Cooperative and competitive regulation of the astrocytic transcriptome by neurons and endothelial cells: Impact on astrocyte maturation

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SUPPORTING FIGURES

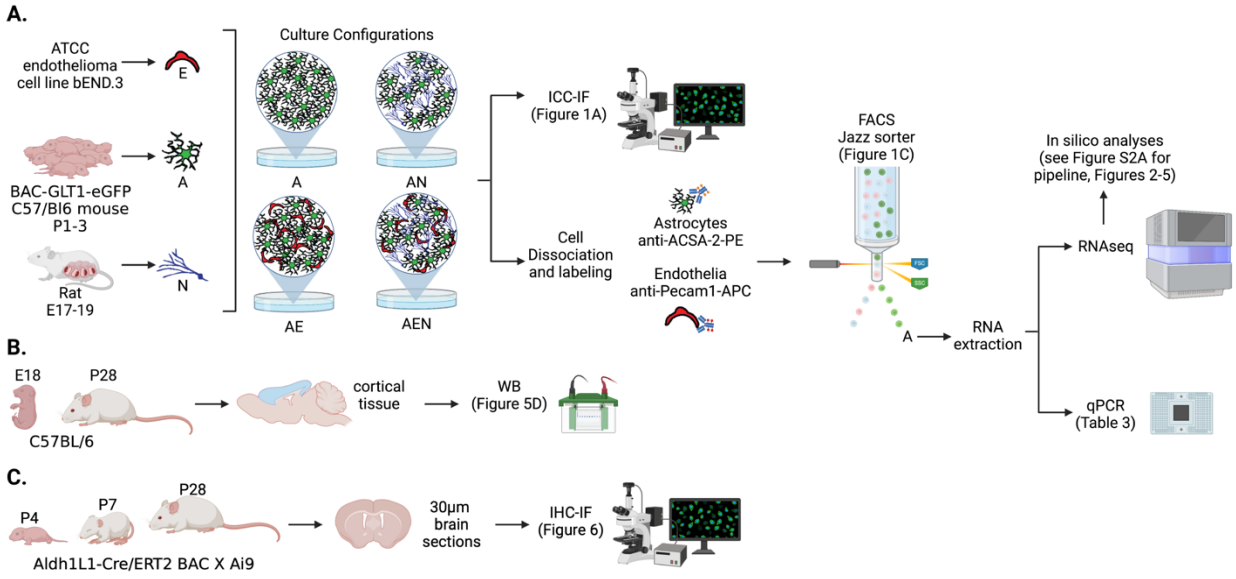


Figure S1. Graphical Timeline. A. Mouse astrocytes were cultured by themselves, in presence of neurons, endothelia, or their combination. After 10 days in culture cells were harvested for immunocytochemistry immunofluorescence (ICC-IF) or dissociated and labelled with anti-astrocyte cell surface antigen 2 (ACSA-2) antibodies or anti-Pecam1 antibodies and separated using fluorescence activated cell sorting (FACS). RNA was then isolated from the sorted astrocytes. RNA was analyzed by qPCR or sequenced. **B.** Cortical tissue was isolated from E19 and P28 mice, resolved in a western blot (WB) and probed with anti-Fibronectin antibodies. **C.** Coronal brain sections were obtained from P4,

P7, and P28 mice and immunohistochemistry immunofluorescence (IHC-IF) was performed for GAT3, FAM107A, and GLT1. See methods sections for details.

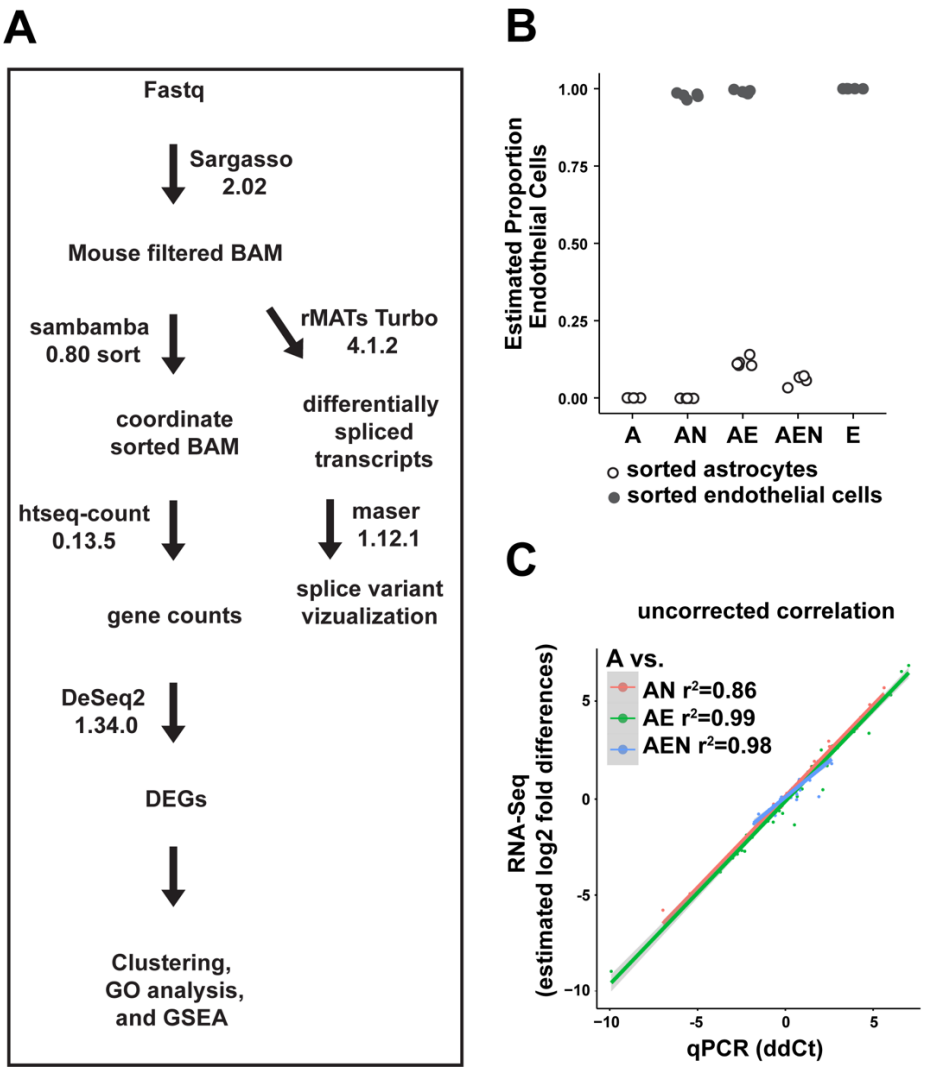
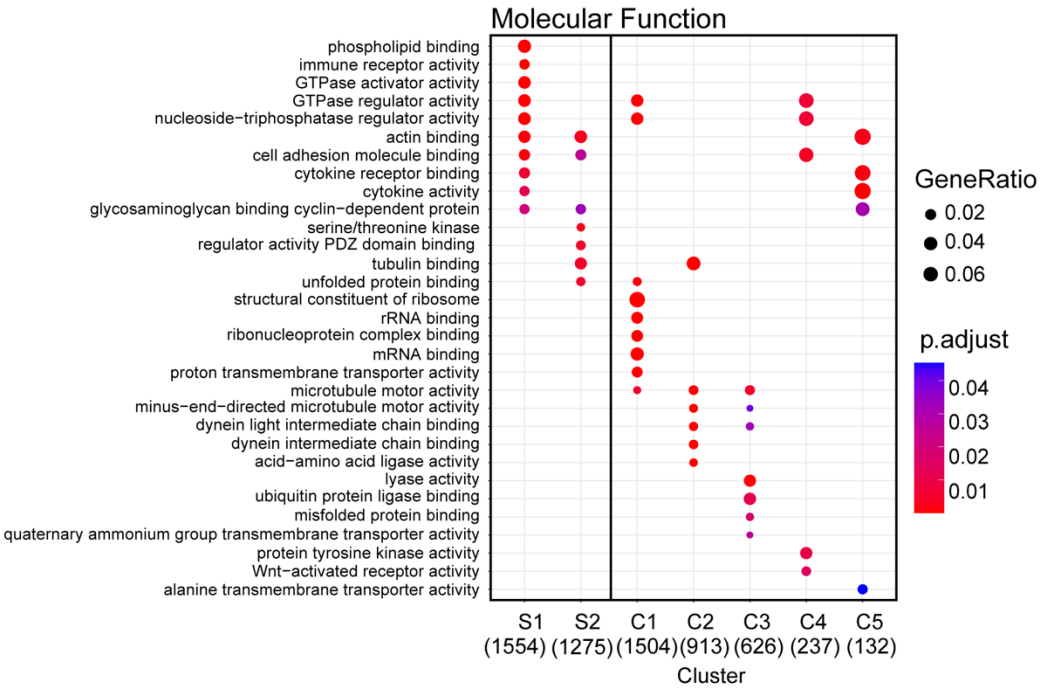


Figure S2. A. Pipeline schematic of RNA sequencing analysis. See methods for steps description. All code that processed the data can be found at GitHub (https://github.com/murailab/Astrocyte_Triple_Co-Culture). **B.** To estimate the percent composition of endothelia in each sample we used the average read counts for each gene in pure astrocytes and pure endothelial samples. As expected, A and AN do not contain

any endothelial-specific sequences. Although we set conservative gates (see Figure 1C), we estimated 12% and 6% of endothelial content in the astrocytes isolated from the AE and the AEN co-cultures respectively. **C.** Correlation of RNAseq uncorrected log_2-fold differences (Y-axis) versus quantitative PCR ddCt data (X-axis).

A


Molecular Function
phospholipid binding
immune receptor activity
GTPase activator activity
GTPase regulator activity
nucleoside−triphosphatase regulator activity
actin binding
cell adhesion molecule binding
cytokine receptor binding
cytokine activity
glycosaminoglycan binding cyclin−dependent protein
serine/threonine kinase
regulator activity PDZ domain binding
tubulin binding
unfolded protein binding
structural constituent of ribosome
rRNA binding
ribonucleoprotein complex binding
mRNA binding
proton transmembrane transporter activity
microtubule motor activity
minus−end−directed microtubule motor activity
dynein light intermediate chain binding
dynein intermediate chain binding
acid−amino acid ligase activity
lyase activity
ubiquitin protein ligase binding
misfolded protein binding
quaternary ammonium group transmembrane transporter activity
protein tyrosine kinase activity
Wnt−activated receptor activity
alanine transmembrane transporter activity
S1 (1554)
S2 (1275)
C1 (1504)
C2 (913)
C3 (626)
C4 (237)
C5 (132)
Cluster
GeneRatio
0.02
0.04
0.06
p.adjust
0.04
0.03
0.02
0.01

B

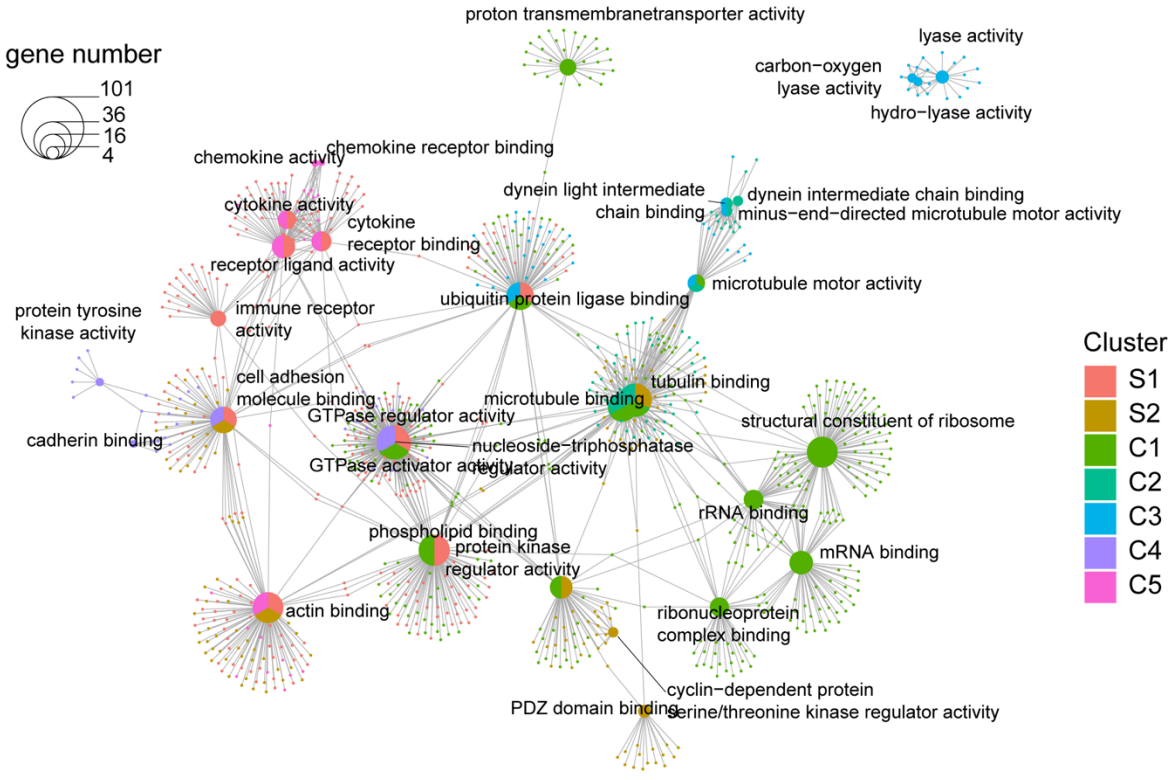

Molecular Function
gene number
101
36
16
4
proton transmembranetransporter activity
lyase activity
carbon−oxygen lyase activity
hydro−lyase activity
chemokine activity
chemokine receptor binding
cytokine activity
cytokine receptor binding
receptor ligand activity
dynein light intermediate chain binding
dynein intermediate chain binding
minus−end−directed microtubule motor activity
microtubule motor activity
ubiquitin protein ligase binding
protein tyrosine kinase activity
immune receptor activity
cell adhesion molecule binding
cadherin binding
GTPase regulator activity
GTPase activator activity
nucleoside−triphosphatase regulator activity
microtubule binding
tubulin binding
structural constituent of ribosome
rRNA binding
mRNA binding
phospholipid binding
protein kinase regulator activity
actin binding
ribonucleoprotein complex binding
cyclin−dependent protein serine/threonine kinase regulator activity
PDZ domain binding
Cluster
S1
S2
C1
C2
C3
C4
C5

Figure S3. A. Gene ontology molecular function enrichment analysis of differentially expressed genes in the clusters assigned in Figure 3B. The color code represents the adjusted p value. The size of the circle represents the gene ratio. **B.** Associated networks for the molecular function enrichment analysis shown in panel A. Each node represents an enrichment term, their size represents the number of genes enriched within that molecular function. The color code represents each gene cluster.

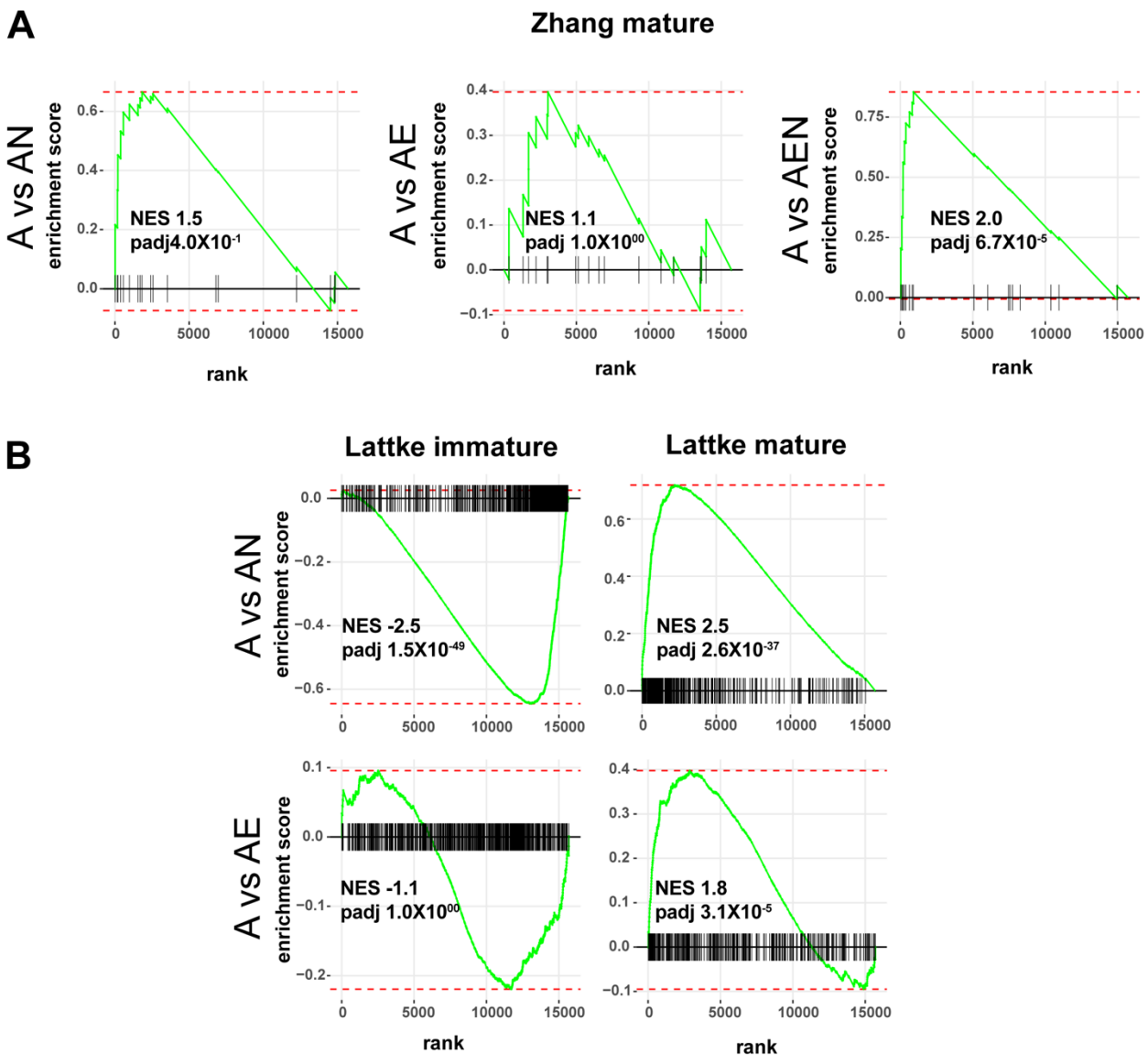


Figure S4. A. Gene set enrichment plots for astrocyte mature markers derived from a custom gene set composed from 18 genes (Zhang et al., 2016). This set of genes was significantly enriched in the co-culture configuration AEN, but not in AN or AE, compared to astrocytes grown in monocultures. Of this gene set we found 10, 0, and 9 genes that were significantly upregulated in the AN, AE, AEN co-culture configurations when compared to astrocytes grown in monoculture. **B.** GSEA of the 678 genes identified as enriched in immature astrocytes (left graphs) or the 359 genes enriched in mature astrocytes (right graphs) (Lattke et al., 2021) in astrocytes grown on their own versus astrocytes grown in the presence of neurons (AN) or endothelia (AE). The normalized enrichment score (NES) and adjusted p values are shown. Each vertical black line in the X-axis represents a gene and indicates its position in the studied gene set. Genes on the far left are enriched in the gene set analyzed, while genes on the far right are underrepresented. The Y-axis represent the enrichment score.

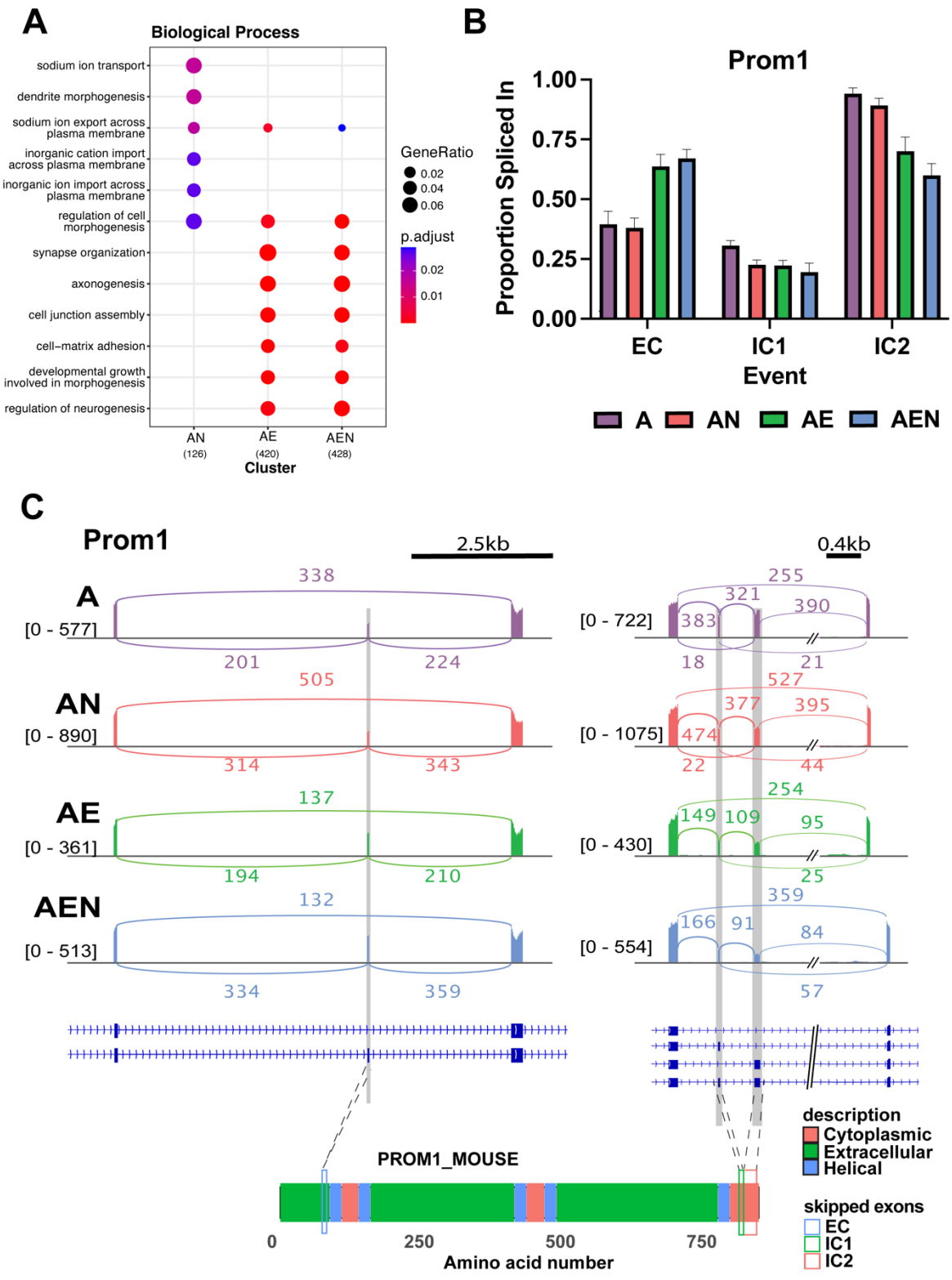


Figure S5. A. Gene ontology biological process enrichment analysis of differentially spliced mRNAs in astrocytes grown as monocultures versus those of astrocytes grown in

the presence of neurons and endothelial cells. The color code represents the adjusted p value. The size of the circles represents the gene ratio. The list with all gene ontology biological processes, the gene ratios, adjusted p values, and gene lists, are found in Table S3. **B.** Alternative splicing in three regions of the prominin 1 (*Prom1*) gene are shown from astrocytes isolated from monocultures (in purple), astrocytes grown in the presence of neurons (in pink), or endothelia (in green), or their combination (in blue). **C.** Sashimi plots representing the raw data of the number of reads mapped to the *Prom1* gene in astrocytes grown in the four different culture configurations, same color code that panel B. The height of the traces represents the number of reads. The gray column highlights differentially spliced exons. The transcript model of the *Prom1* gene in dark blue is from the genome browser Ensembl, boxes represent exons. EC= extracellular, IC1= intracellular region 1, IC2= intracellular region 2 are used to identify the areas in the Prom 1 protein affected by the alternative splicing events.

SUPPORTING TABLES

Table S1. List of all differentially expressed genes in the different culture configurations with full statistical report. Here we report 15,711 genes differentially regulated in the culture configurations. Results of the likelihood-ratio test (LRT) including base mean (column B), log2 fold changes (column C), and adjusted p values (column D) are shown. DESeq2 estimated differences between all culture configurations (columns E to P) are listed.

Table S2. Complete list of the gene ontology biological processes associated with the clusters identified in Figure 3B. Each cluster (cooperative: C1-C2, antagonistic: A1-A3, and redundant: R1-R2) is listed in a different sheet. Cluster, description of gene ontology biological process term, gene ratio (number of genes associated with the term divided by the total number of genes in that cluster), adjusted p-value, and the list of all genes associated with each biological process (gene ID) are listed.

Table S3. Complete list of the gene ontology biological processes and molecular functions associated with the differentially spliced mRNAs. Cluster, identifier, description of gene ontology biological process (BP sheet) or molecular functions (MF sheet) term, gene ratio (number of genes associated with the term over the total number of genes in that cluster), Bg ratio (number of genes included in the gene set that belong to the gene ontology term over the total number of genes identified in the gene set), p-value (raw), adjusted p-value, q value (false discovery rate adjusted p-value), the list of all genes associated with each biological process (gene ID), and count (number of genes differentially spliced associated in that gene ontology category) are listed.