An Isogenic Blood-Brain Barrier Model Comprising Brain Endothelial Cells, Astrocytes and Neurons Derived from Human Induced Pluripotent Stem Cells.

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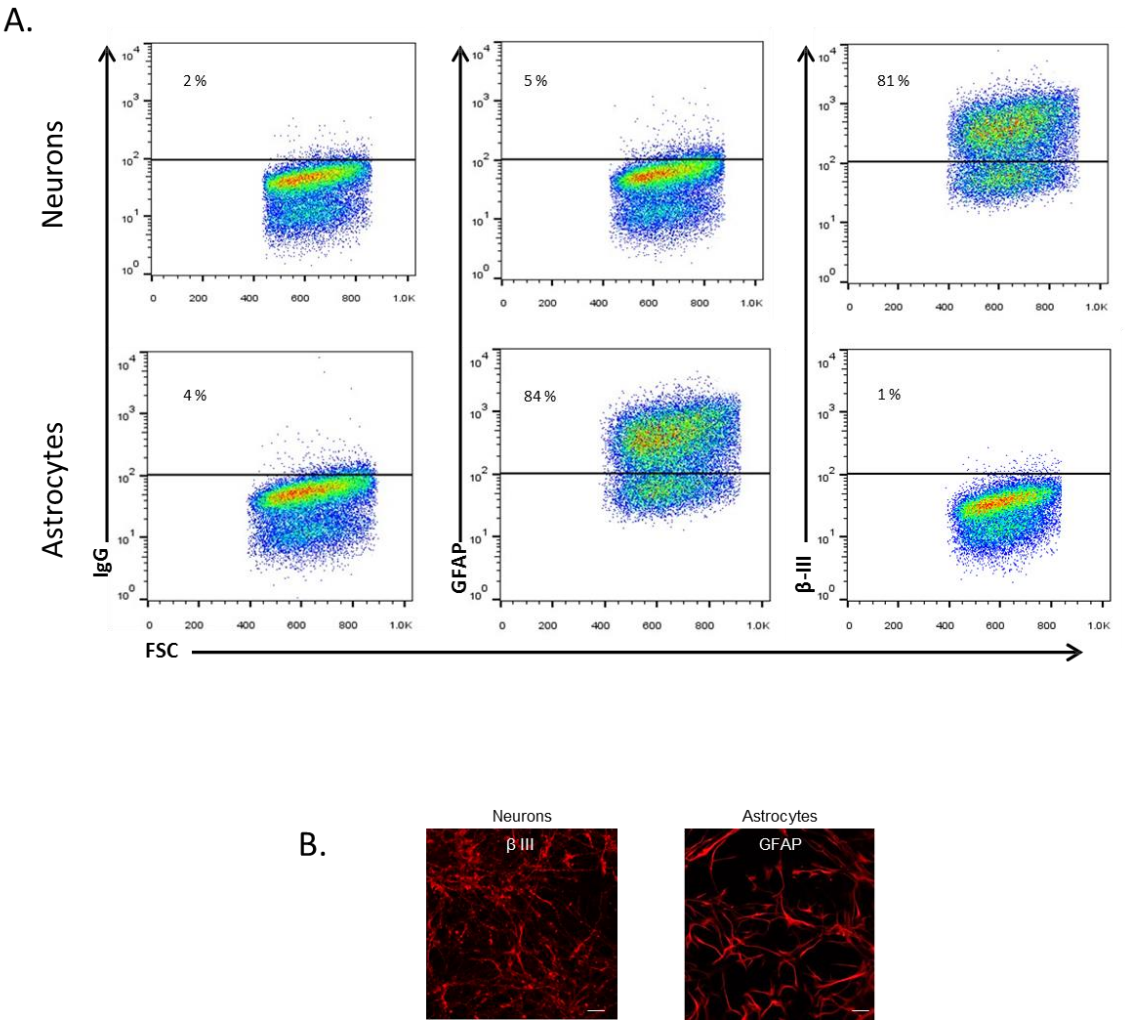
Table S1.

Target Antigen	Antibody Species	Vendor	Clone/Product Number	Dilution*
Pecam-1	Rabbit	Thermo Scientific	RB-10333	1:25
VE-Cadherin	Mouse	Santa Cruz	F-8	1:100
Glut-1	Mouse	Thermo Scientific	SPM498	1:500 1:200 (FC)
Claudin-5	Mouse	Life Technologies	4C3C2	1:200 1:500 (WB)
Occludin	Mouse	Life Technologies	OC-3F10	1:50 1:500 (WB)
P-glycoprotein	Mouse	Thermo Scientific	P170(F4)	1:25 (FC)
Multidrug resistance-asociated protein 1	Mouse	Millipore	MAB4100	1:25 (FC)
Breast Cancer resistance protein	Mouse	Millipore	MAB4155	1:50 1:25 (FC)
Transferrin Receptor	Mouse	R&D	MAB2474	1:200(FC)
Nestin	Mouse	Millipore	10C2	1:500
Beta-III-tubulin	Rabbit	Sigma	T3952	1:500 1:3000 (FC)
Glial fibrillary acidic protein	Rabbit	Dako	ZO334	1:500 1:5000(FC)
Pax-6	Mouse	Developmental Studies	1C8	1:100
S100B	Mouse	Abcam	11178	1:200

*Dilutions given are ideal for immuno-chemistry unless otherwise noted, WB = western blot, FC = flow cytometry.

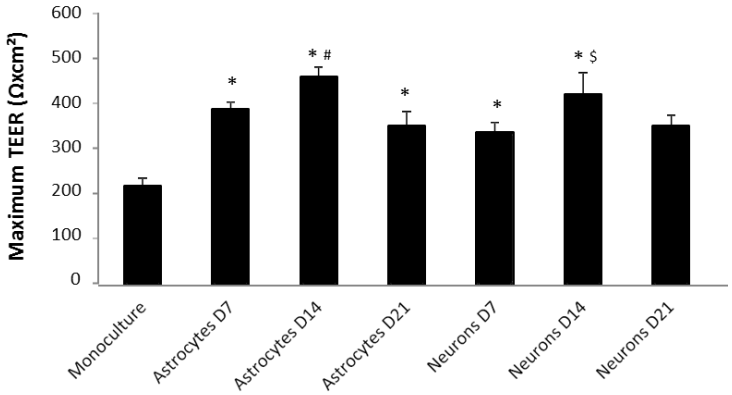
Supplementary Table 1. Antibodies used for Immunocytochemistry, Flow Cytometry, and Western blot.

Supplementary Figure 1



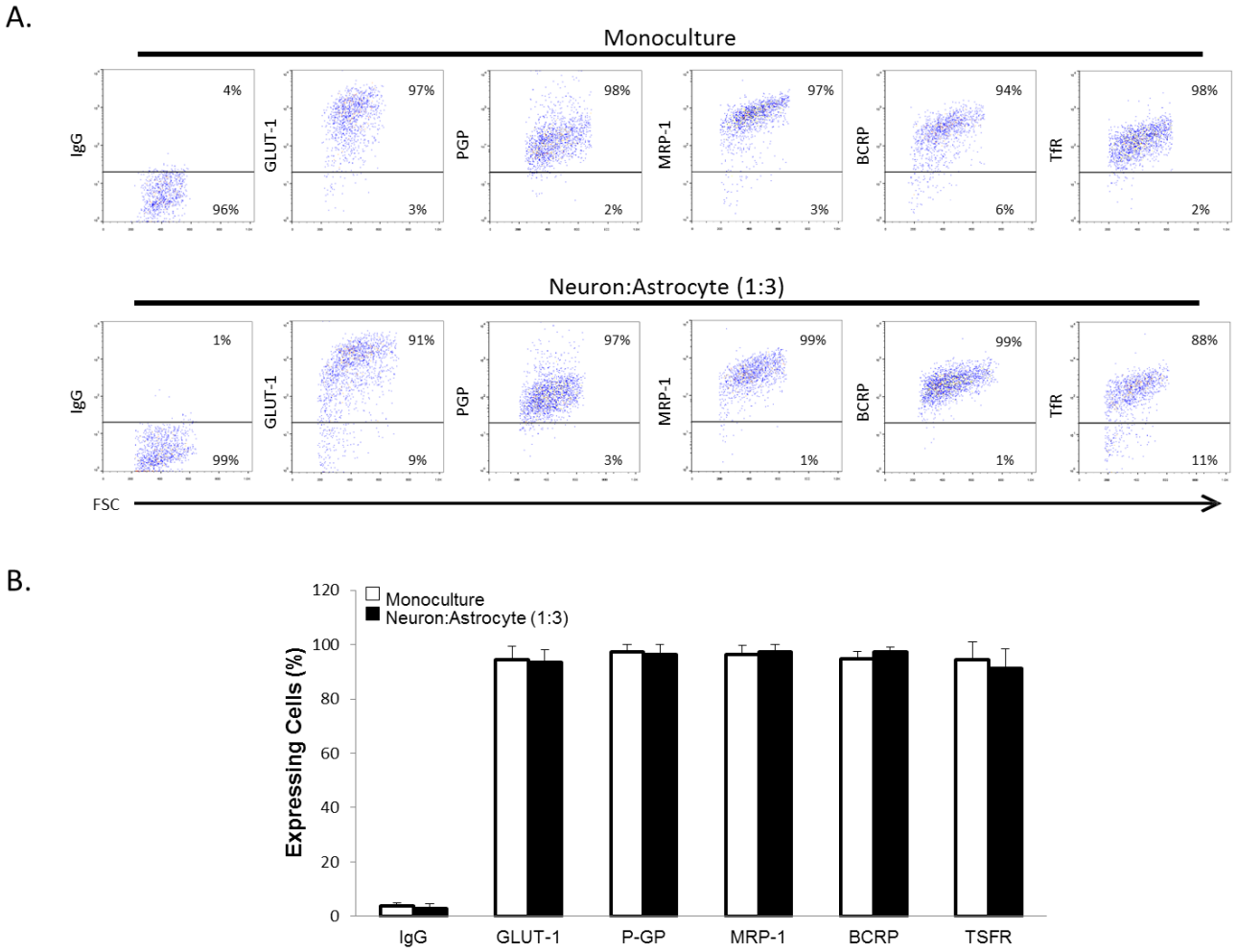
Supplementary Figure 1. Characterization of Day 14 EZ-sphere-derived astrocytes and neurons. (A) Representative flow cytometry density plots from EZ-sphere-derived astrocytes and neurons. Gates were drawn based on rabbit IgG control antibody labeling, and the percentage of total events having immunolabeling above rabbit IgG control is noted. 84% of the differentiated astrocyte population expressed GFAP. 81% of the differentiated neuron population expressed β-III tubulin. (B) CS03n2 EZ-sphere derived neurons and astrocytes continued to express β-III tubulin and GFAP, respectively following 48 h co-culture with CS03n2 iPSC-derived BMECs. Scale bars = 100μm.

Supplementary Figure 2



Supplementary Figure 2. Duration of neuron and astrocyte differentiation that induces maximum barrier tightening. 4.2 EZ-sphere-derived astrocytes and neurons were differentiated for 7 days, 14 days, and 21 days and combined in co-culture with IMR90-4-derived BMECs. TEER measurements were taken at 48 h after the initiation of co-culture. Statistical significance was calculated using ANOVA. *$p<0.05$ vs. monoculture, $^{\#}p<0.05$ vs. astrocytes D7 or D21, $^{\$}p<0.05$ vs. neurons D7 or D21. Values are mean ± SD of three replicates from a single isolation/differentiation, and experiments were repeated for three more additional differentiations for verification of reported statistical trends.

Supplementary Figure 3



Supplementary Figure 3. Flow cytometric analysis of BMECs following co-culture with EZ-sphere-derived neurons and astrocytes (1:3). (A) Representative flow cytometry dot plots from IMR90-4 BMECs in monoculture or after 48 hours of co-culture with 4.2 EZ sphere-derived neuron and astrocytes (1:3). Gates were drawn based on mouse IgG negative control antibody immunolabeling, and the percentage of transporter immunopositive cells is noted in the insets to panel (A) and is compiled in panel (B). Geometric means of the transporter immunopositive populations were extracted from these flow data and are quantitatively compared in Figure 5C. Statistical significance was determined using a Student's t-test. Values are mean ± SD of three independent differentiations.