Human iPSC-derived microglial cells protect neurons from neurodegeneration in long-term cultured adhesion brain organoids

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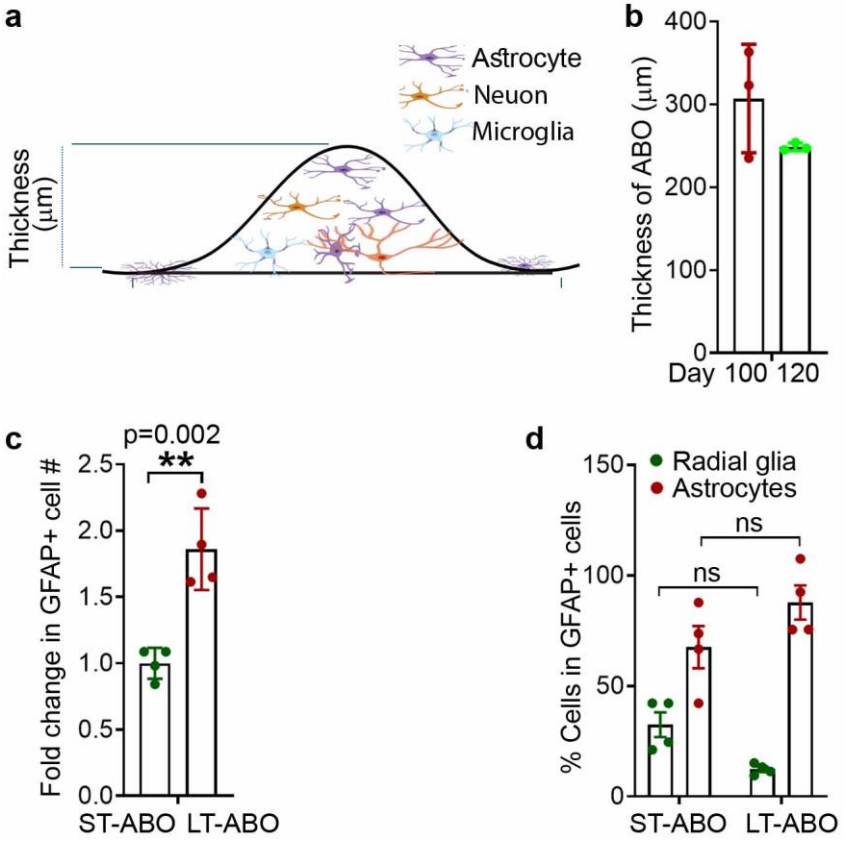
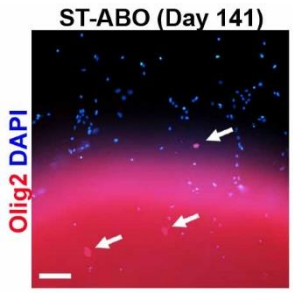
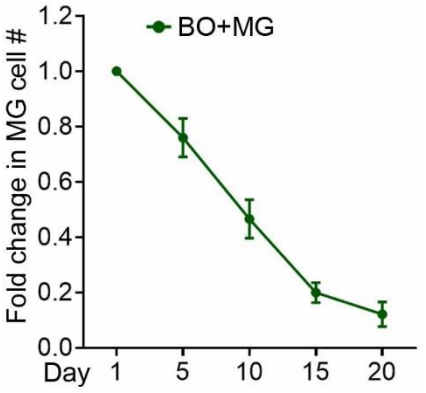


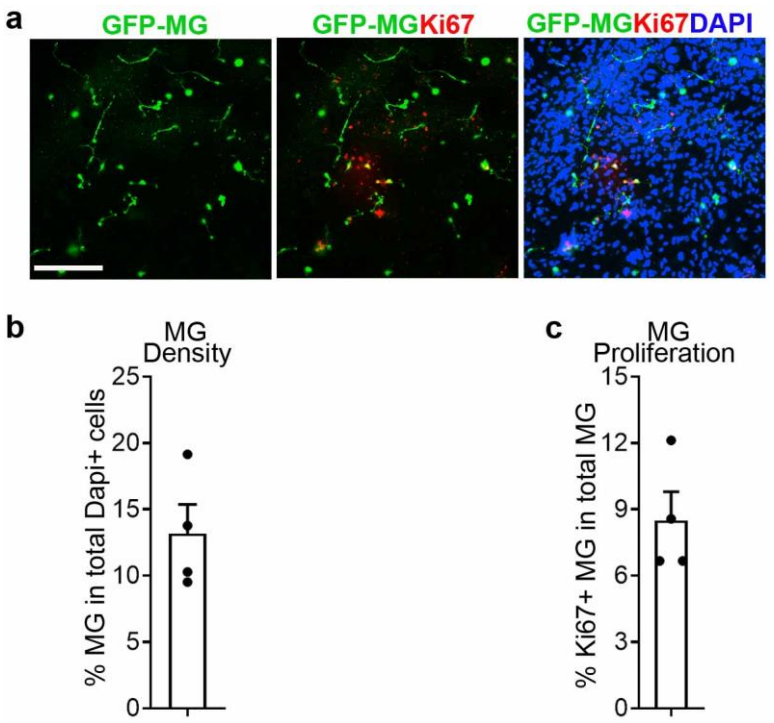
Supplementary Figure 1 Characterization of ABO. a, A schematic showing the shape of the ABO. **b**, The thickness of the core of the ABO. n=3 organoids for each stage. Error bars are SD of the mean. **c**, The fold change in $GFAP^+$ cells in the outer region of the ST-ABO and the LT-ABO. n=4 organoids for the ST-ABO and the LT-ABO, respectively. Error bars are SD of the mean; **p<0.01 by unpaired t-test. **d**, The percentage of the $SOX2^-GFAP^+$ astrocytes and the $SOX2^+GFAP^+$ radial glial cells in $GFAP^+$ cells in the outer region of the ST-ABO and the LT-ABO. n=4 organoids for the ST-ABO and the LT-ABO, respectively. Error bars are SEM of the mean; ns: p>0.05, not statistically significant by two-way ANOVA followed by Tukey's multiple comparison test.



Supplementary Figure 2 Differentiation of OPC in the ST-ABO. Representative image of Olig2 staining in the ST-ABO at day 141 of organoid differentiation. Scale bar, 50 μm.



Supplementary Figure 3 Co-culture of microglia with the conventional brain organoids (BO). The fold change of microglia (MG) cell number (#) in the co-culture with the conventional BO for 20 days. n=3 organoids for each time point. Error bars are SD of the mean.



Supplementary Figure 4 Microglial status in the LT-ABO. a, Imaging of the LT-MG-ABO for the GFP-labeled microglia (GFP-MG) and the proliferative marker Ki67 immunostaining. The merged image with DAPI counterstaining is shown on the right. **b, c**, The density (% of GFP-MG cells in total Dapi+ cells, b) and proliferative rate (% of $Ki67^{+}$ GFP-MG cells in total GFP-MG cells, c) of microglia co-cultured with the LT-ABO. n=4 organoids. Error bars are SD of the mean.

Supplementary Table 1 The antibodies used in this study.

Antibodies	Vender	Catalog number
Goat polyclonal anti-SOX2	R&D	AF2018
Mouse monoclonal anti-TUJ1	Covance	PRB-435P
Rat monoclonal anti-CTIP2	abcam	ab18465
Rabbit monoclonal anti-TBR2	abcam	ab216870
Rabbit polyclonal anti-Olig2	Millipore	AB9610
Mouse monoclonal anti-O4 (IgM)	sigma	O7139
Mouse monoclonal anti-MOG	Millipore	MAB5680
Goat polyclonal anti-SOX10	R&D systems	AF2864-SP
Rat monoclonal anti-MBP	Millipore	MAB386
Chicken polyclonal anti-MAP2	abcam	ab5392
Mouse monoclonal anti-GFAP	Sigma	G3893
Goat polyclonal anti-IBA1	abcam	ab5076
Rabbit monoclonal anti-TREM2	abcam	ab209814
Mouse monoclonal anti-p-Tau (AT8)	Invitrogen	MN1020
Rabbit polyclonal anti-SYN1	synaptic system	106 103
Rabbit polyclonal anti-cleaved caspase 3 (c-Cas3)	Cell Signaling	9661S
Rabbit monoclonal anti-Ki67	Lab vision	RM-9106