

1 ***Escherichia Coli* K1-Colibactin Meningitis Induces Microglial NLRP3/IL-18 Exacerbating**
2 **H3K4me3-Synucleinopathy in Human Inflammatory Gut-Brain Axis**

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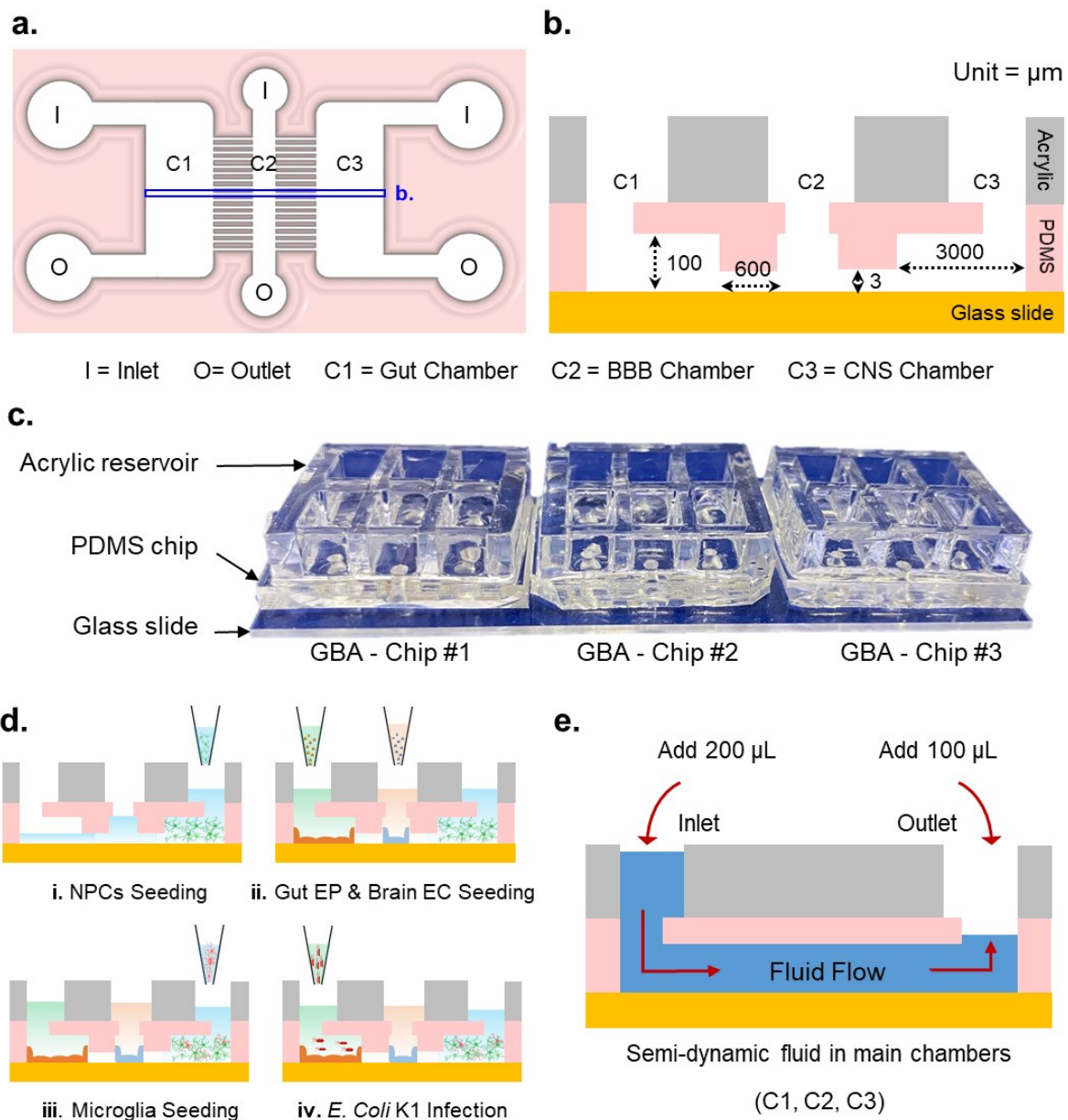
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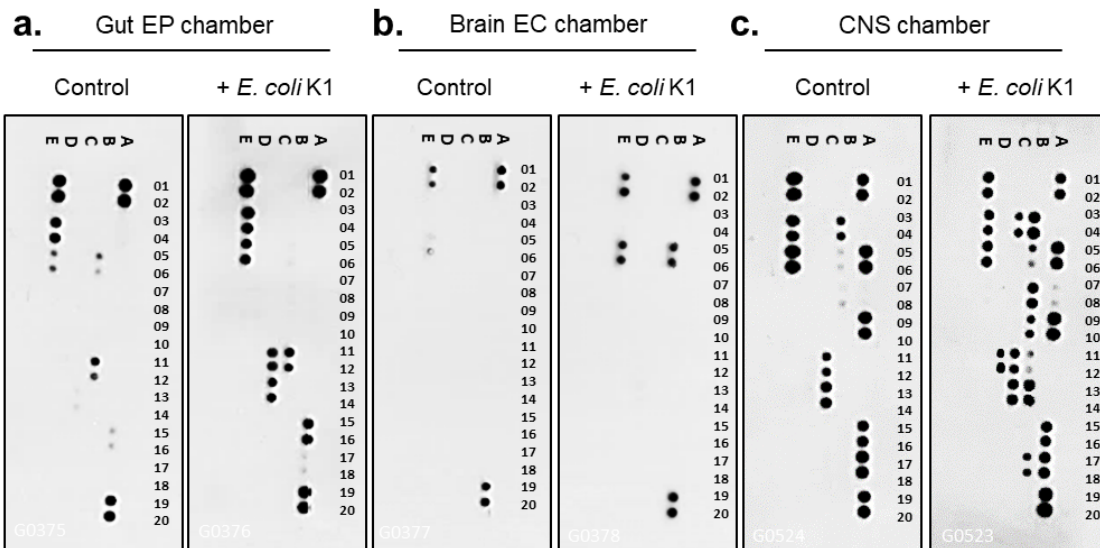
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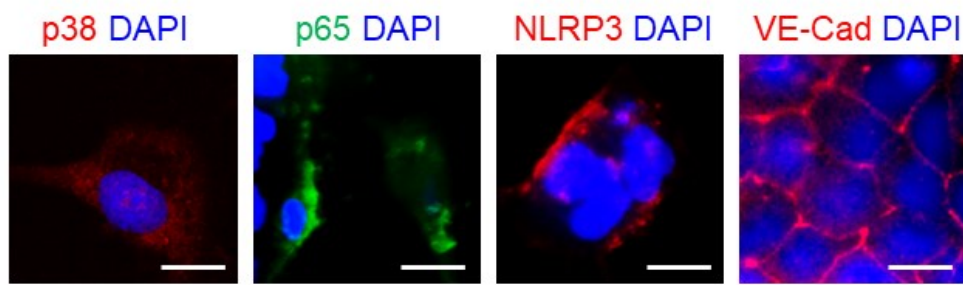
Supplementary Figure 1. Design of microfluidic platform for studying *Escherichia coli* K1 meningitis in gut-brain axis. **(a)** Top view of a GBA microfluidic chip with three main chambers connected by microchannels. **(b)** Cross section view of a GBA microfluidic chip with detailed dimensions. **(c)** An assembled platform with three GBA microfluidic chips on a glass slide. **(d)** A step-by-step procedure of cells seeding for establishing a GBA model. **(e)** Generation of semi-dynamic condition in gut EP, brain EC, and CNS chambers.



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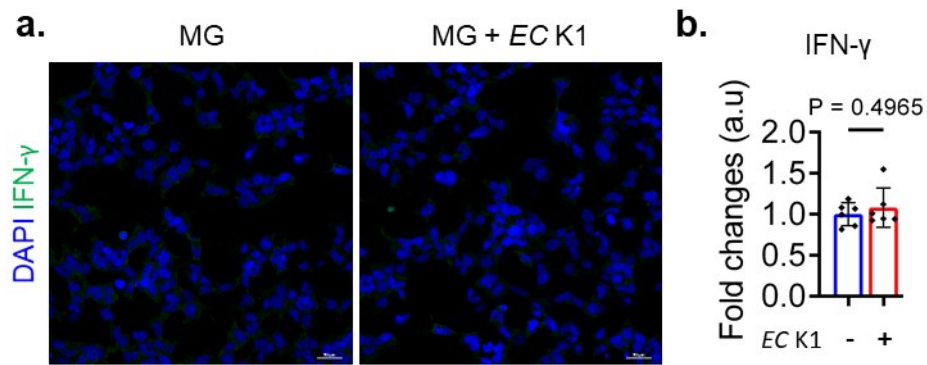
18 **Supplementary Figure 2.** Multiple cytokine detection from conditioned medium harvested in the gut epithelium
 19 chamber (a), the brain endothelium chamber (b), and the CNS chamber.

Co-culture of EP/EC + *E. coli* K1



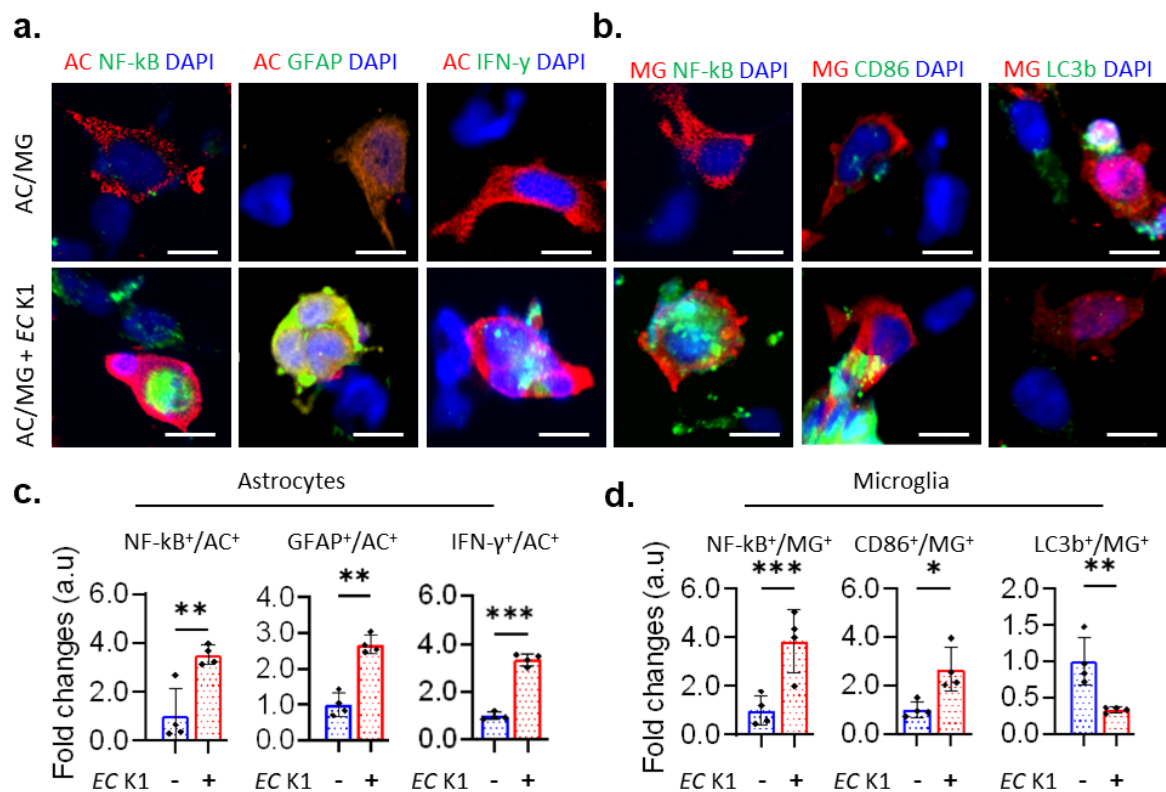
Supplementary Figure 3. Representative images of inflammatory markers in brain endothelium.

Scale bar, 20μm.



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24 **Supplementary Figure 4.** Fluorescent images **(a)** and quantification **(b)** of IFN- γ in microglia single culture
 25 during *E. coli* K1 meningitis. All experiments were repeated six times (n=6) and statistically analyzed by
 26 unpaired t-test. Scale bar, 50 μ m.



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28 **Supplementary Figure 5.** Fluorescent images showing inflammatory responses co-culture model of

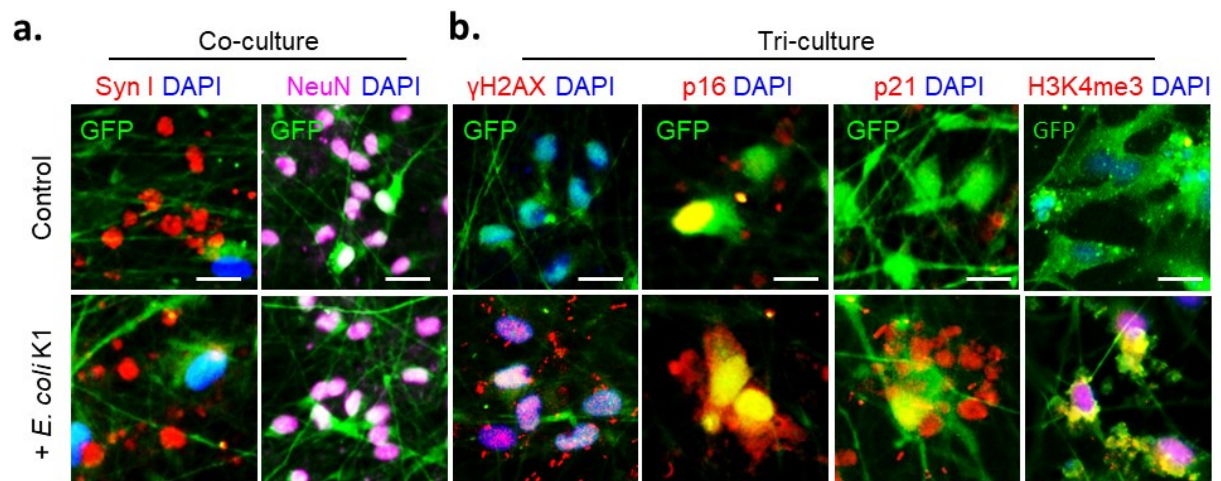
29 astrocytes and microglia during *E. coli* K1 meningitis. **(a&b)** Representative images of astrocytic and microglial

30 markers. **(c&d)** Quantification of markers expressed in microglia and astrocytes. All experiments were

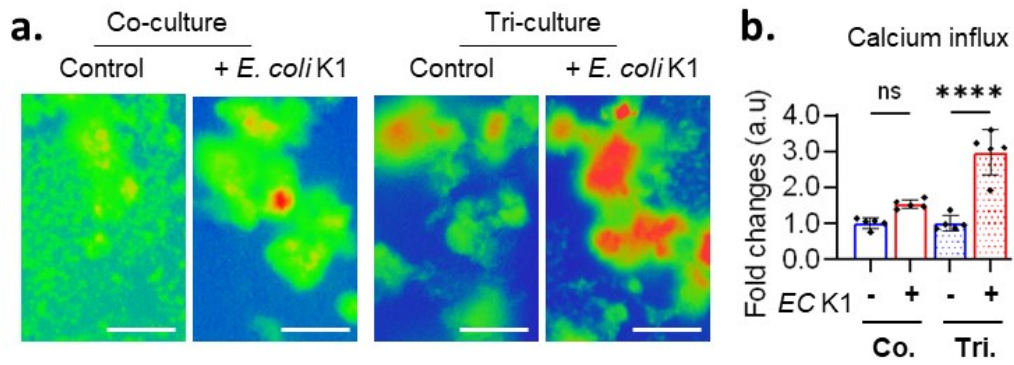
31 repeated four times independently and statistically analyzed by unpaired t-test. The NS, *, **, and ***

32 represented to no significance, p-value < 0.05, p-value < 0.01, and p-value < 0.001, respectively. All

33 experiments were repeated four times (n=4). Scale bar, 50 μ m.

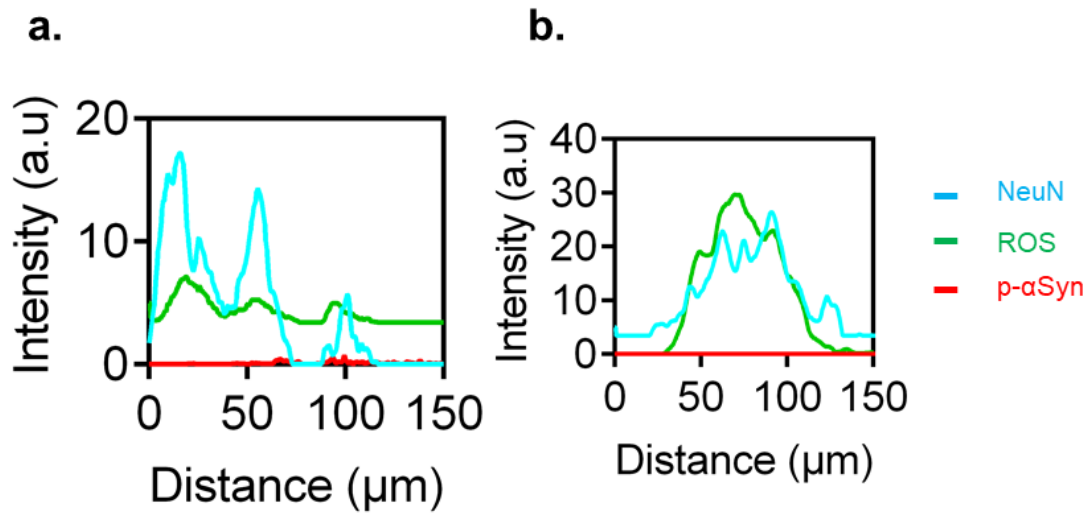


35 **Supplementary Figure 6.** Induction of neuropathological markers in co-culture (**a**) and tri-culture (**b**) during
 36 *E. coli* K1 meningitis. All experiments were repeated four times (n=4). Scale bar, 50 μ m.



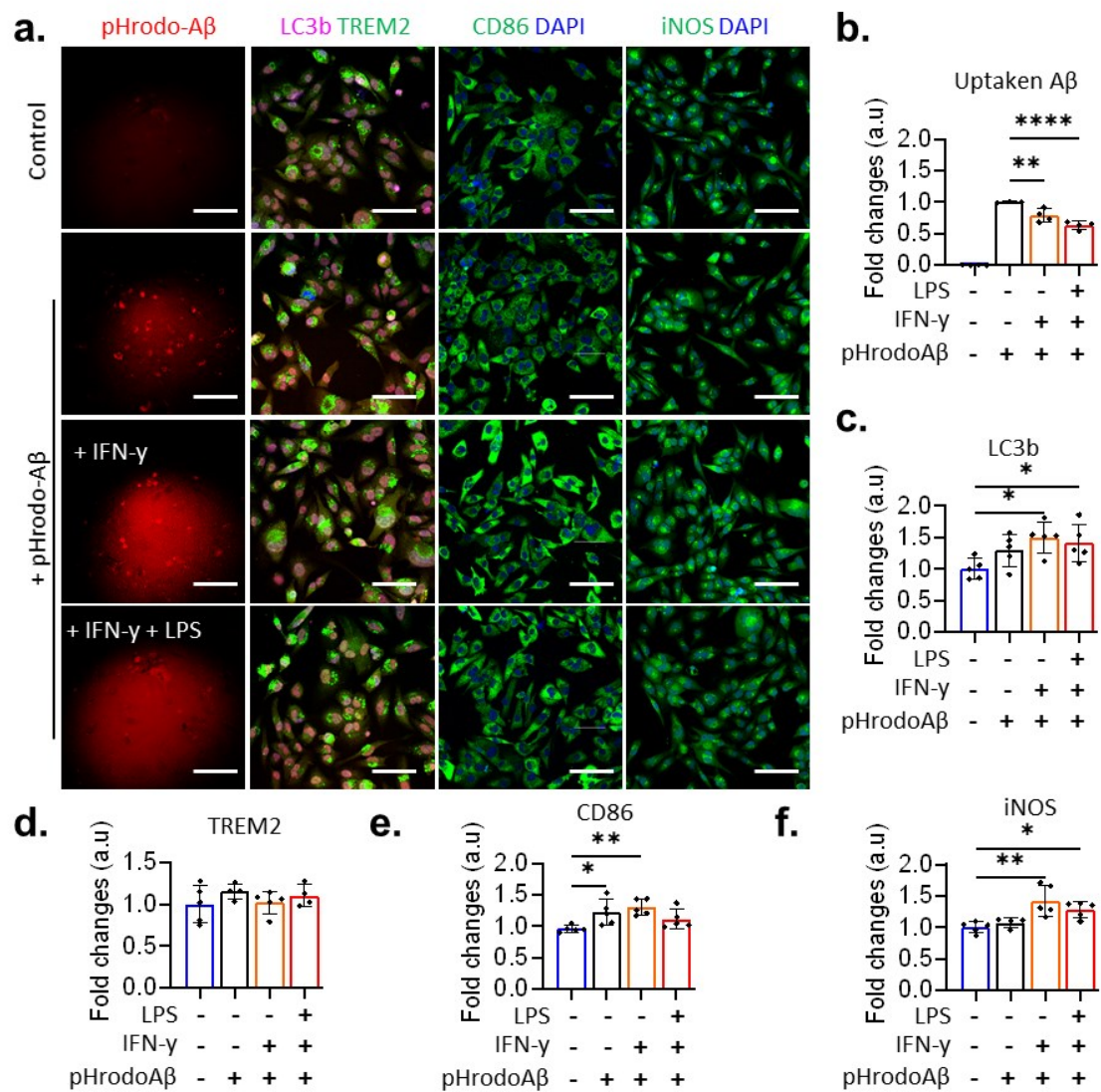
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38 **Supplementary Figure 7.** Mitochondrial calcium influx during *E. coli* K1 meningitis in co-culture and tri-culture
 39 model. **(a)** Representative images and **(b)** quantitative results of calcium influx were assessed and represented
 40 as bar graphs (n=4). All data are presented as mean $\pm$ SD measured by unpaired t-test. The NS, *, **, and ***
 41 represented to no significance, p-value < 0.05, p-value < 0.01, and p-value < 0.001, respectively. Scale bar,
 42 50 μ m.



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44 **Supplementary Figure 8.** Overlap fluorescent signal of NeuN/ROS/p- αSyn in co-culture of hNPC-derived
 45 neurons and astrocytes. Cyan for NeuN, green for ROS, and red for p- αSyn .



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47 **Supplementary Figure 9.** Reduction of microglial autophagy and phagocytosis of neurotoxic proteins, which
 48 worsen detrimental effects of hyper neuroinflammation in microglia. Quantitative results of calcium influx were
 49 assessed and represented as bar graphs (n=4). All data are presented as mean $\pm$ SD measured by One-way
 50 ANOVA followed by post-hoc Tukey test. The NS, *, **, and *** represented to no significance, p-value < 0.05,
 51 p-value < 0.01, and p-value < 0.001, respectively. Scale bar, 50 μ m.

Supplementary Table 1. List of antibodies used in the study

Antibodies	Company	Cat. No.	Dilution
VE-Cadherin polyclonal antibody	In vitrogen	36-1900	1:100
Alpha-synuclein	BD Transduction	610787	1:200
Phosphorylated alpha-synuclein (phospho S129)	Abcam	AB51253	1:200
p65-NFkB	Cell Signaling	6956S	1:200
CD86	Abcam	AB239075	1:300
GFAP	Sigma-Aldrich	AB5541	1:300
γ H2AX	Thermo Fisher Scientific	MA1-2022	1:400
p38 MAPK	Cell signaling	9212S	1:200
p21	Abcam	AB227443	1:100
p16	Sigma-Aldrich	SAB56000308	1:100
iNOS	Thermo Fisher Scientific	PA1-036	1:200
IRF3	Santa Cruz Biotechnology	SC-33641	1:200
NLRP3	Invitrogen	Ma-23919	1:200
H3K4me3 Monoclonal Antibody	Invitrogen	MA5-11199	1:200
NeuN Polyclonal Antibody	In vitrogen	PA5-78499	1:200
Synapsin-1	Abcam	AB254349	1:200
TREM2	Novus Biologicals	NBP1-07101	1:200
LC3b	Santa Cruz Biotechnology	SC-376404	1:200
Goat anti-mouse 2' Ab	Abcam	Ab150116	1:300
Alexa 594	Abcam	Ab150115	1:300
Alexa 647			
Goat anti-rabbit 2' Ab	Abcam	AB150080	1:300
Alexa 594	Abcam	AB150079	1:300
Alexa 647			
Goat anti-chicken 2' Ab	Abcam	AB150171	1:300
Alexa 647			
Donkey anti-Rat 2' Ab	Abcam		1:300
Alexa 647			

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Supplementary Table 2. The human cytokine array coordinates

Position	Row A	Row B	Row C	Row D	Row E
1, 2	Positive control				Positive control
3, 4	CCL1	CXCL11	IL-1ra	IL-13	MIF
5, 6	CCL2	CXCL12	IL-2	IL-16	Serpin E1
7, 8	MIP-1a	G-CFS	IL-4	IL-17A	TNF-α
9, 10	CCL5	GM-CFS	IL-5	IL-17E	TREM-1
11, 12	CD40	ICAM-1	IL-6	IL-18	
13, 14	C5a	IFN-γ	IL-8	IL-21	
15, 16	CXCL1	IL-1α	IL-10	IL-27	
17, 18	CXCL10	IL-1β	IL-12	IL32α	
19, 20	Positive control				Negative control

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