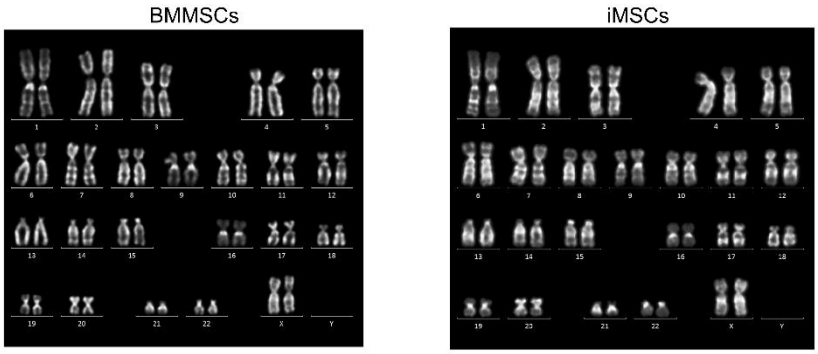

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
A
B
C
D
E

Figure S5. Comparison of secreted factors in BMMSCs and iMSCs

Cytokine array analysis of multiple cytokines secreted from BMMSCs (upper) and iMSCs (lower) with (B) or without (A) treatment with 10 ng/μL of TNF-α. The left panels show representative images of cytokine arrays, and the right panels show the profile of cytokine expression. Because cells were stimulated with TNF-α, data on TNF-α was excluded from the profiles of each cytokine array in Figure (B). (C) Coordinates of cytokine array panel. The cytokines corresponding to the coordinates are listed in Table S1.

A



B

	Chromosome number		Counted nucleus	Modal No.	4n nucleus	Modal karyotype
	45	46				
BMMSCs	2	18	20	46	1/50 (2%)	46,XX
iMSCs	2	18	20	46	6/50 (12%)	46,XX

Figure S6. BMMSCs and iMSCs karyotyping analysis

(A) Representative Q-band analysis results for BMMSCs (left) and iMSCs (right). Most clones showed normal karyotypes. **(B)** Summary of the karyotype analysis. The chromosome number was counted for 20 nuclei, and the tetraploid (4n) nucleus was counted for 50 nuclei for each sample.

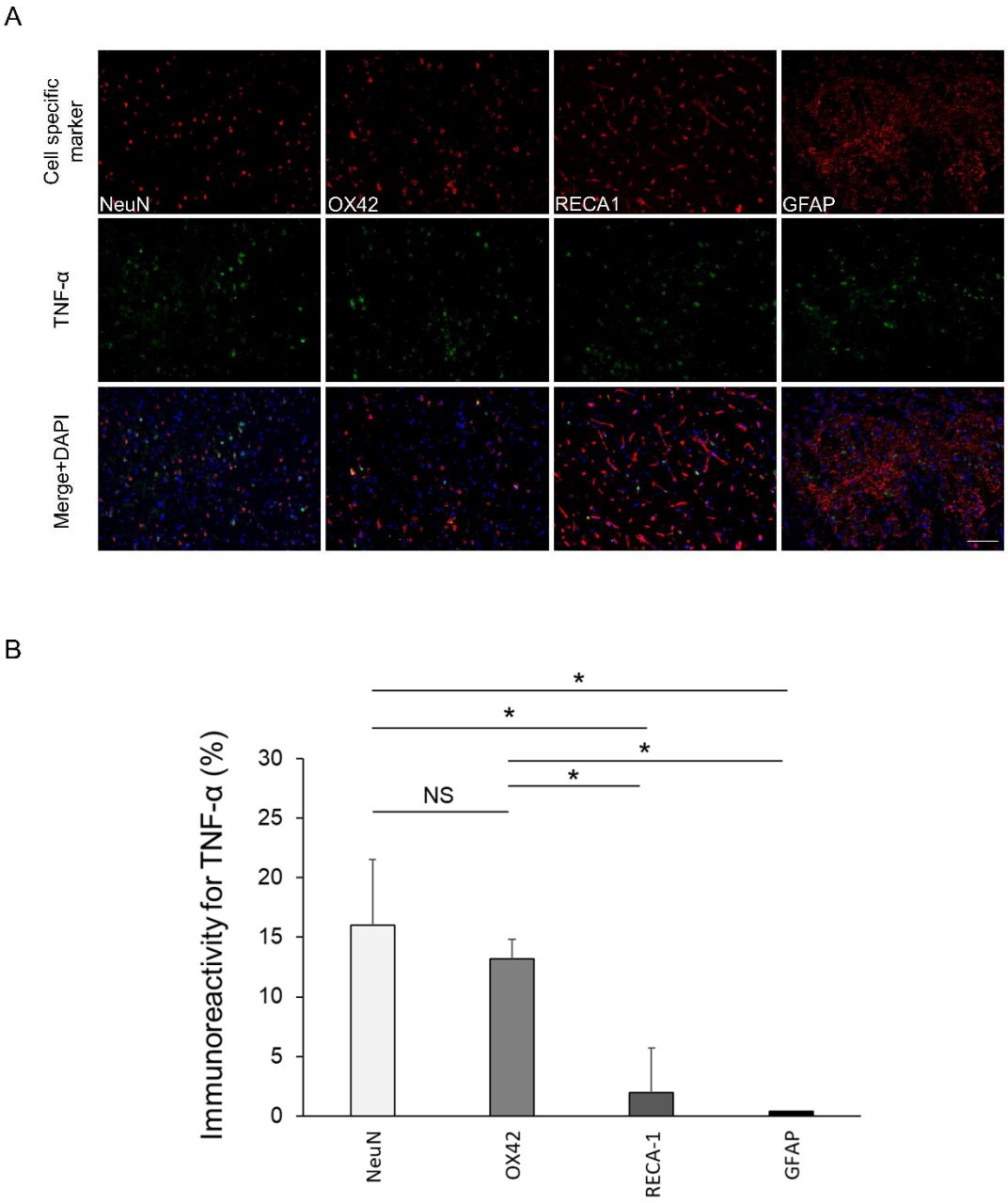


Figure S7. Identification of TNF-α positive cells in the ischemic side of the brain

(A) Immunostaining of neuron ($NeuN^+$, leftmost vertical column, red), microglial cells ($OX42^+$, second vertical column from left, red), endothelial cells ($RECA\text{-}1^+$, third vertical column from left, red), and astrocytes ($GFAP^+$, rightmost vertical column, red) in rat brain

sections. Rat brain sections were also immunostained with TNF-α (middle panels, green). Nuclei were stained in blue with DAPI. Merge overlays of three fluorescence images are shown in the bottom row. Scale bar = 100 μm. **(B)** Percentage of TNF-α^+ cells in the field of view (*$p < 0.01$; $NeuN^+$; n = 5, $OX42^+$; n=4, RECA-1^+; n=5, $GFAP^+$; n=4). NS, not significant.

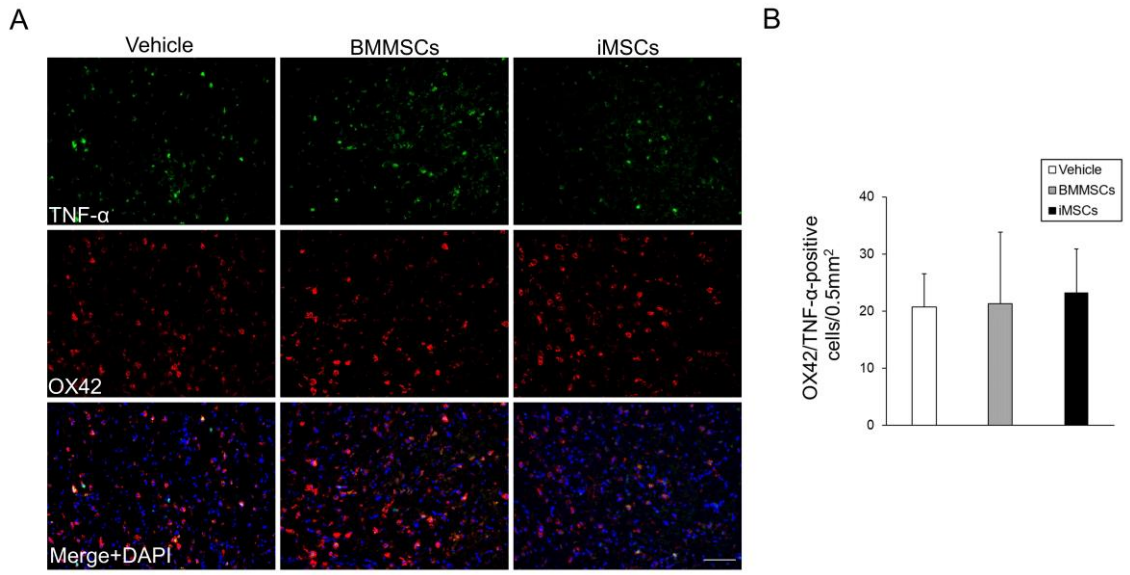


Figure S8. Identification of OX-42/TNF-α double-positive cells in rat brain

(A) Immunostaining of $OX42^{+}$ microglial cells (middle panels, red) with TNF-α (top panels, green) in rat brain sections treated with vehicle (left vertical column), BMMSCs (middle vertical column), and iMSCs (right vertical column). Nuclei were stained in blue with DAPI. Merge overlays of three fluorescence images are shown in the bottom row. Scale bar = 100 μm **(B)** Comparison of OX42/TNF-α-double-positive cells in the vehicle, BMMSC, and iMSC groups (n = 4 for each group).

Table S1. List of cytokine probes corresponding to the panel coordinates of the cytokine array

Coordinate	Target (Alternative name) or Control	Full name
A1, A2	Reference Spot	
A3, A4	C5/C5a	Complement component 5/5a
A5, A6	CD40ligand	Cluster of differentiation 40
A7, A8	G-CSF	Granulocyte colony-stimulating factor
A9, A10	GM-CSF	Granulocyte macrophage colony-stimulating factor
A11, A12	GROa	Growth-related oncogene a
A13, A14	I-309 (CCL1)	C-C Motif chemokine ligand 1
A15, A16	sICAM-1	soluble intercellular adhesion molecule-1
A17, A18	IFN-γ	Interferon-gamma
A19, A20	Reference Spot	
B3, B4	IL-1α	Interleukin-1alpha
B5, B6	IL-1β	Interleukin-1beta
B7, B8	IL-1ra	Interleukin 1 receptor antagonist
B9, B10	IL-2	Interleukin-2
B11, B12	IL-4	Interleukin-4
B13, B14	IL-5	Interleukin-5
B15, B16	IL-6	Interleukin-6
B17, B18	IL-8	Interleukin-8
C3, C4	IL-10	Interleukin-10
C5, C6	IL-12p70	Interleukin 12A and 12B
C7, C8	IL-13	Interleukin-13
C9, C10	IL-16	Interleukin-16
C11, C12	IL-17	Interleukin-17
C13, C14	IL-17E	Interleukin-17E
C15, C16	IL-23	Interleukin-23
C17, C18	IL-27	Interleukin-27
D3, D4	IL-32α	Interleukin-32alfa
D5, D6	IP-10 (CXCL10)	Interferon-gamma inducible protein 10kDa (C-X-C motif chemokine 10)
D7, D8	I-TAC (CXCL11)	IFN-inducible T-cell alpha

		chemoattractant (C-X-C motif chemokine 11)
D9, D10	MCP-1 (CCL2)	Monocyte chemoattractant protein-1 (C-C Motif Chemokine Ligand 2)
D11, D12	MiF	Mesoderm-inducing factor
D13, D14	MIP-1α (CCL3)	Macrophage inflammatory protein-1alfa (C-C Motif Chemokine Ligand 3)
D15, D16	MIP-1β (CCL4)	Macrophage inflammatory protein-1beta (C-C Motif Chemokine Ligand 4)
D17, D18	Serpin E1/PAI-1	Plasminogen activator inhibitor type 1
E1, E2	Reference Spot	
E3, E4	RANTES	Regulated on activation, normal T cell expressed and presumably secreted
E5, E6	SDF-1 (CXCL12)	Stromal cell-derived factor 1 (C-X-C motif chemokine 12)
E7, E8	TNF-α	Tumor necrosis factor-alfa
E9, E10	sTREM-1	Soluble factor triggering receptor Expressed on myeloid cells-1
E19, E20	Negative Control	