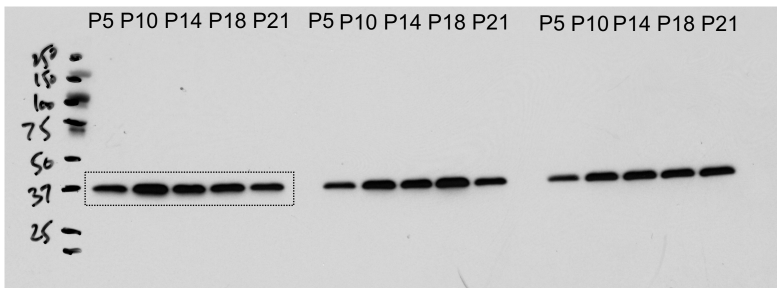
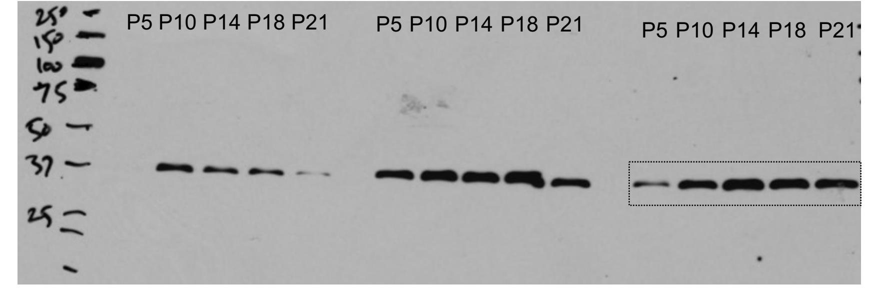
Supplementary Figure 1.

Cortex | Cerebellum

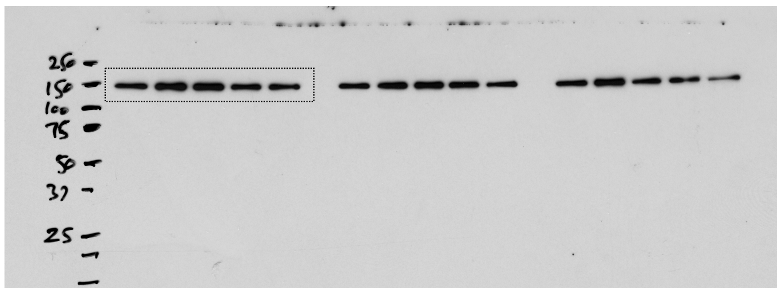
Set 1 Set 2 Set 3 | Set 1 Set 2 Set 3

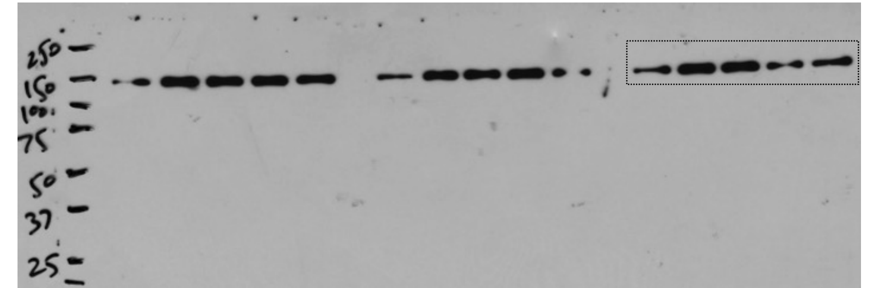
S1PR1



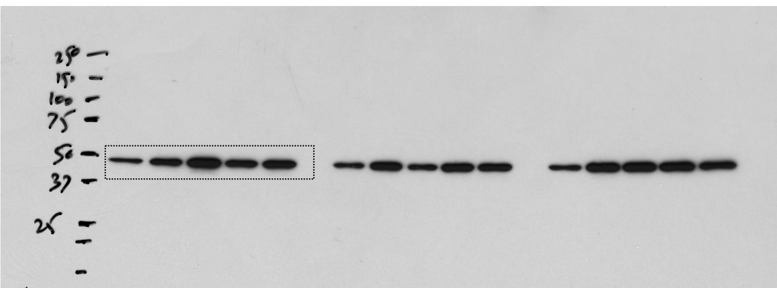


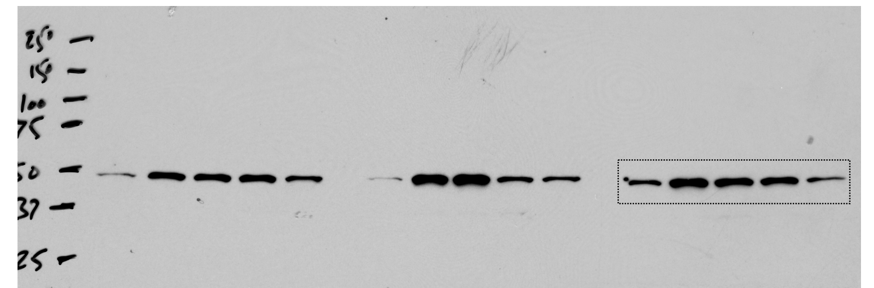
SPARCL1



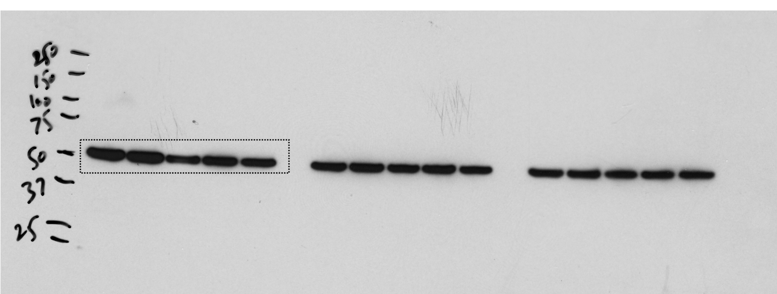


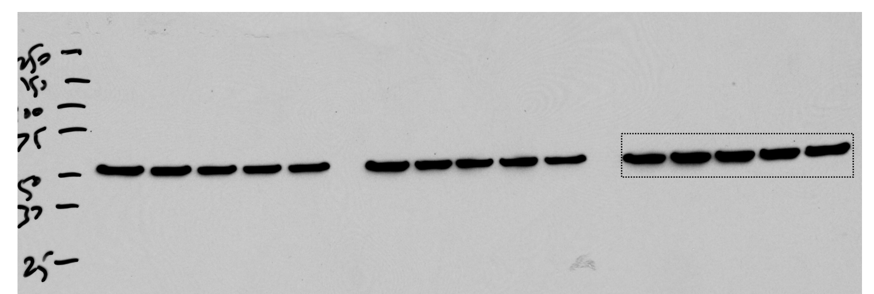
GFAP





Tubulin

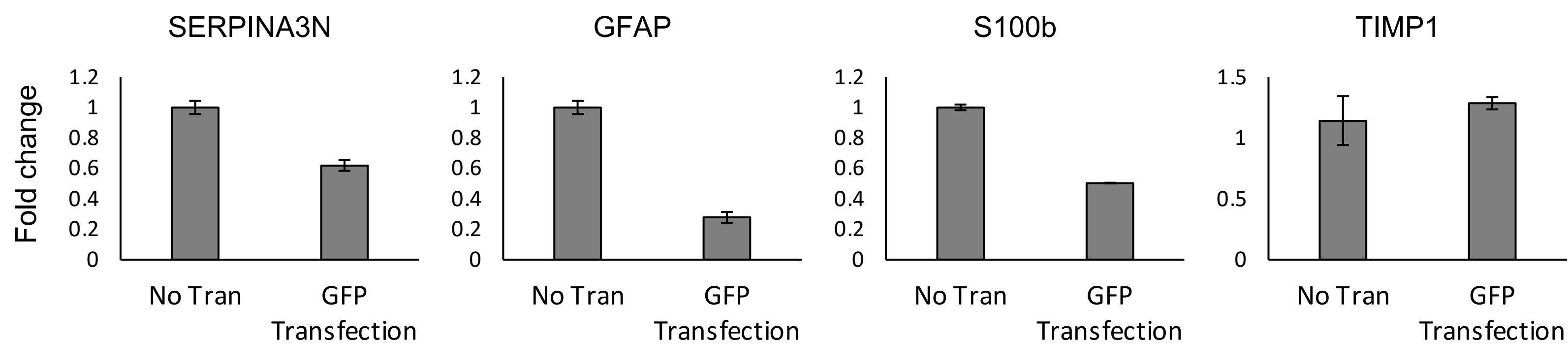




Supplementary Figure 2.

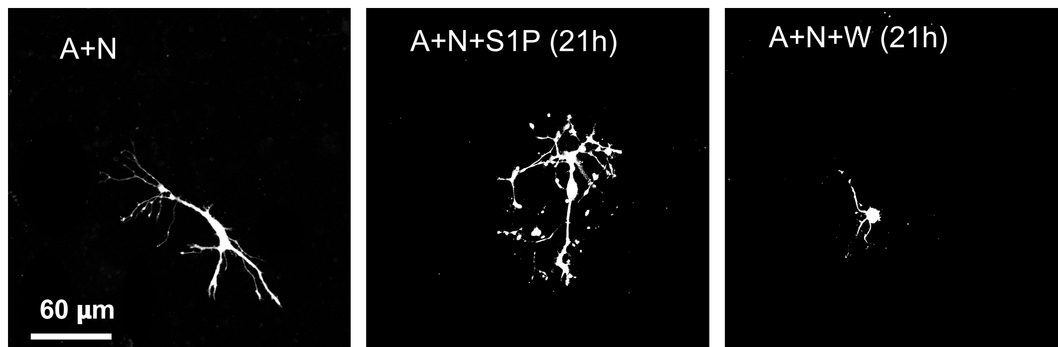
Human Specific Gene Primers	Cycle Threshold (Ct value) in human-Astrocytes	Cycle Threshold (Ct value) in mouse-Neurons
S1PR1	28.66 ± 1.91	Undetected
SPARCL1	28.44 ± 1.12	Undetected
TSP4	26.11 ± 2.82	37.06 ± 0.05
S1PR3	26.15 ± 0.66	38.53 ± 1.53
GLT1	27.62 ± 0.9	Undetected
GLAST	26.10 ± 2.3	36.36-Undetected
NFIX	27.94 ± 0.9	Undetected
GFAP	23.32 ± 0.45	39.92-Undetected
S1PR2	33.20 ± .71	Undetected
S1PR5	33.35 ± .45	Undetected

Supplementary Figure 3.

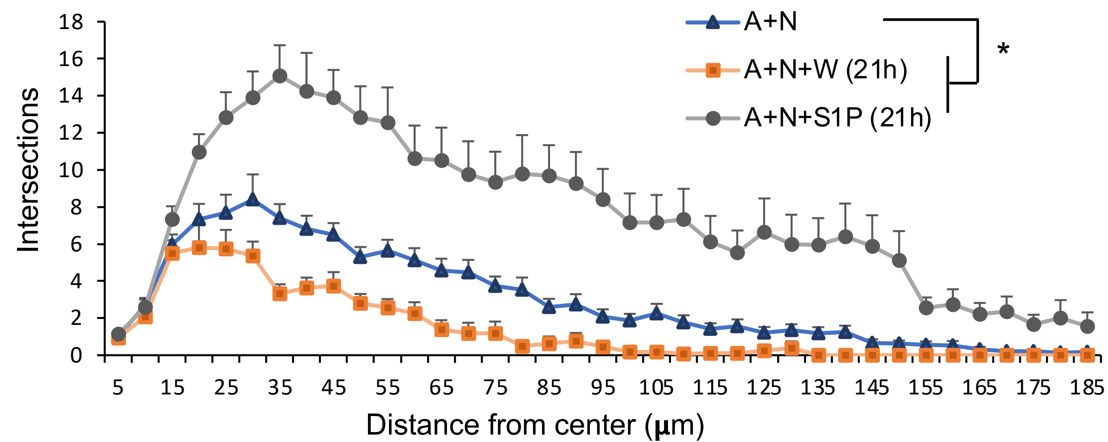


Supplementary Figure 4.

A

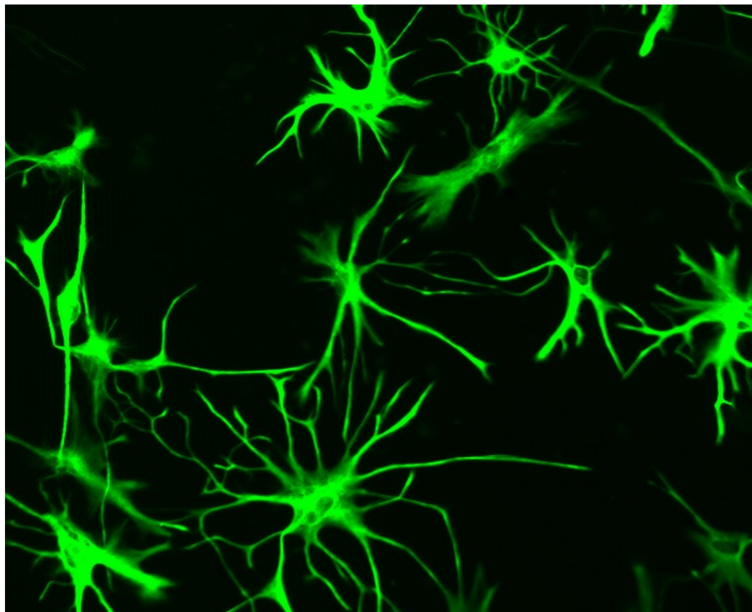


B

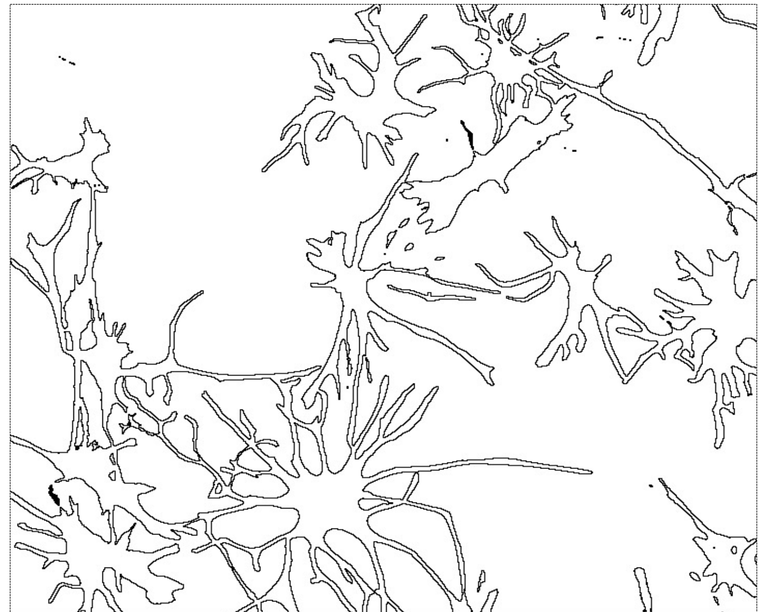


Supplementary Figure 5.

GFAP stained astrocytes



Perimeter tracings of GFAP signal



SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1. Full scanned images of blots of S1PR1, SPARCL1, GFAP and Tubulin from cortical and cerebellar tissues**.** Boxed area represents sections of the blot used in the main figure 1C and D.

Supplementary Figure 2. Authentication of the specificity of human specific qPCR primers used in cocultures of human astrocyte and mouse neurons. A range of cycle threshold values obtained from same amount of template cDNA used either from human astrocytes or mouse neurons for respective human specific primers. Most of these primers either do not detect mouse cDNA or detect signal at very high cycle threshold which often is observed from background noise.

Supplementary Figure 3. Transfection reagent did not affect astrocyte reactivity status. Expression of astrocyte reactivity genes, SERPINA3N, GFAP, S100b and TIMP1, was measured in untransfected or GFP transfected astrocytes cultured in neuronal growth medium. Two days after transfection, cells were Trizol lyzed, RNA was isolated and cDNA was prepared. SYBR green qPCR for indicated genes were run from two independent transfections from one primary cell culture. Data is normalized to GAPDH and represented as fold change relative to untransfected culture conditions.

Supplementary Figure 4. S1P induces astrocyte morphological complexity in neuron co-cultures. (A) Representative images of eYFP transfected astrocytes cocultured with neurons in the presence or absence of 100 nM S1P or 150 nM W146 for 21 hours. (B) Quantitation of astrocyte morphological complexity by Sholl analysis. Data are presented as numbers of total intersections at the indicated distance from the center of the cell body. n= 18-24 images per condition from 3 independent cultures of one primary cell preparation. Data are mean ± SEM; * $p < 0.05$, one way ANOVA followed by Kruskal-Wallis test for the entire curves.

Supplementary Figure 5. Example image of GFAP stained astrocytes cocultured with neurons (left) and tracing of the periphery of GFAP filled processes (right) used in perimeter calculations by FIJI.