

Supplementary Information

Tubular Human Brain Organoids to Model Neuroinflammation

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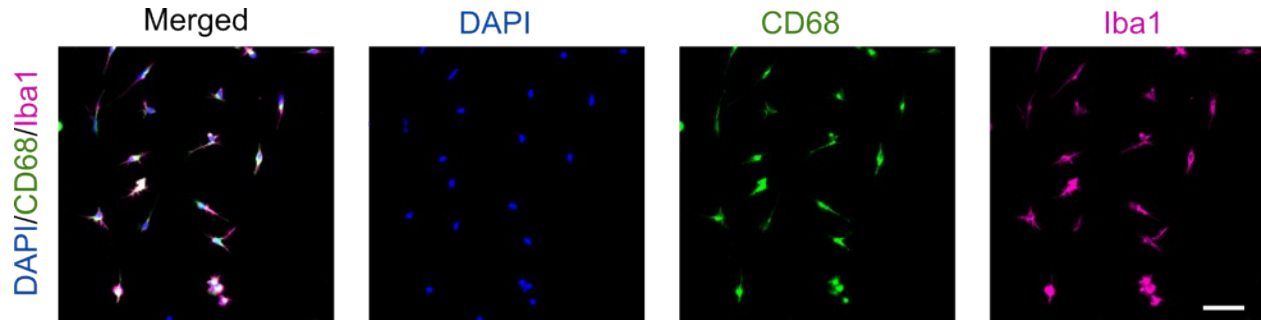


Figure S1 Characterization of induced microglia (iMG). Induced microglia from WA01 cells were stained for CD68 and Iba1 to confirm their identity. Scale bar: 50 μ m.

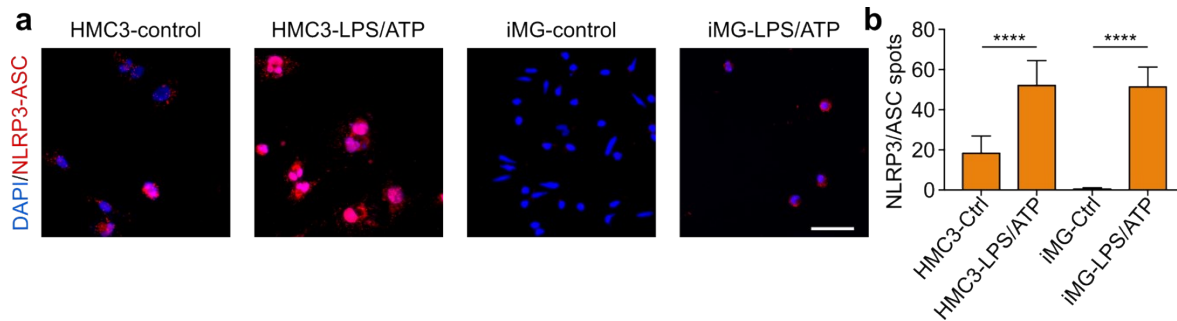


Figure S2 Inflammasome activation level in HMC3 and iMG cells. (a) Colocalization spots of NLRP3/ASC in HMC3 and iMG pre- and post- LPS/ATP treatment. (b) Quantification of baseline and LPS/ATP activated inflammasome levels by NLRP3/ASC colocalization spots per cell. Scale bar: 50 μ m.

Supplementary Tables

Table S1 Antibody used in immunofluorescence staining

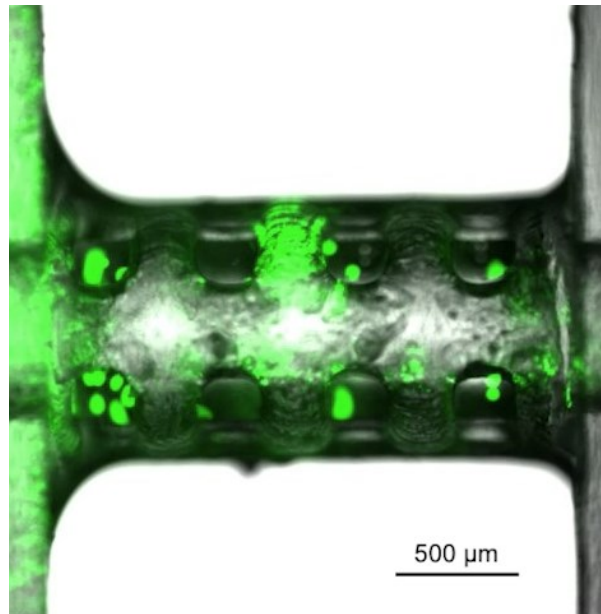
Antigen	Host	Vendor	Catalog#	Dilution
PAX6	Rabbit	Biologend	901301	1:500
MAP2	Chicken	Millipore	AB5543	1:500
GFAP	Rabbit	Abcam	Ab7260	1:500
IBA1	Goat	Abcam	Ab5076	1:500
CD68	Mouse	Thermo Fisher	MA5-13324	1:100
NLRP3	Mouse	Novus	NBP1-97601	1:200
ASC	Rabbit	Adipogen	AG-25B-0006	1:200

Table S2 Primer sequences for qPCR analysis

Gene	Primer sequence
PAX6 Fwd	AGT TCT TCG CAA CCT GGC TA
PAX6 Rev	ATT CTC TCC CCC TCC TTC CT
SOX2 Fwd	GGA TAA GTA CAC GCT GCC CG
SOX2 Rev	ATG TGC GCG TAA CTG TCC AT
FOXC1 Fwd	CGT TCA GCT ACA ACG CGC TCA T
FOXC1 Rev	CAG ATT GTG GCG GAT GGA GTT C
TBR1 Fwd	GAC TCA GTT CAT CGC CGT CA
TBR1 Rev	TCG TGT CAT AAT TAT CCC GAA ATC C
CTIP2 Fwd	CAG AGC AGC AAG CTC ACG
CTIP2 Rev	GGT GCT GTA GAC GCT GAA GG
TUJ1 Fwd	CTC AGG GGC CTT TGG ACA TC
TUJ1 Rev	CAG GCA GTC GCA GTT TTC AC
NKX2.1 Fwd	GGA GGG AGC TGG GGA GAG G
NKX2.1 Rev	ATT TTC GCG GAG GGC GGT CG
OTX-2 Fwd	TTA AAA TCT CTG CCA TGG AAA
OTX-2 Rev	AGA ACA AAA ACC CGT GCC TT
En2 Fwd	GAA CCC GAA CAA AGA GGA CA
En2 Rev	CGC TTG TTC TGG AAC CAA AT
Brachyury Fwd	CCC GTC TCC TTC AGC AAA GTC
Brachyury Rev	TGG ATT CGA GGC TCA TAC TTA TGC
SOX17 Fwd	AGA TGC TGG GCA AGT CGT
SOX17 Rev	GCT TCA GCC GCT TCA CC
HIF1a Fwd	TGC TCA TCA GTT GCC ACT TC
HIF1a Rev	AAA ACA TTG CGA CCA CCT TC
GAPDH Fwd	GAC AGT CAG CCG CAT CTT CT
GAPDH Rev	AAA TGA GCC CCA GCC TTC TC

Table S3 Forebrain protocol medium compositions

Ingredients	Concentrations
Forebrain Medium I (Day 1 – Day 6)	
DMEM/F12	1X
KOSR	20%
GlutaMax	1X
MEM-NEAA	1X
β-mercaptoethanol	1X
Dorsomorphin	2 μM
A-83-01	2 μM
Y-27632	5 μM
Penn/Strep	1X
Forebrain Medium II (Day 7 -Day 14)	
DMEM/F12	1X
N2 supplement	1X
GlutaMAX	1X
MEM-NEAA	1X
CHIR-99021	1 μM
SB431542	1 μM
Penn/Strep	1X
Forebrain Medium III (Day 14+)	
Neuralbasal medium	1X
GlutaMAX	1X
MEM-NEAA	1X
N2	1X
B27	1X
Penn/Strep	1X
Ascorbic Acid	0.2 mM
cAMP	0.5 mM
BDNF	20 ng/mL
GDNF	20 ng/mL



Video S1. Perfusion flow indicated by fluorescent beads inside the inner lumen of the tubular organoid device. Flow inside the inner lumen of the tubular organoid device as demonstrated by flushing of fluorescent beads. Scale bar: 500 μm .