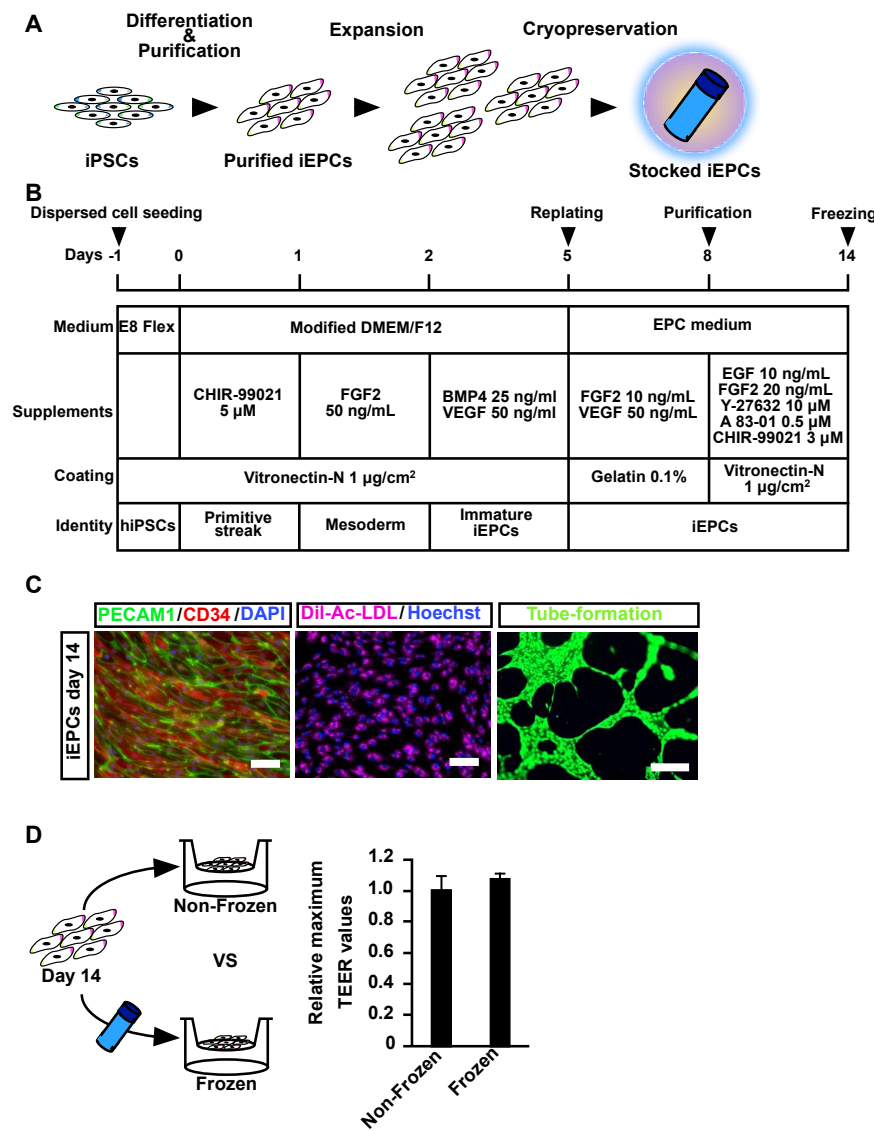


Additional file S1

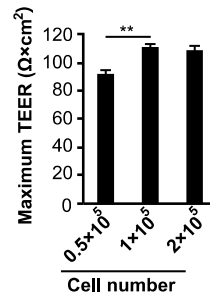
Generation of Brain Microvascular Endothelial-like Cells from Human iPS Cell-Derived Endothelial Progenitor Cells Using TGF-β Receptor Inhibitor, Laminin 511 Fragment, and Neuronal Cell Culture Supplements

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Components

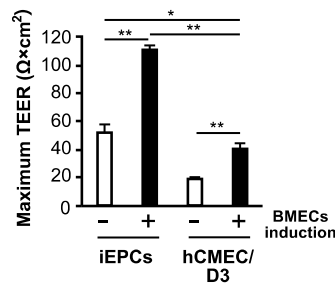


**Figure S1. Effect of freeze–thawing on the physical barrier function of iEPCs**  
(A and B) Scheme for the differentiation of iEPCs. (C) Immunofluorescence analyses of PECAM1 (green), CD34 (red), and Dil-Ac-LDL uptake and tube formation assays of 610B1-derived iEPCs. DAPI = blue (immunofluorescence analyses); Hoechst 33342 (Dil-Ac-LDL uptake assay) = blue; Dil-Ac-LDL = pink; calcein = green. Scale bars = 100 μm (immunofluorescence analyses and Dil-Ac-LDL uptake assay) and 500 μm (tube formation assay). (D) Measurement of TEER values of frozen and non-frozen iEPCs ( $7 \times 10^5$  cells/insert) on day 14. Data are presented as the mean  $\pm$  SD ( $n = 3$ ; Student's  $t$ -test).



**Figure S2. Effect of seeding cell number on TEER values**

Thawed iEPCs were seeded at  $0.5 \times 10^5$ ,  $1 \times 10^5$ , and  $2 \times 10^5$  cells/insert and cultured in HE-SFM supplemented with 20 ng/mL FGF2, penicillin–streptomycin solution, 7.5% B-27 supplement, and 1  $\mu$ M A 83-01 on cell culture inserts coated with a coating solution consisting of fibronectin, collagen type IV, and 10  $\mu$ g/mL LN511F. Measurement of TEER values of iEPCs over a long period (from day 2 to 10) and the maximum value among them is shown. Data are presented as the mean  $\pm$  SD ( $n = 3$ ;  $**p < 0.01$ ; Tukey's HSD test;  $1 \times 10^5$  group vs. others).



**Figure S3. TEER values of hCMEC/D3 cultured under BMEC-inducing conditions**

Thawed iEPCs or hCMEC/D3 cells ( $1 \times 10^5$  cells/insert) were cultured under the same conditions as Fig. 3A and B. Measurement of TEER values from day 2 to 10 and the maximum value among them is shown. Data are presented as the mean  $\pm$  SD ( $n = 3$ ;  $*p < 0.05$ ,  $**p < 0.01$ ; Tukey's HSD test).