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Supplemental Information

Efficient conversion of human induced pluripotent stem cells into microglia by defined transcription factors

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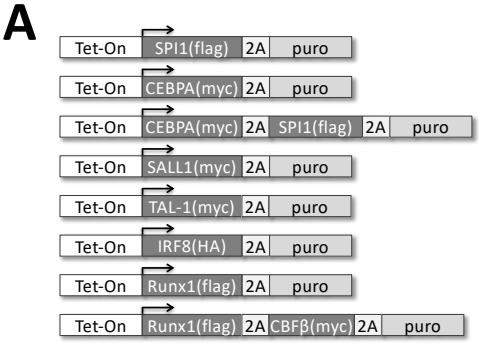
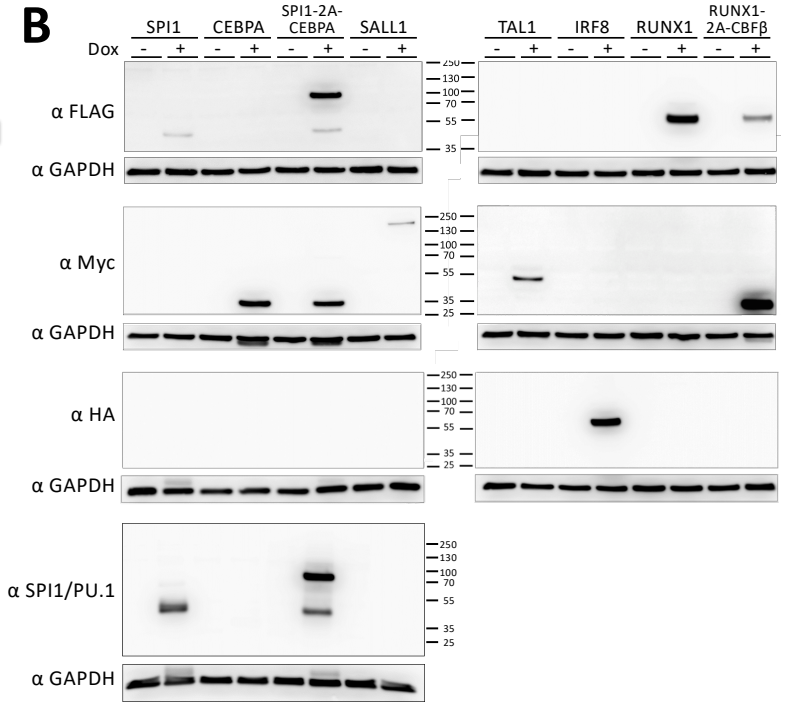
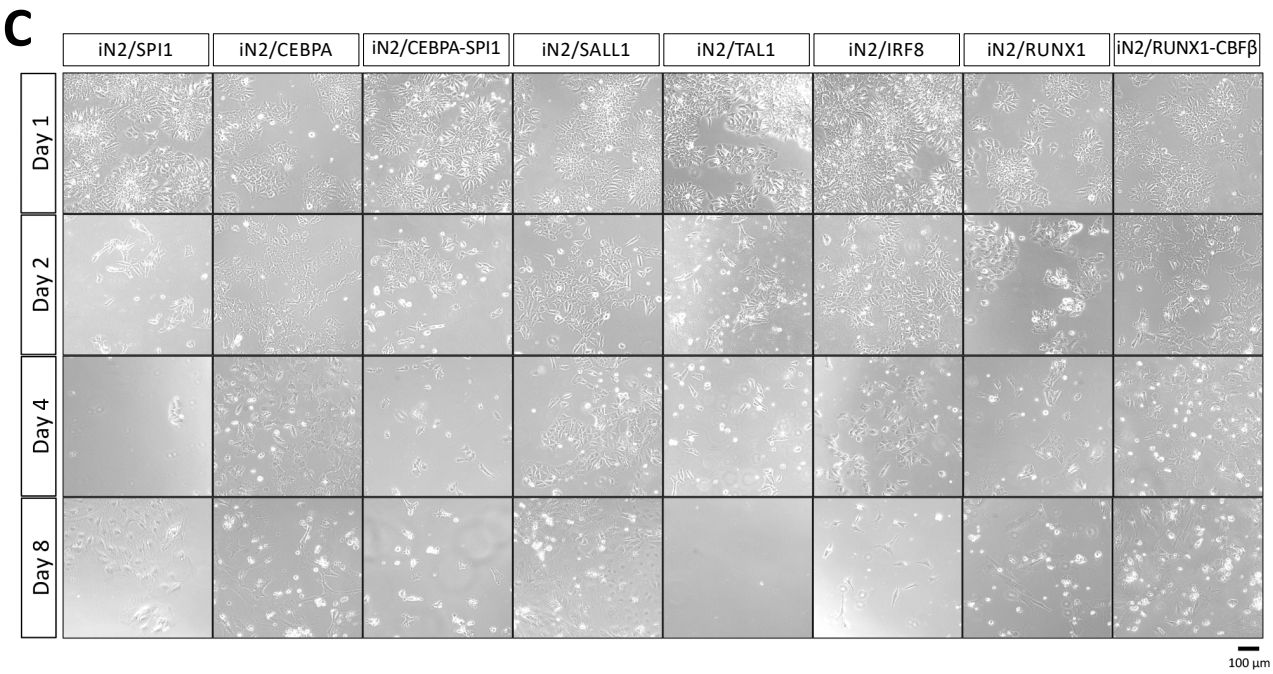


Predicted protein		Amino acids (a.a.)/ molecular weight (kD)#	
SPI1(flag)		305/35.0	
CEBPA(myc)		268/28.7	
CEBPA(myc)	SPI1(flag)	266/28.4	311/35.7
SALL1(myc)		1353/143.6	
TAL1(myc)		360/37.4	
IRF8(HA)		454/51.4	
RUNX1(flag)		507/54.8	
RUNX1(flag)	CBFβ(myc)	508/54.9	223/26.0

M.W. predicted by SnapGene software





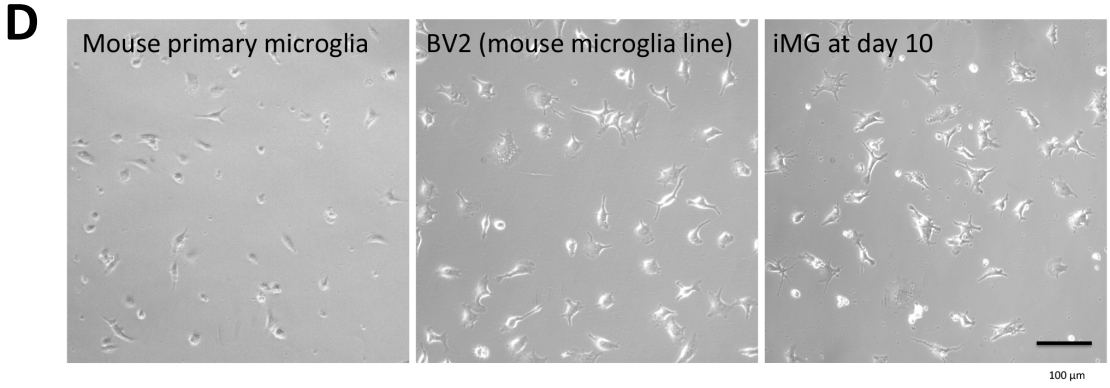


Figure S1. Induction of microglia-like cells from human iPSCs. Related to Figure 1.
(A) Eight constructs were constructed that expressed the candidate transcription factors involved in defining microglial cell fate during embryogenesis; the choice was based on a review of the literature. The expected molecular weights (MWs) of the expressed proteins are shown in right panels. (B) N2-iPSCs were transduced with lentivirus expressing both rtTA and one of the tetO promoter-driven pro-microglaial gene linked with puromycin resistant by T2A. Expression of each pro-microglial genes was induced using doxycyclin on day 0. Puromycin selection for 24 hr was started on day 1, and lysates of the surviving cells were harvested on day 3. These lysates were then subjected to Western blotting using anti-flag, myc or HA antibodies. GAPDH was used as a loading control. (C) Representative phase contrast images that display the morphological changes of N2-iPSCs after induction with the various different candidate genes on days 1, 2, 4 and 8. Scale bar, 100 μm. (D) Induced microglia on day 10 (right panel) show a microglia-like morphology that resembles that of mouse primary microglia (left panel) and the BV2 cell line (middle panel). Scale bar, 100 μm.

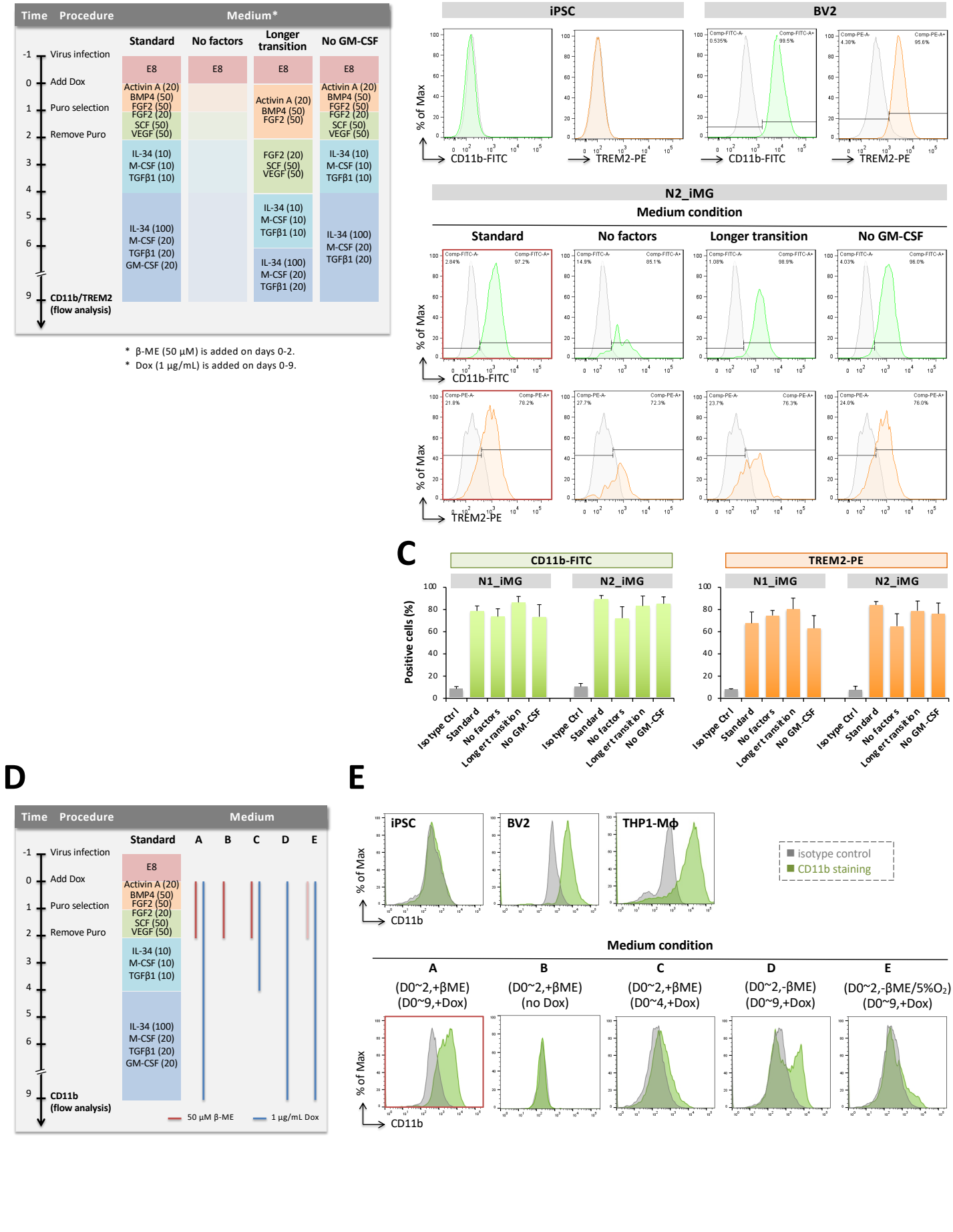

A
Time
Procedure
Medium*
Standard
No factors
Longer transition
No GM-CSF
-1
Virus infection
0
Add Dox
1
Puro selection
2
Remove Puro
3
4
5
6
9
CD11b/TREM2 (flow analysis)
E8
Activin A (20)
BMP4 (50)
FGF2 (50)
FGF2 (20)
SCF (50)
VEGF (50)
IL-34 (10)
M-CSF (10)
TGFβ1 (10)
IL-34 (100)
M-CSF (20)
TGFβ1 (20)
GM-CSF (20)
* β-ME (50 μM) is added on days 0-2.
* Dox (1 μg/mL) is added on days 0-9.
B
iPSC
BV2
% of Max
CD11b-FITC
TREM2-PE
N2_iMG
Medium condition
Standard
No factors
Longer transition
No GM-CSF
C
CD11b-FITC
TREM2-PE
N1_iMG
N2_iMG
Positive cells (%)
Isotype Ctrl
Standard
No factors
Longer transition
No GM-CSF
D
Time
Procedure
Medium
Standard
A
B
C
D
E
-1
Virus infection
0
Add Dox
1
Puro selection
2
Remove Puro
3
4
5
6
9
CD11b (flow analysis)
50 μM β-ME
1 μg/mL Dox
E
iPSC
BV2
THP1-Mϕ
% of Max
CD11b
isotype control
CD11b staining
Medium condition
A
(D0~2,+βME)
(D0~9,+Dox)
B
(D0~2,+βME)
(no Dox)
C
(D0~2,+βME)
(D0~4,+Dox)
D
(D0~2,-βME)
(D0~9,+Dox)
E
(D0~2,-βME/5%O2)
(D0~9,+Dox)

Figure S2. Identification of the basal media and growth factors required for induction and differentiation of microglia-like cells from human iPSCs.
(A) Various growth factors were tested to determine whether they brought about iMG cell induction and differentiation. The numbers in parenthesis indicate the concentration of human recombinant proteins used in ng/ml. (B) Flow cytometry analyses showing CD11b and TREM2 expression in cells grown in the four different medium listed in (A). Green, CD11b antibody conjugated to FITC; orange, TREM2 antibody conjugated to PE; gray, isotype control. (C) Quantification of the CD11b+ or TREM2+ positive cells present among N2-iMGs cultured in the different medium. Data are presented as means ± SEM (n = 4 batches of independent differentiation). (D) Various basal media, with different duration of treatment of β-mercaptoethanol (β-ME) and doxycycline (Dox), were tested to determine whether they brought about iMG cell induction and differentiation. The numbers in parenthesis indicate the concentration of human recombinant proteins used in ng/ml. (E) Flow cytometry analyses showing CD11b expression in cells grown in the five different medium A-E listed in (D). The greatest number of CD11b+ cells were present under the condition identified by the red frame and therefore we used these induction conditions for all follow-up experiments. Green, CD11b antibody conjugated to FITC; gray, isotype control. The figure is representative of three-independent experiments (n = 3).

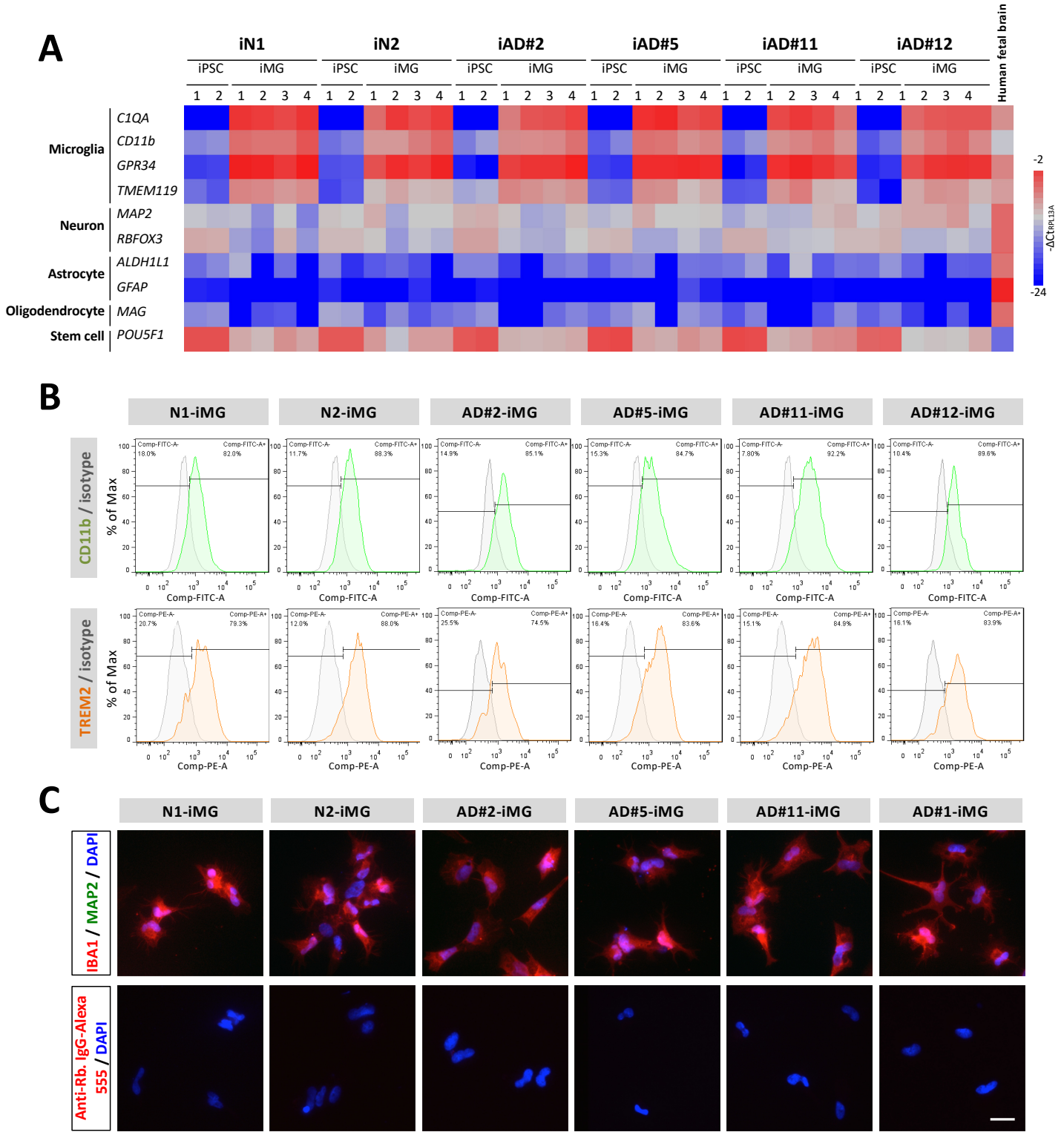
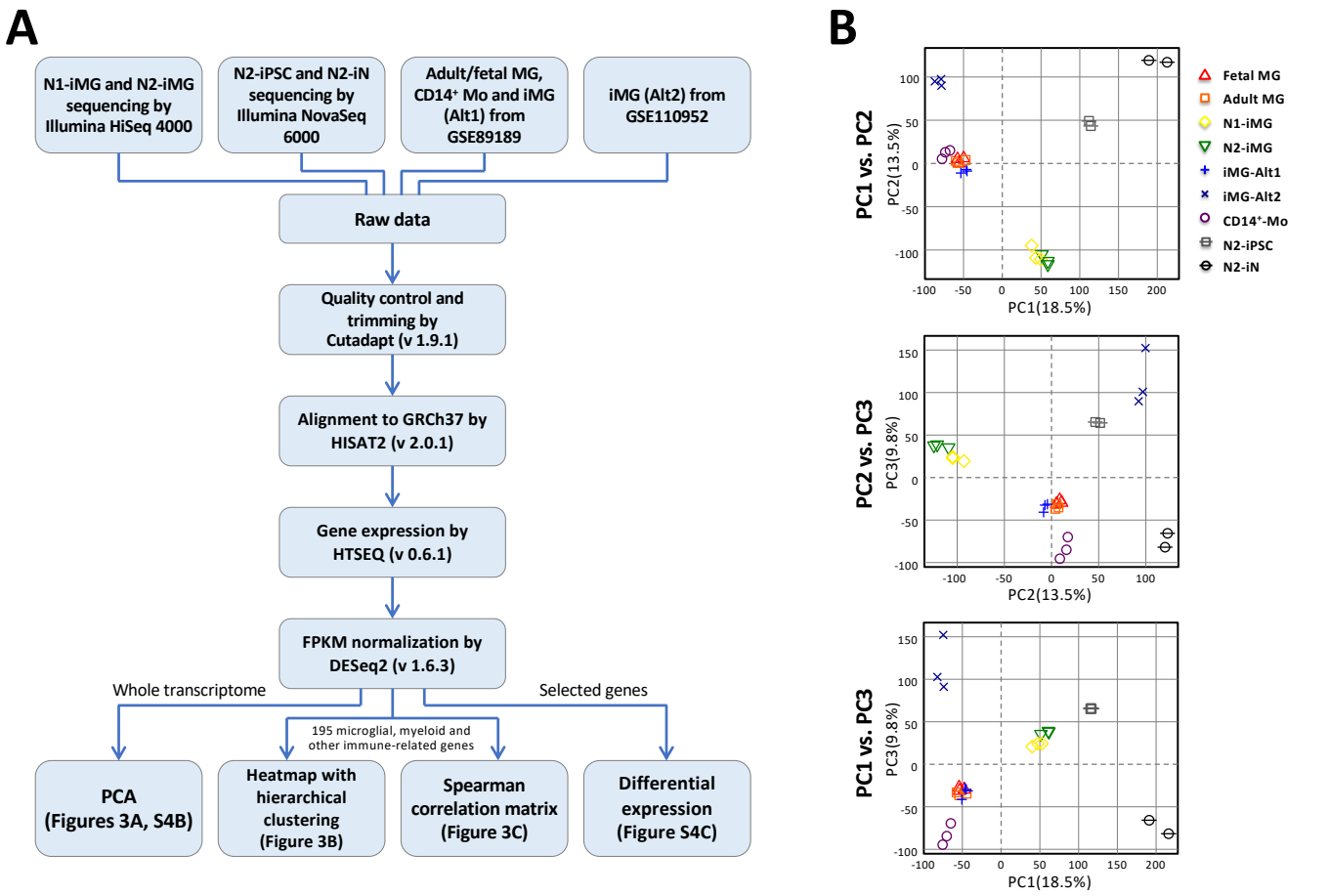

A
iN1
iN2
iAD#2
iAD#5
iAD#11
iAD#12
iPSC
iMG
Human fetal brain
Microglia
C1QA
CD11b
GPR34
TMEM119
Neuron
MAP2
RBFOX3
Astrocyte
ALDH1L1
GFAP
Oligodendrocyte
MAG
Stem cell
POU5F1
-2
-24
-ΔCtRPL13A
B
N1-iMG
N2-iMG
AD#2-iMG
AD#5-iMG
AD#11-iMG
AD#12-iMG
CD11b / isotype
TREM2 / isotype
% of Max
Comp-FITC-A
Comp-PE-A
C
N1-iMG
N2-iMG
AD#2-iMG
AD#5-iMG
AD#11-iMG
AD#1-iMG
IBA1 / MAP2 / DAPI
Anti-Rb. IgG-Alexa 555 / DAPI

Figure S3. Differentiation of six hiPSC lines into microglia-like cells. Related to Figure 2.
(A) Comparative heatmap depiction of differential gene expression of iPSCs and iMGs differentiated from six iPSC lines using real-time RT-PCR. iPSC and iMG group contained two and four independent experiments, respectively. Levels of the gene expression indicated on the left side of the panel are normalized to RPL13A mRNA levels as an internal control. (B) Flow cytometry analyses showing the expression of microglial surface markers, CD11b and TREM2, in six iPSC line-derived iMG cells after 9 days of differentiation. Green, CD11b antibody conjugated to FITC; orange, TREM2 antibody conjugated to PE; gray, isotype control. The figure is representative of three-independent experiments (n = 5). (C) Representative images of 6 lines of iMG cells immunostained for microglia and neuronal markers, IBA1 and MAP2, respectively. Scale bar, 20 μm.


A
N1-iMG and N2-iMG sequencing by Illumina HiSeq 4000
N2-iPSC and N2-iN sequencing by Illumina NovaSeq 6000
Adult/fetal MG, CD14+ Mo and iMG (Alt1) from GSE89189
iMG (Alt2) from GSE110952
Raw data
Quality control and trimming by Cutadapt (v 1.9.1)
Alignment to GRCh37 by HISAT2 (v 2.0.1)
Gene expression by HTSEQ (v 0.6.1)
FPKM normalization by DESeq2 (v 1.6.3)
Whole transcriptome
Selected genes
195 microglial, myeloid and other immune-related genes
PCA (Figures 3A, S4B)
Heatmap with hierarchical clustering (Figure 3B)
Spearman correlation matrix (Figure 3C)
Differential expression (Figure S4C)
B
PC1 vs. PC2
PC2(13.5%)
PC1(18.5%)
PC2 vs. PC3
PC3(9.8%)
PC2(13.5%)
PC1 vs. PC3
PC3(9.8%)
PC1(18.5%)
Fetal MG
Adult MG
N1-iMG
N2-iMG
iMG-Alt1
iMG-Alt2
CD14+-Mo
N2-iPSC
N2-iN

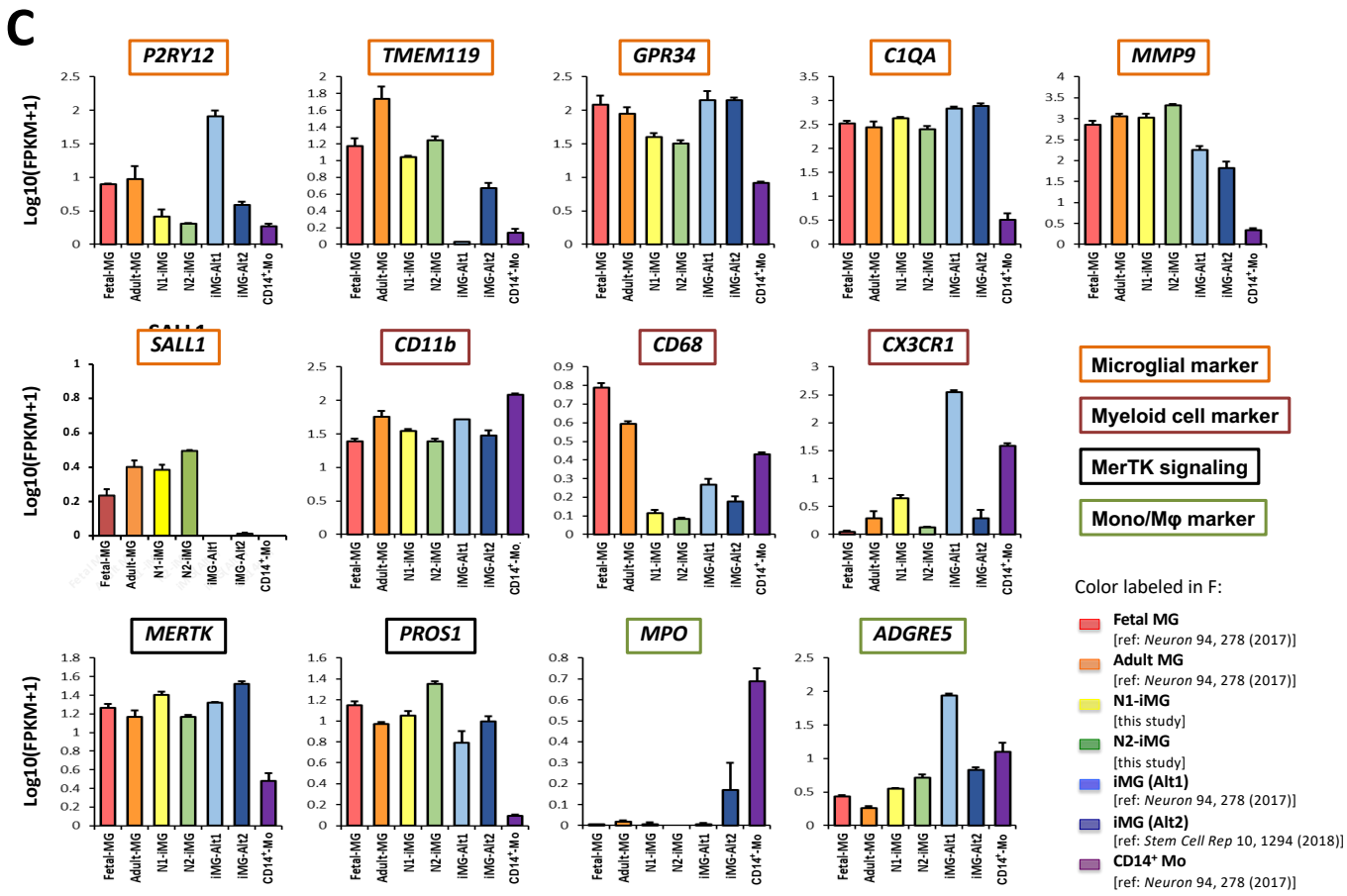

C
P2RY12
TMEM119
GPR34
C1QA
MMP9
SALL1
CD11b
CD68
CX3CR1
MERTK
PROS1
MPO
ADGRE5
Log10(FPKM+1)
Fetal-MG
Adult-MG
N1-iMG
N2-iMG
iMG-Alt1
iMG-Alt2
CD14+-Mo
Microglial marker
Myeloid cell marker
MerTK signaling
Mono/Mφ marker
Color labeled in F:
Fetal MG [ref: Neuron 94, 278 (2017)]
Adult MG [ref: Neuron 94, 278 (2017)]
N1-iMG [this study]
N2-iMG [this study]
iMG (Alt1) [ref: Neuron 94, 278 (2017)]
iMG (Alt2) [ref: Stem Cell Rep 10, 1294 (2018)]
CD14+ Mo [ref: Neuron 94, 278 (2017)]

Figure S4. RNA-seq analysis. Related to Figure 3.
(A) Workflow of RNA-seq analysis. (B) PCA analysis of different cells by whole transcriptome sequencing of protein coding gene expression. (C) Bar graphs of the expression of various microglia-specific genes, as well as other myeloid genes, in D11-12 iMG cells, fetal MG cells, adult MG cells, iMG cells obtained by two other methods [iMG (Alt1) and iMG (Alt2)], and CD14+ peripheral blood monocytes shown as [Log10 (FPKM + 1)]. These results were derived from the RNA-seq results and are presented as mean ± SEM (n = 3 RNA-seq data sets).

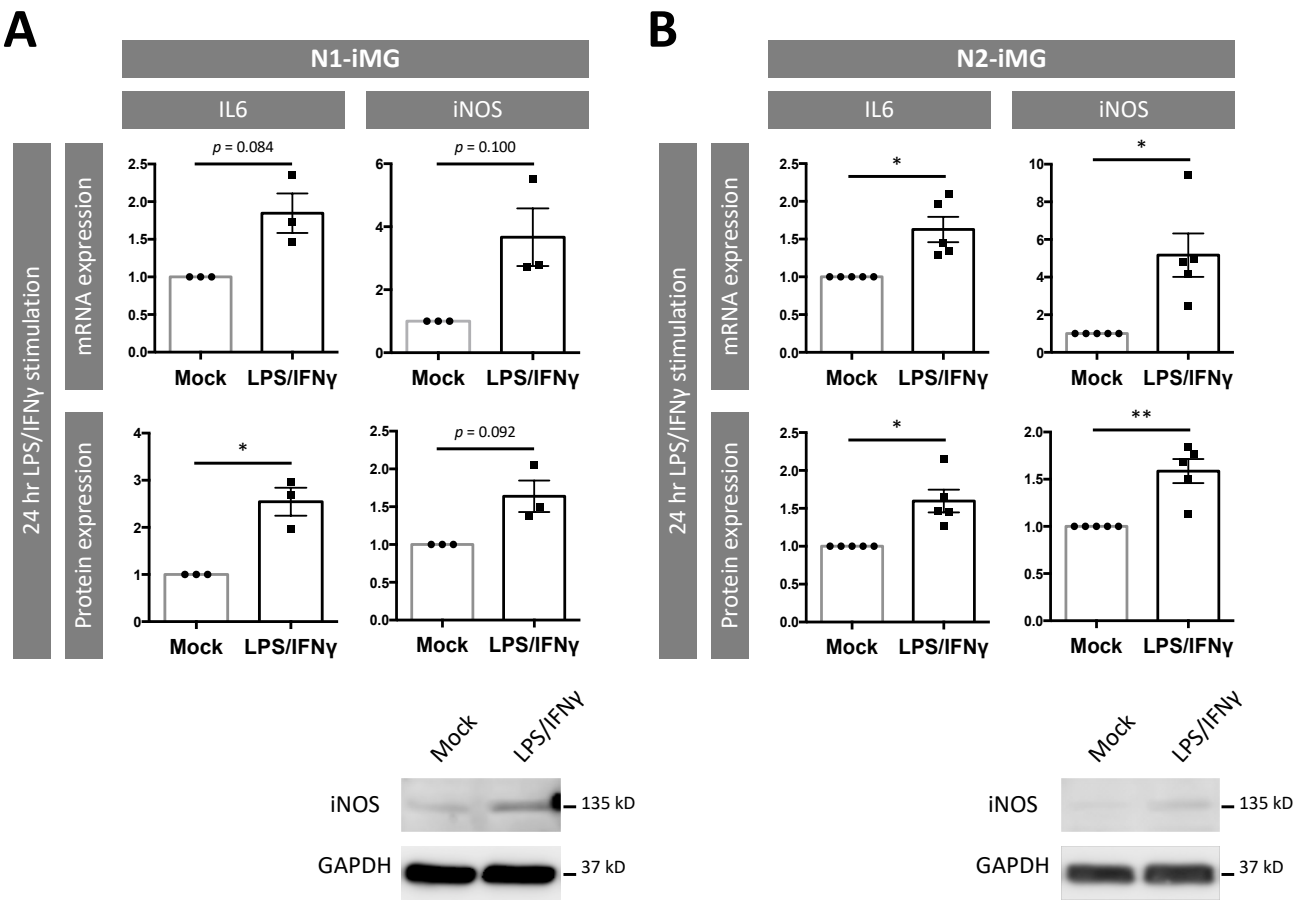


Figure S5. iMG cells exhibit appropriate physiological responses to LPS and IFNγ challenge. Related to Figure 4.

Analyses of inflammation-related gene expression at mRNA and protein levels in N1-iMG (A) and N2-iMG (B) cells after LPS (100 ng/mL) and IFNγ (20 ng/mL) stimulation for 24 hr. IL6 protein in supernatant was detected by ELISA. iNOS protein in cells was analyzed by western blotting. Data are presented as means ± SEM (n = 3 and 5 independent differentiations for N1-iMG and N2-iMG, respectively). *$p < 0.05$, **$p < 0.01$ when compared to mock-treatment group by the ratio paired *t*-test.

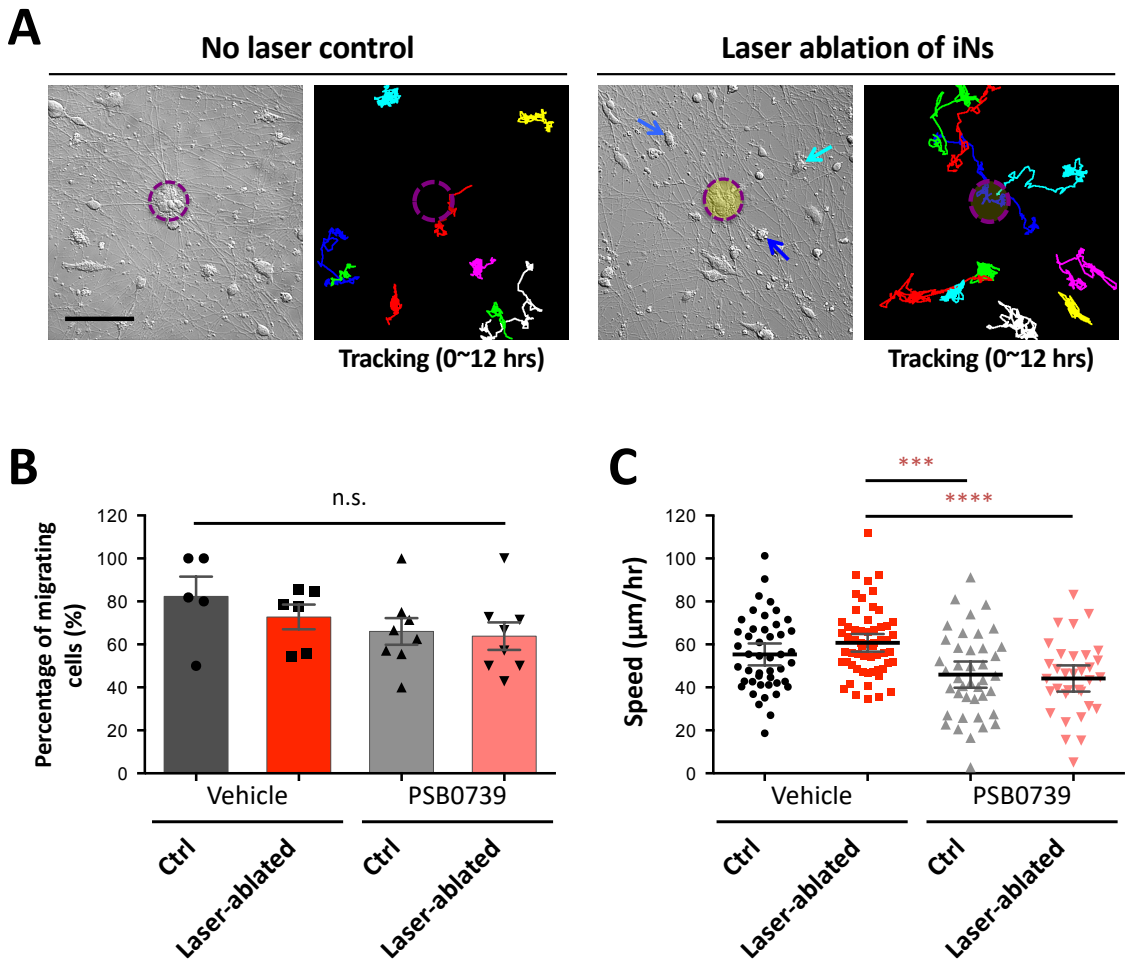


Figure S6. iMG cells respond to and migrate toward an injured neuron cluster. Related to Figure 7, Movies S6 and S7.

(A) An example of the time-lapse imaging of co-cultures with or without laser-induced neuronal injury. The 405-nm laser light was first applied to the region of interest (magenta circles) for 5 min, and the time-lapse DIC imaging was recorded for 12 hours. Blue arrows in the DIC images mark iMG cell migrating towards the central iN cluster. The images on the right panel show the result of cell traces, which are presented as a color-coded trajectory for each cell over the 12-hr recording time. Scale bar, 100 μm. (B) The percentage of migrating iMG cells in the control or laser-ablation group over a 12-hr recording period. Data are presented as means ± SEM (n = 5-8 independent fields in each group). n.s., not significant by one-way ANOVA. (C) The speed of migration of the iMG cells in the control or laser-ablation group over a 12-hr recording period. Data are presented as means ± SEM (n = 33-58 cells from 5-8 fields in each group). ***$p < 0.001$, ****$p < 0.0001$ by one-way ANOVA with Tukey's multiple comparisons.

Supplemental Movies

Movie S1. A representative image of 3D reconstruction of iMG phagocytosis of latex beads (red). The plasma membrane and nuclei of the live cells were stained with CellMask deep red (green) and Hoechst 33342 (blue), respectively. Scale bar, 10 μm. (Related to Figure 4B)

Movie S2. A representative image of 3D reconstruction of iMG phagocytosis of TAMRA-labeled fibrillar Aβ (fAβ, red). The plasma membrane and nuclei of the live cells were stained with CellMask deep red (green) and Hoechst 33342 (blue), respectively. Scale bar, 10 μm. (Related to Figure 4C)

Movie S3. An example of the time-lapse DIC imaging of iMG cultures recorded for 12 hours. Scale bar, 50 μm. (Related to Figure 6D)

Movie S4. An example of the time-lapse DIC imaging of iMG cultures with iNs recorded for 12 hours. Scale bar, 50 μm. (Related to Figure 6D)

Movie S5. The cell death of iNs was monitored using propidium iodide (PI, red) staining. Scale bar, 20 μm. (Related to Figure 7B)

Movie S6. An example of the time-lapse DIC imaging of iMG-iN co-cultures without laser-induced neuronal injury. Scale bar, 50 μm. (Related to Figure 7C)

Movie S7. An example of the time-lapse DIC imaging of iMG-iN co-cultures with laser-induced neuronal injury in the selected region (magenta circles). Scale bar, 50 μm. (Related to Figure 7C)

Supplemental Tables

Table S1. iMG differentiation efficiency using different combination of TFs. Related to Figure 1.

S1-1. Flow cytometry with anti-CD11b antibody

	CD11b+ (%, mean ± SEM)	**Replicate** (independent diff., n)
Isotype Ctrl (N.C.)*	2.84 ± 1.42	5
BV2 (P.C.)#	82.70 ± 5.35	5
iN2/SPI1$	6.06 ± 0.97	5
iN2/SPI1;CEBPA	58.43 ± 6.94	4
iN2/SPI1;IRF8	7.56 ± 0.77	3
iN2/SPI1;IRF8;TAL1	6.09 ± 0.99	3
iN2/SPI1;RUNX1	6.58 ± 0.87	3
iN2/SPI1;SALL1	7.22 ± 1.14	3
iN2/SPI1;TAL1	7.83 ± 1.15	3
iN2/RUNX1	6.19 ± 2.05	5
iN2/RUNX1;CBFβ	4.47 ± 1.19	3
iN2/RUNX1;IRF8	8.77 ± 3.19	3
iN2/RUNX1;TAL1	7.46 ± 1.77	3
iN2/SALL1	8.63 ± 1.25	5
iN2/SALL1;IRF8	8.37 ± 2.29	3
iN2/SALL1;RUNX1	10.60 ± 3.80	3
iN2/SALL1;TAL1	7.23 ± 1.34	3
iN2/CEBPA	5.42 ± 0.74	3
iN2/IRF8	6.03 ± 0.54	5
iN2/TAL1	5.41 ± 0.35	5
iN2/TAL1;IRF8	7.92 ± 2.25	3

* BV2 labeled with FITC conjugated rat IgG2b kappa antibody (isotype control) was used as negative control (N.C.).
# BV2 labeled with FITC conjugated anti-CD11b antibody was used as positive control (P.C.).
$ iN2 (hiPSC line) extopically expressing the indicated transcription factors (TFs).

S1-2. Immunocytochemistry with anti-IBA1 antibody

	IBA1+ (%, mean ± SEM)	**Replicate** (independent diff., n)
2nd Ab (N.C.)**	0	7
BV2 (P.C.)	95.42 ± 1.58	7
iN2/SPI1	2.55 ± 1.72	7
iN2/SPI1;CEBPA	75.90 ± 3.74	7
iN2/SPI1;IRF8	0	3
iN2/SPI1;IRF8;TAL1	0	3
iN2/SPI1;RUNX1	0	3
iN2/SPI1;SALL1	0	3
iN2/SPI1;TAL1	0	3
iN2/RUNX1	0	4
iN2/RUNX1;CBFβ	0	7
iN2/RUNX1;IRF8	0	3
iN2/RUNX1;TAL1	0	3
iN2/SALL1	0	6
iN2/SALL1;IRF8	0	3
iN2/SALL1;RUNX1	0	3
iN2/SALL1;TAL1	0	3
iN2/CEBPA	0	3
iN2/IRF8	0.01 ± 0.01	6
iN2/TAL1	0	4
iN2/TAL1;IRF8	0	3

** BV2 labeled with 2nd antibody was used as negative control (N.C.).

Table S2. The primers and TaqMan® probes for qPCR analysis. Related to Figures 3, 4 and 6.

Gene	Forward primer (5' - 3')	Reverse primer (5' - 3')	Probe
ADGRE5	ACGCATGAAGCTGAATTGG	GTTCTGGATGGAGAGGATGC	#78
ALDH1L1	CGGTGGGGGAAGATCAGT	ATCCAGGGCCTCAATGGT	#63
C1QA	GAGCATCCAGTTGGAGTTGAC	ACACAGAGCACCAGCCAT	#13
CD11B	GGCTCTGCTTCCTGTTTGG	GGCAATGTCACTATCCTCTTGA	#66
CD68	CCTCAGCTTTGGATTCATGC	GAGCCGAGAATGTCCACTGT	#67
CX3CR1	CCCTGGAAGGTGCTGTTATC	TCCATGAGATTGGACTGGAA	#60
GFAP	AGAGGGACAATCTGGCACA	CAGCCTCAGGTTGGTTTCAT	#29
GPR34	CGGTGAAAGGTTGCGACTAT	GGTCGCTATGATTGGTTATAAAGC	#52
IL-6	GATGAGTACAAAAGTCCTGATCCA	CTGCAGCCACTGGTTCTGT	#40
INOS	ATTCAGCTGTGCCTTCAACC	CATTGCCAAACGTACTGGTC	#66
ITGAL	GCTGAGAGCCAGATGATCG	GAGCACTCCACTTCATGCAC	#78
MAG	CCTTCAACCTGTCTGTGGAGTT	CGGGTTGGACTTCACCAC	#63
MAP2	CCTGTGTTAAGCGGAAAACC	AGAGACTTTGTCCTTTGCCTGT	#62
MMP9	GAACCAATCTCACCGACAGG	GCCACCCGAGTGTAACCATA	#6
MPO	CGTCAACTGCGAGACCAG	GTCATTGGGCGGGATCTT	#66
P2RY12	TGACAAAAATCCAGGGTAGTGA	CGTCAGTAAAGTCTTGAGTGCTCTT	#17
POU5F1(OCT4)	CTTCGCAAGCCCTCATTTC	GAGAAGGCGAAATCCGAAG	#60
RBFOX3(NEUN)	CCCTCCGACCCTACAGAGA	CCACGTCTAAAATTTTTCCGAAT	#66
RPL13A	CAAGCGGATGAACACCAAC	TGTGGGGCAGCATACCTC	#28
TNFα	CAGCCTCTTCTCCTTCCTGAT	GCCAGAGGGCTGATTAGAGA	#29
TMEM119	AGTCCTGTACGCCAAGGAAC	AGGAGCAGCAACAGAAGGAT	#75

Table S3. List of 195 selected microglia/monocyte/macrophage-related genes (Bennett et al., 2016; Dong et al., 2013) in clustering heatmap. Related to Figure 3.

ABCG1	CD6	EIF2S1	HLA-A	JUNB	NFE2	RUNX1	TGFB1
ABHD15	CD7	ELMO1	HLA-B	KCNK6	NFKBIZ	SALL1	TGFBR1
ACP2	CDKN2D	ENTPD1	HLA-C	KCTD11	NKG7	SERINC3	TLR9
ACVR1	CEBPD	EPB42	HLA-DOA	KLF4	NOTCH2	SFT2D1	TMEM106A
ADAM8	CFH	EPSTI1	HLA-DPB1	KLHL21	NR4A2	SFT2D2	TMEM119
ADI1	CLEC4A	ETV5	HLA-DQA2	LAG3	NUMB	SIRPA	TMEM14C
AIF1	CLEC4D	F11R	HLA-DQB1-AS1	LAP3	NXF1	SLC15A3	TMEM163
AKT3	CMTM6	FAM102B	HLA-DRB9	LDHB	OLFML2B	SLC17A9	TMEM185B
ANG	CMTM7	FAM105A	HLA-E	LIPN	OLR1	SLC39A1	TMEM204
APOL3	CNR2	FAM110A	HLA-H	LMAN1	OPHN1	SLC40A1	TMEM258
APP	COMMD8	FAM91A1	ICAM3	LYPD3	ORAI1	SLC46A1	TMEM55B
ARHGAP12	COMMD9	FCAR	IDO1	MAP2	P2RY6	SLC46A2	TNFAIP3
ASB2	COMT	FCGR1B	IFI44L	MAPK1	PAX6	SLC7A7	TNFRSF17
ASGR2	CR1L	FCGR1C	IGJ	MB21D2	POLR2G	SLC7A8	TNFSF10
ATRAID	CRIP1	FMNL1	IL10	MERTK	PPARG	SMOX	TP53
BIN1	CRISPLD2	FSCN1	IL1A	MMP9	PROS1	SOX2	TSPAN3
BLNK	CRYBB1	G6PC3	IL6R	MN1	PTAFR	SPARC	TSPAN7
BTG1	CSF1R	GAPDH	IRF7	MPI	PTGS2	SRGAP2	UBASH3B
C1QA	CSF2RB	GAPT	ITGA6	MPO	RARA	STK17B	ZFP36
C9orf72	CXCL16	GBP4	ITGA9	MX2	RCAN1	STX11	ZSWIM4
CASP8	CYB5A	GBP5	ITGAM	MYLIP	RGL1	SYNGR1	
CBFB	CYB5B	GNLY	ITGB2	NDUFA3	RGS2	TANC2	
CCL4	DCTPP1	GOLM1	ITGB5	NDUFA7	RHOB	TARDBP	
CD164	DNAJB4	GPR157	ITM2B	NDUFC2	RIPK2	TBCB	
CD276	DYNLRB1	HFE	ITPRIPL1	NECAP1	RPS27L	TGFA	

Table S4. Antibodies used in this study.

Antibodies (flow cytometry)	Host Species	Target Species	Vendor	Cat. No.	Dilution
Human BD Fc Block	N/A	Human	BD Biosciences	564220	1:20
CD11b-FITC	Rat	Human, Mouse	BioLegend	101206	1:100
TREM2-PE	Rat	Human, Mouse	R&D Systems	FAB17291P	1:50
Isotype control-FITC	Rat	N/A	invitrogen	11-4031-82	1:100
Isotype control-PE	Rat	N/A	invitrogen	12-4031-82	1:50

Antibodies (immunofluorescent staining)	Host Species	Target Species	Vendor	Cat. No.	Dilution
IBA1	Rabbit	Human, Mouse, Rat, etc	Wako	019-19741	1:1000
MAP2	Mouse	Human, Mouse, Rat, etc	Millipore	AB5622	1:1000
FLAG	Rabbit	N/A	SIGMA	F7425	1:1000
MYC	Mouse	Human	SIGMA	M5546	1:1000
Mouse-Alexa 488	Goat	Mouse	Invitrogen	A11001	1:500
Rabbit-Cy5	Goat	Rabbit	Invitrogen	A10523	1:500

Antibodies (western)	Host Species	Target Species	Vendor	Cat. No.	Dilution
FLAG	Rabbit	N/A	SIGMA	F7425	1:5000
MYC	Mouse	Human	SIGMA	M5546	1:5000
HA	Mouse	N/A	SIGMA	H9658	1:5000
iNOS	Rabbit	Human, Mouse	Invitrogen	PA3-030A	1:1000
PU.1	Rabbit	Human	Abcam	ab76543	1:3000
GAPDH	Mouse	Human, Mouse, Rat, etc	proteintech	60004-1	1:10000
Mouse-HRP	Goat	Mouse	Bethyl	A90-216P	1:5000
Rabbit-HRP	Goat	Rabbit	Bethyl	A120-201P	1:5000

Chemicals for nucleus staining	Vendor	Cat. No.	Dilution		
DAPI	SIGMA	D9542	1:1000		
Propidium iodide	SIGMA	P-4170	1:1000		

Supplemental Experimental Procedures

Cell culture

The two human induced pluripotent stem cell (hiPSC) lines, NTUH-iPSC-01-05 and NTUH-iPSC-02-02 (abbreviated to iN1 and iN2, respectively), were purchased from Bioresource Collection and Research Center of Food Industry Research and Development Institute, Taiwan. The four AD-iPSC lines were generated by the Brain Research Center of National Yang-Ming University, Taiwan using non-integrating Sendai virus (Wu et al., 2019) and are available to other researchers via Biobank of Taipei Veterans General Hospital, Taiwan. The uses of these hiPSC lines followed the Policy Instructions of the Ethics of Human Embryo and Embryonic Stem Cell Research guidelines in Taiwan. In addition, approval from the Institutional Review Boards of National Yang-Ming University was obtained. Human iPSCs were routinely maintained in Essential 8™ (E8) medium (Gibco, USA) on vitronectin (VTN-N, Gibco, USA) coated dishes at 37°C in a 5% CO_2 incubator. Cells were passaged when the culture reached 85% confluency, typically every 4 days. For passaging, the hiPSCs were washed once with Dulbecco's Phosphate-Buffered Saline (DPBS) without calcium and magnesium (Gibco) and then treated with DPBS/EDTA (0.5 mM UltraPure EDTA in DPBS) for 3-4 minutes at 37°C. When the cells started to separate and round up, they were removed from the dish by gently pipetting. An appropriate number of cells was then transferred to a new culture dish at a passaging ratio of 1:10.

The mouse microglial cell line BV2 and human embryonic kidney 293FT (HEK293FT) cells were maintained in Dulbecco's modified Eagle medium (DMEM, Gibco) containing 10% fetal bovine serum (FBS, Biological Industries, USA) and 1% penicillin-streptomycin-glutamine (PSG, Gibco) at 37°C in a 5% CO_2 incubator. Medium was renewed every two days or when the medium had begun to turn yellow. Cells were passaged upon reaching 85% confluency, typically every 4 days. For subculture, the cells were washed once with DPBS together with TrypLE™ express enzyme (Gibco). When the cells started to separate and round up, they were removed from the dish by gently pipetting. The passaging ratio was routinely 1:10.

The human monocytic cell line THP-1 was maintained in suspension using RPMI-1640 medium (Gibco) containing 10% FBS and 1% PSG at 37°C n a 5% CO_2 Incubator. Fresh medium was added every two days or when the medium began to turn yellow. Cells were subcultured every 4 days by centrifugation and then resuspended at 1.5 x 10^5 viable cells/ml. To induce the THP-1 monocytes to turn into macrophages, 1.5 x 10^6 cells were seeded into a 10-cm Petri dish containing serum free RPMI-1640 medium supplemented with 10 ng/ml phorbol 12-myristate 13-acetate (PMA, Sigma) for 6 hours. After cells had attached to the Petri-dish, the medium was replaced with fresh RPMI-1640 containing 10% FBS and 1% PSG. These cells were regarded as M0 macrophages and then subjected to further analyses.

Lentiviral plasmid construction

The lentiviral vector backbone pTetO-Ngn2-puro was a gift from Marius Wernig (Addgene plasmid #52047). The open reading frames of human SPI1, SALL1, TAL1, IRF8 and RUNX1 were amplified from various constructs by PCR to include flanking EcoRI and XbaI sites. The open reading frame of CBFβ was amplified from the RNA of THP-1 by RT-PCR to include flanking SbfI and XbaI sites. The open reading frame of CEBPA was amplified from the THP-1 RNA by RT-PCR to include flanking EcoRI and XbaI sites, or flanking EcoRI and SbfI sites with a T2A linking peptide sequence. SPI1, SALL1, TAL1, IRF8, RUNX1 and CEBPA were introduced to the EcoRI and XbaI sites of the pTetO-T2A-Puro backbone. CBFβ was introduced to SbfI and XbaI sites of the pTetO-RUNX1-T2A-Puro backbone to generate the pTetO-RUNX1-T2A-CBFβ-T2A-Puro construct. CEBPA was introduced to EcoRI and SbfI sites of pTetO-SPI1-T2A-Puro backbone to generate the pTetO-CEBPA-T2A-SPI1-T2A-Puro construct. The integrity of the vector was verified by restriction enzyme digestion, and the correct sequence of inserted fragment was confirmed by Sanger sequencing.

Lentivirus generation and infection

HEK293FT cells were seeded at 5x10^6 cells in a 10-cm dish and incubated overnight. Cells were co-transfected with 6 μg of lentiviral construct with 5 μg of the packaging plasmid pCMV-Δ8.91 and 1 μg of the envelope plasmid pVSV-G; this was done using Lipofectamine 3000 (Invitrogen) by following the manufacturer's instructions. Two batches of virus-containing supernatant were collected at 24 and 72 hours after transfection. The viral supernatant was filtered through a 0.45-μm filter (low protein binding) to remove cell debris and then concentrated 20 fold using an Amicon Ultra-15 Centrifugal Filter Unit (MWCO 3 kDa,

Merck). The virus concentrates were separated into aliquotes of 100 µl and stored at -80°C until use. HiPSCs were seeded in 12-well plate one day before infection and should be exhibiting 50% to 60% confluence at the time of transduction. For infection, the culture was replaced with fresh medium containing the appropriate lentivirus and 8 µg/ml polybrene. Twenty-four hours after infection, the infection medium was replaced with the fresh E8 medium. All experiments using infected hiPSCs were started after two passages to allow cell adaptation.

Differentiation of microglia-like cells from hiPSCs

HiPSCs that had been infected with both FUW-rtTA and pTetO-CEBPA-T2A-SPI1-T2A-Puro were seeded on day -1. On day 0, the cells had reached at least 70% confluency; at this point the culture medium was replaced with DMEM/F12 (Gibco) supplemented with N2 (Gibco) and non-essential amino acids (NEAA, Gibco). The medium also contained human bone morphogenic protein-4 (BMP4, 50 ng/ml, PeproTech), basic fibroblast growth factor (bFGF/FGF2 50 ng/ml, PeproTech) and activin-A (20 ng/ml, PeproTech). Doxycycline (2 µg/ml, Sigma) was added to promote TetO downstream gene expression and retained in the medium until the end of the experiment. On day 1, the medium was replaced with DMEM/F12/N2/NEAA containing human vascular endothelial growth factor (VEGF, 50 ng/ml, PeproTech), stem cell factor / KIT ligand (SCF, 50 ng/ml, PeproTech) and FGF2 (20 ng/ml). Puromycin (1 µg/ml, Gibco) was also added for 24 hours to select for successfully infected cells. On day 2, the medium was replaced with DMEM/F12/N2/NEAA containing human IL-34 (10 ng/ml, PeproTech), macrophage colony-stimulating factor (M-CSF, 10 ng/ml, PeproTech) and transforming growth factor-β 1 (TGFβ-1, 10 ng/ml, PeproTech). Two days later, the medium was replaced with DMEM/F12/N2/NEAA containing human IL-34 (100 ng/ml), M-CSF (20 ng/ml) and TGFβ-1 (20 ng/ml). After day 4, half medium was removed and replaced with fresh DMEM/F12/N2/NEAA containing human IL-34 (100 ng/ml), M-CSF (20 ng/ml), granulocyte M-CSF (GM-CSF, 20 ng/ml, PeproTech) and TGFβ-1 (20 ng/ml); this was repeated every 3 days from this point onwards. Cells were assayed on days 9, day 12 or day 15 in most experiments (Figure S10).

Differentiation of neuronal cells from hiPSCs

HiPSC-derived neurons were generated using a published protocol (Zhang et al., 2013) with modifications. HiPSCs that had been infected with FUW-rtTA and pTetO-Ngn2-puro were seeded on day -1. On day 0, when the cells reached at least 70% confluency, the culture medium was replaced with DMEM/F12 supplemented with N2, NEAA, human brain-derived neurotrophic factor (BDNF, 10 ng/ml, PeproTech), human neurotrophin-3 (NT-3, 10 ng/ml, PeproTech) and mouse laminin (0.2 µg/ml, Gibco). Doxycycline (2 µg/ml) was added to induce TetO-regulated Ngn2 expression and retained in the medium until the end of the experiment. On day 1, puromycin (1 µg/ml) was added to kill any uninfected cells. On day 2, puromycin was removed and the cells were replated in Neurobasal medium supplemented with B-27 supplement (Gibco), GlutaMAX (Gibco), mouse laminin (0.2 µg/ml), human BDNF (10 ng/ml) and NT-3 (10 ng/ml) on poly-L-lysine and laminin coated dishes (~10^5 cells/mL). On day 4, cytosine arabinoside (Ara-C, 2 µM, Sigma) was added to the medium to inhibit non-neuronal cell proliferation and was then removed on day 6. After day 6, 50% of the medium was changed every 4 days. Induced neurons were assayed between days 14 to 20 in most experiments.

Co-culture of hiPSC-derived microglia-like (iMG) cells and neurons (iNs)

For co-culture, the iNs and iMGs were cultured separately as described above until day 4. After day 4, the two cells were co-cultured under a set of conditions suitable for both iNs and iMG cells. Briefly, iMG cells were detached from plate with EDTA. Next 1x10^5 iMG cells were gently seeded onto the monolayer of a neuronal culture in DMEM/F12/N2/B27/NEAA/GlutaMAX that contained IL-34 (100 ng/ml), M-CSF (20 ng/ml), TGFβ-1 (20 ng/ml), BDNF (10 ng/ml), NT3 (10 ng/ml) and doxycycline (2 µg/mL). Half medium was changed for fresh DMEM/F12/N2/B27/NEAA/GlutaMAX containing human IL-34 (100 ng/ml), M-CSF (20 ng/ml), GM-CSF (20 ng/ml), TGFβ-1 (20 ng/ml), BDNF (10 ng/ml), NT3 (10 ng/ml) and doxycycline (2 µg/mL) every 3 days (Figure S10). For the comparison of gene expression, the iMG cells cultured alone or in co-culture was isolated by the positive selection of $CD11b^+$ cells using an immunomagnetic separation kit (Miltenyi Biotec), according to the manufacturer's instruction, and then subjected to RT-qPCR.

Western blotting

The infected hiPSCs after 3-days of doxycycline treatment and 1-day of puromycin selection were

lysed in RIPA buffer (50 mM Tris-HCl, pH 8.0, 150 mM NaCl, 1% Nonidet P-40 (NP-40), 1% sodium deoxycholate and 0.1% SDS) supplemented with protease inhibitor cocktail (Roche). Lysate (20 µg) was separated by electrophoresis on 10% SDS-polyacrylamide gel in tricine-glycine buffer and transferred to a polyvinylidine difluoride (PVDF) membrane (Millipore). The membrane was blocked with 5% skimmed milk in PBST (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2HPO_4 and 0.1% Tween-20) and then incubated with primary antibody solution at 4°C overnight. After washing with PBST, the membrane was incubated with horseradish peroxidase (HRP)-conjugated secondary antibody for 1 hr at room temperature. After washing again with PBST, the signals were developed using Immobilon Western Chemiluminescent HRP substrate (Millipore) and detected using a LAS3000 imaging system (Fujifilm, Japan). All antibodies and dilutions used in Western blotting are listed in Table S4.

Immunocytochemistry

The cells cultured on coverslips were fixed with 4% paraformaldehyde (PFA) in PBS (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2HPO_4, pH 7.2-7.4) at room temperature for 10 minutes and then made permeable with 0.1% Triton X-100 in PBS for 5 minutes. Next the cells were blocked using PBS containing 3% bovine serum albumin (BSA, Sigma) and 3% goat serum (Gibco) at room temperature for 1 hour and then the appropriate primary antibodies were applied at the indicated concentrations (Table S4) overnight at 4°C. Next the cells were washed in PBS three times and incubated with the appropriate secondary antibody and DAPI at room temperature for 2 hours. After washing, the coverslips were mounted in the Fluoromount-G solution (Invitrogen). The fluorescent images were acquired using a Zeiss Axio Observer inverted microscope fitted with an Andor Zyla cMOS camera. All images were then processed using ImageJ software (NIH, Bethesda, MD, USA, https://imagej.nih.gov/ij) (Schneider et al., 2012). All antibodies and dilutions used in immunocytochemistry are listed in Table S4.

Flow cytometry

Cells were dissociated with EDTA into a single cell suspension and then blocked with Human Fc Block (BD Biosciences, USA) on ice for 20 minutes. For detection of microglial surface markers, the cells were incubated with pre-conjugated anti CD11b-FITC (M1/70, 1:100, BioLegend), anti CX3CR1 (C-X3-C motif chemokine receptor 1)-Alexa Fluo 647 (1:20, BioLegend), anti TREM2 (triggering receptor expressed on myeloid cells 2)-PE (1:50, R&D Systems) or isotype control antibodies (1:50, BioLegend) for 30 minutes at 4°C, washed twice with FACS buffer (DPBS with 1% FBS and 0.1% sodium azide), resuspended in propidium iodide (PI) containing FACS buffer and then analyzed using a BD FACSCanto analyzer. The results were further analyzed using FlowJo software. Briefly, the cells were first gated for PI-negative (for live cells) and proper FSC and SSC signals, and then the signals of gated cells were analyzed in the FITC (525/40 nm) and PE (585/42 nm) filters.

Magnetic activated cell sorting (MACS)

The iMG cells for RNA sequencing and in co-culture were isolated by the positive selection of $CD11b^+$ cells using an immunomagnetic separation kit (Miltenyi Biotec), according to the manufacturer's instructions. The cells isolated by the MACS method were washed twice with PBS and then quickly processed for RNA isolation.

RNA isolation and RT-qPCR analysis

Total RNA was isolated using a Tissue Total RNA Mini Kit (Geneaid, Taiwan) by following the manufacturer's instructions. In-column DNase I digestion was performed to remove genomic DNA contamination. Reverse transcription was then performed using Superscript IV (Invitrogen) and $Oligo(dT)_{20}$ primers by following the manufacturer's instructions. The cDNA was stored at -20°C until used for qPCR analysis. TaqMan Real-time polymerase chain reactions (PCRs) were carried out using FastStart Universal Probe Master Mix (ROX) (Roche) and the StepOnePlus™ real-time PCR system (Thermo Fisher Scientific). Primers were intron-spanning and designed using the *Universal ProbeLibrary Assay Design Center* (Roche). The primers and probes for the qPCR analysis are listed in Table S2.

RNA sequencing

Total RNA from each sample was extracted using a Total RNA Mini Kit (Geneaid) and then quantified and its purity assessed using an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA),

NanoDrop (Thermo Fisher Scientific) and by electrophoresis on 1% agarose gels, respectively. Next, 1 µg of total RNA with a RIN value above 7 was used to prepare the appropriate libraries. Next generation sequencing library preparation was constructed according to the manufacturer's protocol (NEBNext® Ultra™ RNA Library Prep Kit for Illumina®). The poly(A) mRNA isolation was performed using either a NEBNext Poly(A) mRNA Magnetic Isolation Module (NEB) or a Ribo-Zero™ rRNA removal Kit (illumina). The mRNA fragmentation and priming was performed using NEBNext RNA First Strand Synthesis Reaction Buffer and NEBNext Random Primers. First strand cDNA was synthesized using ProtoScript II Reverse Transcriptase and the second-strand cDNA was synthesized using Second Strand Synthesis Enzyme Mix. The purified double-stranded cDNA obtained by AxyPrep Mag PCR Clean-up (Axygen) was then treated with End Prep Enzyme Mix to repair both ends and add a dA-tail; this was done in a single reaction, and was followed by a T-A ligation to add adaptors to both ends. Size selection of Adaptor-ligated DNA was then performed using AxyPrep Mag PCR Clean-up (Axygen), and fragments of ~360 bp (with the approximate insert size being 300 bp) were recovered. Each sample was then amplified by PCR for 11 cycles using the P5 and P7 primers, with both primers carrying sequences that are able to anneal with the flow cell in order to perform bridge PCR and P7 primer carrying a six-base index allowing for multiplexing. The PCR products were cleaned up using AxyPrep Mag PCR Clean-up (Axygen), validated using an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA), and quantified by Qubit 2.0 Fluorometer (Invitrogen, Carlsbad, CA, USA). Then libraries with different indices were multiplexed and loaded on an Illumina instrument according to manufacturer's instructions (Illumina, San Diego, CA, USA). N1-iMG and N2-iMG libraries were sequenced using the Illumina HiSeq 4000 system; N2-iPSC and N2-iN libraries were sequenced using the Illumina NovaSeq 6000 system. Sequencing was carried out using a 2x150bp paired-end (PE) configuration; image analysis and base calling were conducted by the Control Software (HCS) + OLB + GAPipeline-1.6 (Illumina) on the instrument. The RNA sequencing (RNA-seq) was processed and analyzed by GENEWIZ (South Plainfield, NJ, USA).

Transcriptome analysis

To compare the transcriptome profile of our iMGs with that of human primary microglia to support the microglial identity, RNA-seq data for human adult microglia, human fetal primary microglia, $CD14^+$ monocytes and iPSC-derived microglia obtained using other differentiation protocols were retrieved from NCBI GEO (GSE89189 and GSE110952) (Abud et al., 2017; Brownjohn et al., 2018). Reads from the datasets of GSE89189 and GSE110952 were mapped and counted using the same methods and databases as for our data. Briefly, reads were first mapped to a reference genome of human (GRCh37; hg19) using HISAT2 (version 2.0.1). Per-gene expression levels (read counts) were quantitated using HTSEQ (v 0.6.1), and the fragments per kilobase of transcript per million mapped reads (FPKM) was calculated by the formula "total exon fragments / (mapped reads [millions] x exon length [KB])". FPKM from different experiments was then normalized using Median of ratios method (DESeq2, v 1.6.3). Principal component analysis, heatmap with hierarchical clustering and Spearman correlation matrix were performed using R version 3.1.2. The workflow of RNA-seq data analysis is shown in Figure S5A. The data discussed in this publication have been deposited in NCBI's Gene Expression Omnibus (Edgar et al., 2002) and the accessible through GEO Series accession number GSE163984 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE163984).

Inflammation assay

The iMG culture medium on days 9-12 was replaced with basal media without IL34, M-CSF, GM-CSF and TGF-β for 6 hr, and then the cells were stimulated with lipopolysaccharides (LPS, 100 ng/ml, Sigma) and interferon γ (IFNγ, 20 ng/ml, PeproTech) for 6 or 24 hr. Next the conditioned medium (CM) was harvested and centrifuged in the presence of the protease inhibitor cocktail (Roche) and stored at -80°C until analysis. IL6 protein in CM was measured via a sensitive fluorescence based sandwich enzyme-linked immunosorbent assay (ELISA) that used a kit (Biolegend). The detailed procedure was performed according to the manufacturer's protocol. The cell lysates were prepared with RB buffer (Geneaid), and total RNA from either the mock treatment or the LPS/IFN-γ treatment was collected and assessed by RT-qPCR for expression of inflammatory genes.

Phagocytosis assay

Aqueous red-orange fluorescent latex beads (Sigma) were pre-opsonized in 50% FBS/DPBS at 37°C for 1 hr. Fibrillar fluorescent amyloid-beta (TAMRA-fAβ) was generated as described previously (Stine et al., 2011). Briefly, fluorescently labeled Aβ1-42 peptide (AnaSpec, Fremont, CA, USA) was first dissolved in

NH_4OH (0.1%) to 1 mg/ml, further diluted to 100 µg/ml using sterile endotoxin-free water, vortexed thoroughly, and incubated at 37°C for 7 days. TAMRA-fAβ was thoroughly mixed prior to cell exposure. For the phagocytosis assay, cells were pre-exposed to DMSO, cytochalasin (10 µM, Sigma) or Mer THR inhibitor (UNC569, 10 µM, Sigma) for 1 hr and further incubated with either pre-opsonized latex beads (20 microns per cell) or TAMRA-fAβ (5 µM) for 1 hr. Next they were washed twice with DPBS/1% FBS to remove the remaining extracellular latex beads or TAMRA-fAβ. Samples were then labeled with FITC-conjugated anti-CD11b antibody and analyzed using a BD FACSCanto analyzer (BD Biosciences, USA). The results were further analyzed using FlowJo software (FlowJo, Ashland, OR, USA). The cells were first gated for PI-negative (for live cells) and proper FSC and SSC signals, and then the signals of CD11b and subtracts (latex beads or TAMRA-fAβ) were detected in the FITC (525/40 nm) and PE (585/42 nm) filters, respectively.

Calcium imaging analysis

For calcium imaging, iMG cells, pre-exposed to DMSO or PSB0739 (50 µM, Tocris) for 1 hr, were incubated with 5 µM Fura-2/AM (Molecular Probes) calcium dye in loading buffer (150 mM NaCl, 5 mM glucose, 10 mM HEPES, 1 mM MgCl2, 5 mM KCl and 2 mM CaCl2 at pH 7.4) at 37°C for 30 minutes. After incubation, the dye was washed out three times using loading buffer before the cells were used for experiments. The baseline Ca^{2+} signal was measured for 5 min and then either adenosine 5'-diphosphate (ADP, 50 µM, Sigma) or adenosine 5'-triphosphatein (ATP, 100 µM, Sigma) were introduced as a steady flow after the baseline measurement. The fast switching excitation wavelengths of 340 nm and 380 nm were provided by a monochromator (Polychrome IV, TILL Photonics GmbH Gräfelfing, Germany), and fluorescence images were acquired through a CCD camera (Micromax YHS1300, Roper Scientific, Trenton, NJ, U.S.A.) attached to a fluorescence microscope (IX70, Olympus, Tokyo, Japan) at a sampling rate of 0.5 Hz. The monochromator, CCD camera, and image acquisition were controlled by MetaFluor (Molecular Devices, Downingtown, PA, USA). Data analysis was performed using ImageJ software (NIH, Bethesda, MD, USA).

Transwell migration assay

iMG cells ($5.5x10^4$ cells/well) were pre-exposed to DMSO or PSB0739 for 1 hr at 37°C in 5% CO_2 cell culture incubator. The cells were then washed three times with basal medium and added to the inner compartment of a FluoroBlok™ Transwell chamber (8 µm polycarbonate inserts in 24 wells; Corning) containing either ADP (50 µM) or ATP (100 µM) in the bottom chamber. The transwells were then incubated at 37°C in a 5% CO_2 incubator. After 6 hrs, the cells were washed three times with PBS and fixed in 4% PFA for 15 min at room temperature. Next the cells were stained with DAPI for 10 min in order to visualize nuclei of cells. Fluorescent images of the cells were captured using a Zeiss inverted microscope fitted with a Andor Zyla cMOS camera. Data analysis was further performed using ImageJ software.

Laser ablation and time-lapse imaging

iNs and iMGs were co-cultured on PLL/vitronectin/laminin coated 18-mm coverslips from day 4. On days 9-11, the coverslips were transferred to the Ludin Chamber (Life Imaging Services, Swithland) and covered with CO_2-independent medium (Gibco) supplemented with N2, B27, NEAA, GlutaMAX, PSG, IL-34 (100 ng/ml), M-CSF (20 ng/ml), GM-CSF (20 ng/ml), TGFβ-1 (20 ng/ml), BDNF (10 ng/ml), NT3 (10 ng/ml) and doxycycline (2 µg/mL). Propidium iodide (PI, 1 µM, Sigma) was then added to label the dead cells. For imaging, each selected field contained a clump of 10-20 neuronal (iN) cell bodies; this clump was central to the selected field and was surround by iN and iMG cells. For laser ablation, the central neuronal clump was exposed to a 408-nm wavelength laser beam for 5 minutes to induce cell death. To document the responses of iN and iMG cells toward the laser induced cell death, images were acquired using a Nikon inverted microscope with a 20x objective lens over 12 hours with a frame taken every 5 minutes. Cell movement and location was tracked using the Manual Tracking plugin of imageJ. The iMG cells with a migration distance exceeding two cell bodies were defined as migrating iMGs and these were then included into the tracking results. The distances of each iMG cell body relative to the center of its neuronal clumps on each time point were calculated.

Statistics

Data are shown as means ± SEM. Statistical significance was assessed using *t*-test or one-way ANOVA together with the appropriate *post hoc* test as indicated. All tests were done using GraphPad Prism software (San Diego, CA, USA). Values are indicated in the figures as * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ and n.s. (not significant).

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