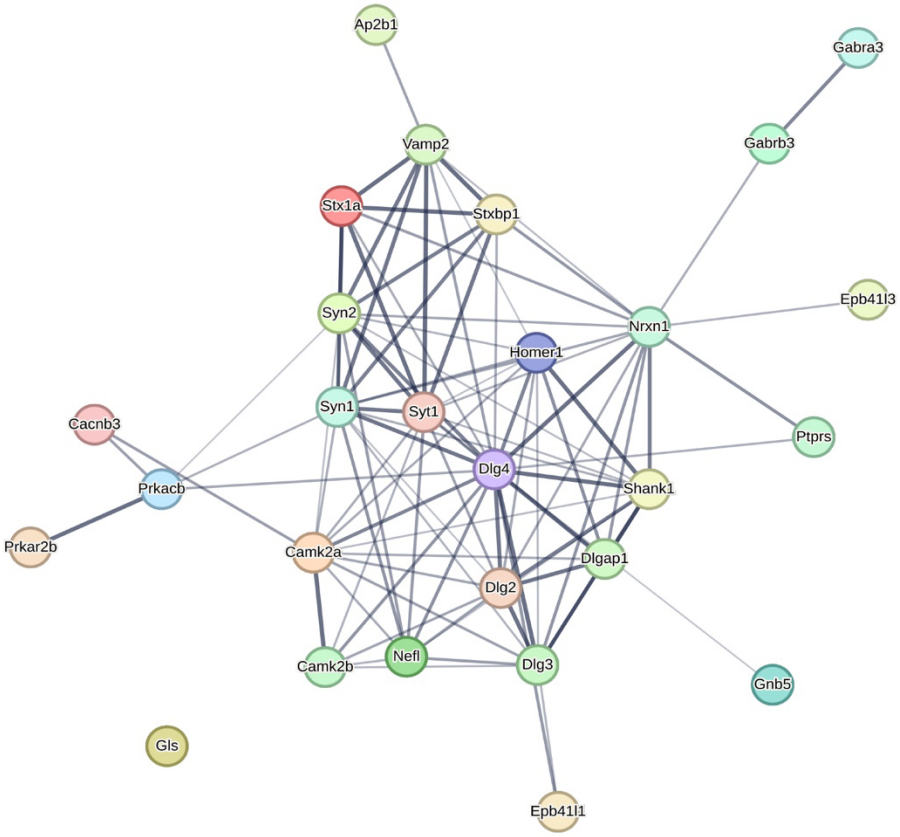
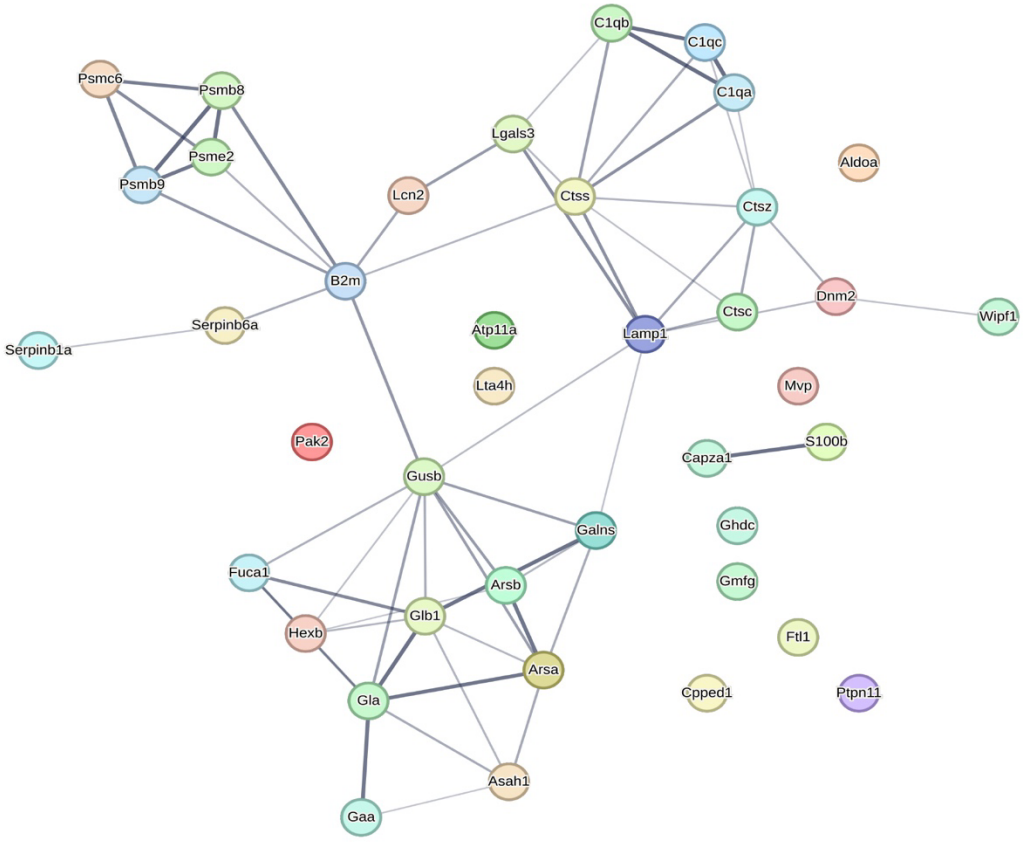
Supplementary Materials:

a

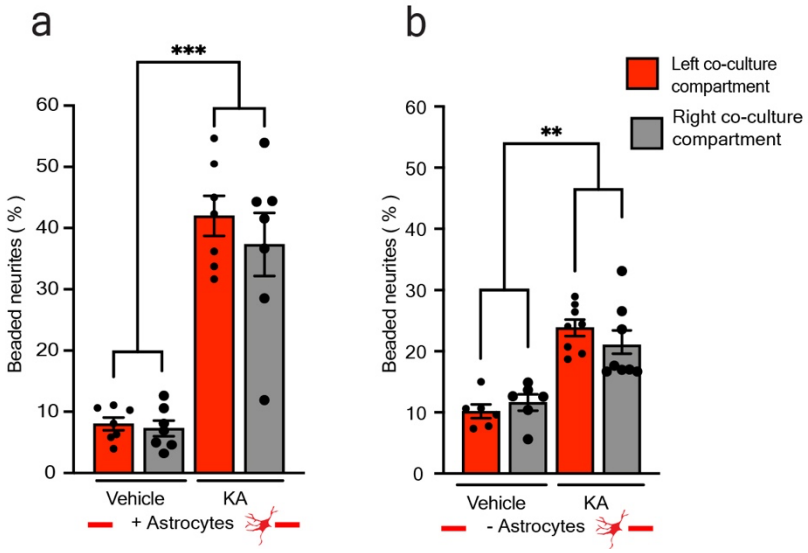


b



Supplementary Fig. 1. String analysis of the Reactome pathway of the cellular populations in different compartments of the microfluidic device.

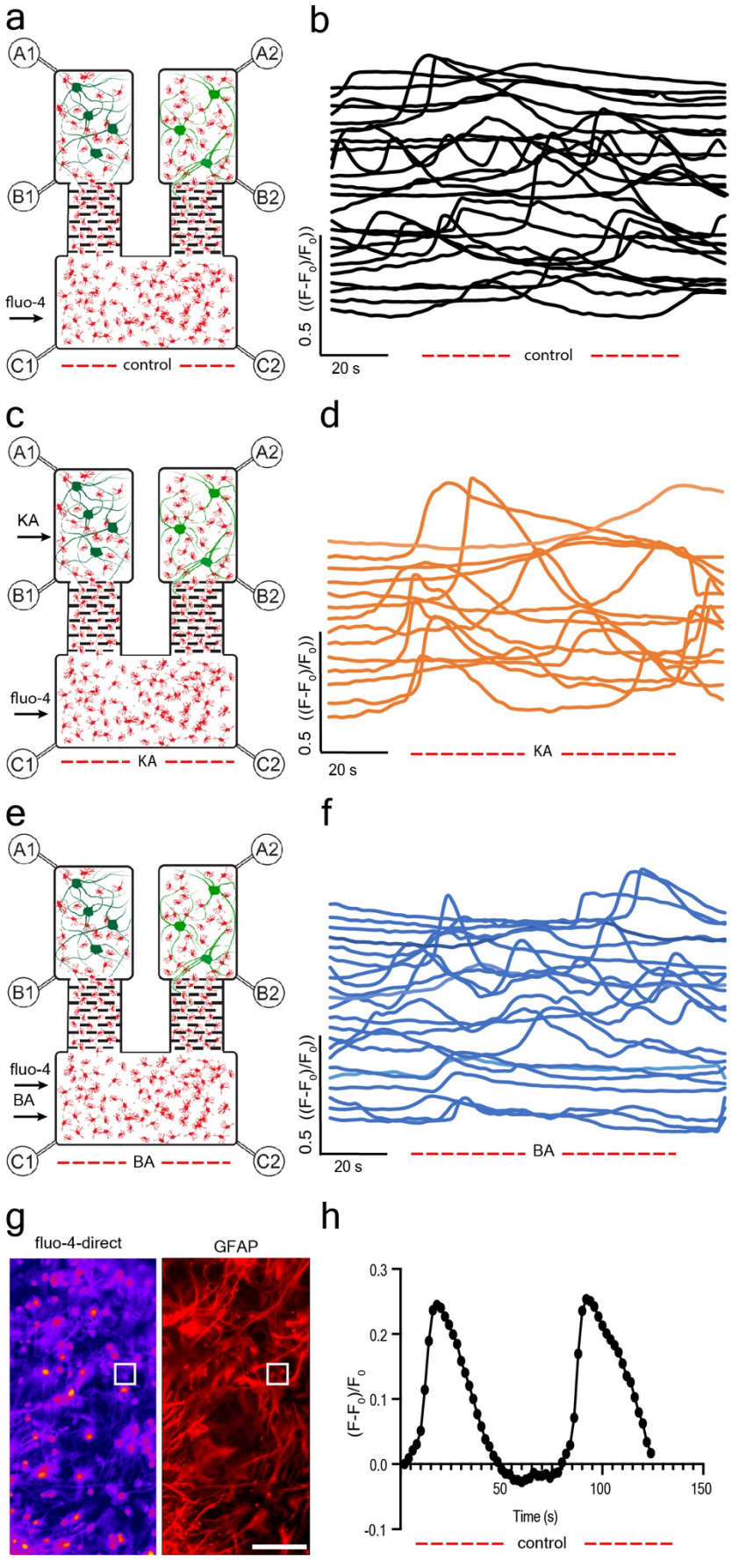
(a) STRING interaction network of significantly enhanced proteins expressed ($-\log_{10}$P value ≥5) in the co-culture compartments at 7 DIV that involved with neurotransmitter receptors, transmission across chemical synapse, and neuronal system.
(b) STRING interaction network of significantly enhanced proteins expressed ($-\log_{10}$P value ≥5) in the astrocyte-only compartments at 7 DIV that involved in innate immune system and neutrophil degranulation.



Supplementary Fig. 2. Kainic Acid (KA) treatment to the left co-culture compartment for 15 minutes caused a substantial loss of neurite integrity in neurons of the left and right co-culture compartments at 6 hours post-treatment in the presence of astrocytes.

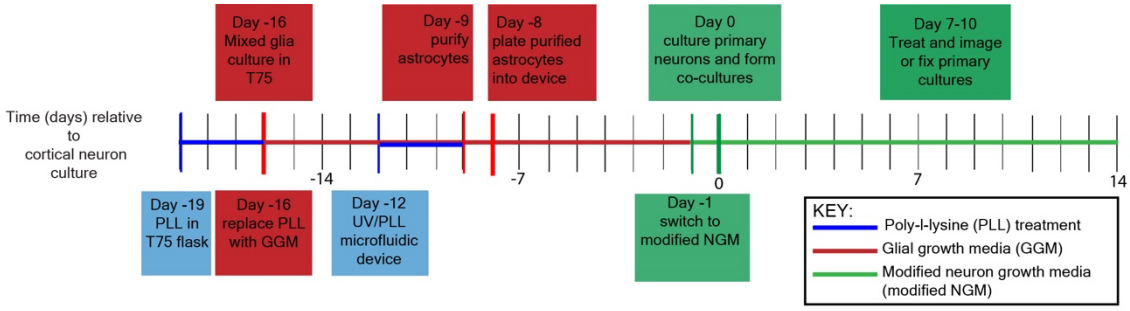
(a) Percentage of beaded axons in a mm^2 ROI in the left and right co-culture compartments at 6 hours, following a 15-minute KA treatment to the left co-culture compartment in the presence of astrocytes; ***$p<0.001$; one-way ANOVA with Tukey's post hoc test. Values represent mean ± SEM; n=3.

(b) Percentage of beaded axons in a mm^2 ROI in the left and right co-culture compartments at 6 hours, following a 15-minute KA treatment to the left co-culture compartment in the absence of astrocytes; **$p<0.01$; one-way ANOVA with Tukey's post hoc test. Values represent mean ± SEM; n=3.


a
A1
A2
B1
B2
fluo-4
C1
control
C2
b
0.5 ((F-F0)/F0))
20 s
control
c
A1
A2
KA
B1
B2
fluo-4
C1
KA
C2
d
0.5 ((F-F0)/F0))
20 s
KA
e
A1
A2
B1
B2
fluo-4
BA
C1
BA
C2
f
0.5 ((F-F0)/F0))
20 s
BA
g
fluo-4-direct
GFAP
h
0.3
0.2
(F-F0)/F0
0.1
0.0
-0.1
50
100
150
Time (s)
control

Supplementary Fig. 3. Effect of KA (1 mM) or BA (1 μM) on intracellular free calcium in astrocytes, measured with fluo-4-direct.

(a) Schematic diagram of microfluidic device populated with astrocytes and neurons. Arrows indicate addition of fluo-4-direct to the astrocyte-only compartment.
(b) Changes in fluorescence intensity of fluo-4-direct ($F\text{-}F_0/F_0$) over time, representing dynamic changes in free intracellular calcium levels in individual astrocytes of the astrocyte-only compartment.
(c) Schematic diagram of microfluidic device populated with astrocytes and neurons. Arrows indicate addition of KA (1 mM) to the left co-culture compartment, and addition of fluo-4-direct to the astrocyte-only compartment.
(d) Changes in fluorescence intensity of fluo-4-direct ($F\text{-}F_0/F_0$) in response to the addition of KA (1 mM) to the left co-culture compartment.
(e) Schematic diagram of microfluidic device populated with astrocytes and neurons. Arrows indicates addition of fluo-4-direct and subsequently BA, to the astrocyte-only compartment.
(f) Changes in fluorescence intensity of fluo-4-direct ($F\text{-}F_0/F_0$) in response to the addition of BA (1 μM) to the astrocytes-only compartment.
(g) Fluo-4-direct in live untreated astrocytes, and GFAP immunolabelling of the same astrocyte-only compartment. White box indicates ROI around an individual astrocyte soma. Scale bar = 20 μm.
(h) Dynamic changes in free intracellular calcium over time in the single untreated astrocyte soma from the ROI outlined in (g).



Supplementary Fig. 4. Timeline in days, for the preparation of the microfluidic device, the derivation, culture and plating of primary astrocytes and neurons, and maintenance of co-cultures in this microfluidic device.

Table S1: Proteins significantly ($-\log_{10}$P value ≥ 5) enriched in the astrocyte-only compartments identified via DAVID Reactome pathway analysis. Proteins are clustered by function in the innate immune system and neutrophil granulation.

Innate immune system		Neutrophil degranulation	
Asah1	Hexb	Fuca1	Ctss
Ftl1	Serpbinb1a	Ctsz	Lamp1
Lta4h	Lgals3	Lcn2	Lta4h
Gaa	Gusb	Atp11a	Gmfg
Gmfg	Psmc6	Asah1	Lgals3
Atp11a	Cpped1	Serpinb1a	hexb
Psmb9	C1qb	Aldoa	Ctss
C1qa	Serpinb6a	Gla	Lamp1
C1qc	Psmb8	Gusb	Lta4h
C1qb	Arsa	Gaa	Gmfg
Lamp1	Ctsz	Serpinb6a	Arsb
B2m	Ptpn11	Cpped1	Galns
Wipf1	Psme2	Mvp	B2m
S100b	Mvp	Glb1	Ctsc
Lcn2	Aldoa	Lgals3	Arsa
Glb1	Ghdc	hexb	Ghdc
Arsb	Fuca1		
Pak2	Ctss		
Capza1	Ctsc		
Gla	Dnm2		

Table S2: Proteins significantly ($-\log_{10}$P value ≥ 5) enriched in the co-culture compartments identified via DAVID Reactome pathway analysis. Proteins are clustered by involvement in neuronal system, the chemical synapse, and the Neurotransmitter Receptor.

Nervous System	Synaptic transmission	Post-synaptic receptors
Stx1a	Gnb5	Dlg2
Dlg3	Stx1a	Camk2a
Dlgap1	Camk2a	Gabra3
Gabrb3	Nefl	Prkar2b
Camk2a	Vamp2	Dlg4
Gabra3	Epb4111	Epb41l4
Vamp2	Prkacb	Dlg3
Syn1	Syt1	Gabrb3
Ep41l3	Camk2b	Ap2b1
Ap2b1	Prkar2b	Mef2c
Gnb5	Syn2	Prkacb
Dlg4	Cacnb3	Gnb5
Prkacb	Syn1	Nefl
Dlg2	Dlg4	Camk2b
Nrxn1	Ap2b1	
Epb4111	Gabra3	
Ptprs	Dlg3	
Homer1	Stx1bp1	
Camk2b	Gls	
Prkar2b	Dlg2	
Stxbp1	Gabrb3	
Gls		
Syn2		
Syt1		
Shank1		
Cacb3		
Nefl		