

Supplementary Table 1. List of primary and secondary antibodies used for immunocytochemistry

Name	Source	Dilution
DAPI	Thermo Scientific, Cat. no. 62248	1:1000
OCT4	Cell Signaling Technology, Cat. no. 9656	1:300
NANOG	Cell Signaling Technology, Cat. no. 9656	1:300
TRA-1-60	Cell Signaling Technology, Cat. no. 9656	1:300
SOX17	R&D systems, Cat. no. SC022	1:100
GATA4	R&D systems, Cat. no. SC022	1:100
BRACHYURY	R&D systems, Cat. no. SC022	1:100
HAND1	R&D systems, Cat. no. SC022	1:100
OTX2	R&D systems, Cat. no. SC022	1:100
SOX1	R&D systems, Cat. no. SC022	1:100
CTIP2	Millipore, Cat. no. MABE145	1:400
SATB2	Abcam, Cat. no. ab51502	1:300
TMEM119	Proteintech, Cat. no. 66948	1:500
IBA1	Cell Signaling Technology, Cat. no. 17198	1:500
MAP2	EnCor, Cat. no. CPCA-MAP2	1:500
GFAP	Cell Signaling Technology, Cat. no. 3670	1:500

Name	Source	Dilution
Donkey anti-Rabbit IgG	Invitrogen, Cat. no. A21206	1:1000
Donkey anti-Rabbit IgG	Invitrogen, Cat. no. A10042	1:1000
Goat anti-Mouse IgM	Invitrogen, Cat. no. A21043	1:1000
Goat anti-Mouse IgG	Invitrogen, Cat. no. A11001	1:1000
Goat anti-Mouse IgG	Invitrogen, Cat. no. A11003	1:1000
Goat anti-Chicken IgG	Invitrogen, Cat. no. A21449	1:1000

Supplementary Table 2. Stepwise medium compositions for generating microglia-containing cerebral organoids

Day	Medium phase	CO medium composition	MG medium composition
0	Pre-induction	mTeSR Plus (STEMCELL Technologies, Cat. No. 100-0276) with 10 μ M ROCK inhibitor (Y-27632; Selleckchem, Cat. No. S1049)	
1 - 7	Early induction	CO-1: DMEM/F12 (Corning, Cat. No. 10-0990-CV) with 10% KOSR (Gibco, Cat. No. 10828028), 1X P/S (Gibco, Cat. No. 15140122), 1X NEAA (Gibco, Cat. No. 11140050), 1X Glutamax (Gibco, Cat. No. 35050061), Dorsomorphin (2 μ M; (Selleckchem, Cat. No. S7840), A83-01 (2 μ M)	MG-1: mTeSR Plus (STEMCELL Technologies, Cat. no. 100-0276) with SCF (20 ng/ml; STEMCELL Technologies, Cat. No. 78062.1), BMP4 (50 ng/ml; PeproTech, Cat. No. 120-05), VEGF (50 ng/ml; PeproTech, Cat. No. 100-20)
8 - 14	Commitment	CO-2: DMEM/F12 (Corning, Cat. No. 10-0990-CV), 1X P/S (Gibco, Cat. No. 15140122), 1X NEAA (Gibco, Cat. No. 11140050), 1X Glutamax (Gibco, Cat. No. 35050061), 1X N2 supplement (Gibco, Cat. No. 17502048), CHIR99021 (1 μ M; Sigma-Aldrich, Cat. No. SML1046), Cyclopamine (2 μ M; Selleckchem, Cat. No. S1146), SB431542 (1 μ M; Tocris, Cat. No. 1614)	MG-2: VIVO 15 (Lonza, Cat. No. 02-060F) with IL-3 (25 ng/ml; STEMCELL Technologies, Cat. No. 78040.1), M-CSF (100 ng/ml; PeproTech, Cat. No. 300-25)
15 - 28	Early differentiation	CO-3: DMEM/F12: Neurobasal medium (1:1; Corning, Cat. No. 10-0990-CV, Gibco, Cat. No. 21103049), 1X P/S (Gibco, Cat. No. 15140122), 1X NEAA (Gibco, Cat. No. 11140050), 1X Glutamax (Gibco, Cat. No. 35050061), 0.1 mM β -mercaptoethanol (Gibco, Cat, No. 21985023), 1X N2 supplement (Gibco, Cat. No. 17502048), 1X B27 supplement (Gibco, Cat. No. 17504044), human Insulin (2.5	MG-2: (same as above)

		µg/ml; (Sigma-Aldrich, Cat. No. A1895601)	
29 - 69	Mid-stage development	CO-3: (same as above)	MG-3: Advanced DMEM/F12 (Gibco, Cat. No. 12634010) with IL-34 (100 ng/ml; PeproTech, Cat. No. 200-34), GM-CSF (10 ng/ml; PeproTech, Cat. No. 300-03)
≥70	Late-stage development	CO-4: Neurobasal Gibco, Cat. No. 21103049), 1X P/S (Gibco, Cat. No. 15140122), 0.1 mM β-mercaptoethanol (Gibco, Cat, No. 21985023), 1X B27 supplement (Gibco, Cat. No. 17504044), 1 µM cAMP (Selleckchem, Cat. No. S7858), 0.2 mM ascorbic acid (Sigma-Aldrich, Cat. No. A0278), BDNF (20 ng/ml; PeproTech, Cat. No. 450-02), and GDNF (20 ng/ml; PeproTech, Cat. No. 450-10)	MG-3: (same as above)

*Media mixed at a 1:1 ratio.

Supplementary Table 3. Primer sequences used for quantitative PCR analysis

Gene	Sequences (5'-3')	Usage
<i>FOXG1</i>	F-ACTGAACCCCAACAAAACC R-TTCTGAGCCGAGAATGTCC	qRT-PCR
<i>CTIP2</i>	F-TCCAGCTACATTTCACAACA R-GCTCCAGGTAGATGCGGAAG	qRT-PCR
<i>TBR1</i>	F-CACTGGAGGTTTCAAGGAGG R-TCTTGGCGCATCCAGTGAGC	qRT-PCR
<i>MAP2</i>	F-CAGGAGACAGAGATGAGAATTCC R-CAGGAGTGATGGCAGTAGAC	qRT-PCR
<i>GFAP</i>	F-AACCTGCAGATTCGAGGGGG R-CAGGCAGCTAACCGCGA	qRT-PCR
<i>GRIN1</i>	F-GAGAAGGAGAACATCACCGAC R-GTCCCCATCCTCATTGAACTC	qRT-PCR
<i>GRIA2A</i>	F-CGCTGTCATATTCCTGGCTAG R-GCACTGTCCCAAATCGAAAAG	qRT-PCR
<i>P2RY12</i>	F-AACGAGGGGTGTAGGTAAAG R-TTCAGCAGTGCAGTCAAAG	qRT-PCR
<i>TREM2</i>	F-ATCTCCAGGGCTGAGAGAC R-AGGAGAAGCAGTGTTTCAGG	qRT-PCR
<i>TMEM119</i>	F-TTCCTGGATGGGATAGTG R-GAAGGACGATGGGTAATAG	qRT-PCR
<i>CD68</i>	F-TCAACTGCCACTCACAGTCC R-TGGGCAGAACTGGTGAATCC	qRT-PCR
<i>IL1β</i>	F-AGCTACGAATCTCCGACCAC R-CGTTATCCCATGTGTGAAGAA	qRT-PCR
<i>TNFα</i>	F-CCTCTCTCTAATCAGCCCTCTG R-GAGGACCTGGGAGTAGATGAG	qRT-PCR
<i>CCL2</i>	F-CAGCCAGATGCAATCAATGCC R-TGGAATCCTGAACCCACTTCT	qRT-PCR
<i>β-actin</i>	F-TCAAGATCATTGCTCCTCCTGAG R-ACATCTGCTGGAAGGTGGACA	qRT-PCR

F, forward primer; R, reverse primer; qRT-PCR, quantitative RT-PCR.