Supplementary Information

Cryopreservation of brain endothelial cells derived from human induced pluripotent stem cells is enhanced by ROCK inhibition

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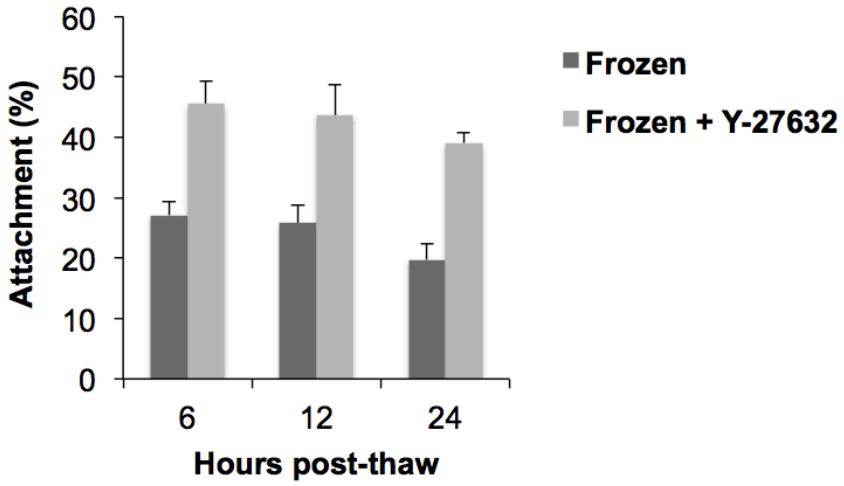
Inventory of Supplementary Information

Supplementary Table S1: Primer sequences for qPCR experiments.

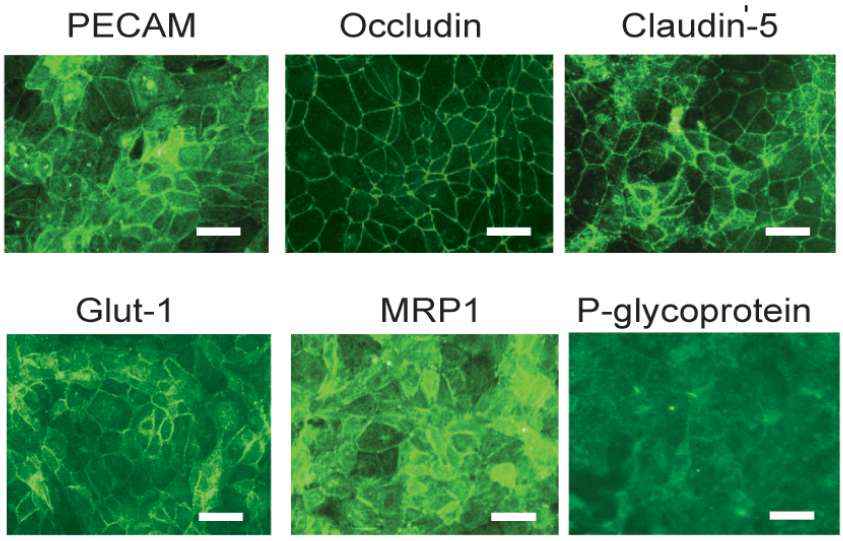
Target	Forward sequence (5'-3')	Reverse sequence (5'-3')
AGER	GTAGATTCTGCCTCTGAACTC	CTTCACAGATACTCCCTTCTC
β-actin	CATGTACGTTGCTATCCAGGC	CTCCTTAATGTCACGCACGAT
BCRP	TCATTTAGTTTGTCAGTGGGTG	CAGGTATGTGAAAAGCAGGAAT
GLUT-1	AACTCTTCAGCCAGGGTCCAC	CACAGTGAAGATGATGAAGAC
INSR	TGTTCATCCTCTGATTCTCTG	GCTTAGATGTTCCCAAAGTC
LDLR	GCCATTGTCGTCTTTATGTC	AAACACATACCCATCAACGA
MRP-1	CTCTATCTCTCCCGACATGACC	AGCAGACGATCCACAGCAAAA
Occludin	GACTTCAGGCAGCCTCGTTAC	GCCAGTTGTGTAGTCTGTCTCA
P-glycoprotein	TTGCTGCTTACATTCAGGTTTCA	AGCCTATCTCCTGTCGCATTA
STRA6	TTTGGAATCGTGCTCTCCG	AAGGTGAGTAAGCAGGACAAG
TFRC	GCACAGCTCTCCTATTGAAAC	GGTATCCCTCTAGCCATTCAG
VE-Cad	GGTCAAACTGCCCATACTTG	CGCAATAGACAAGGACATAACAC
ZO-1	ACCAGTAAGTCGTCCTGATCC	TCGGCCAAATCTTCTCACTCC

Supplementary Table S2: Immunocytochemistry antibodies.

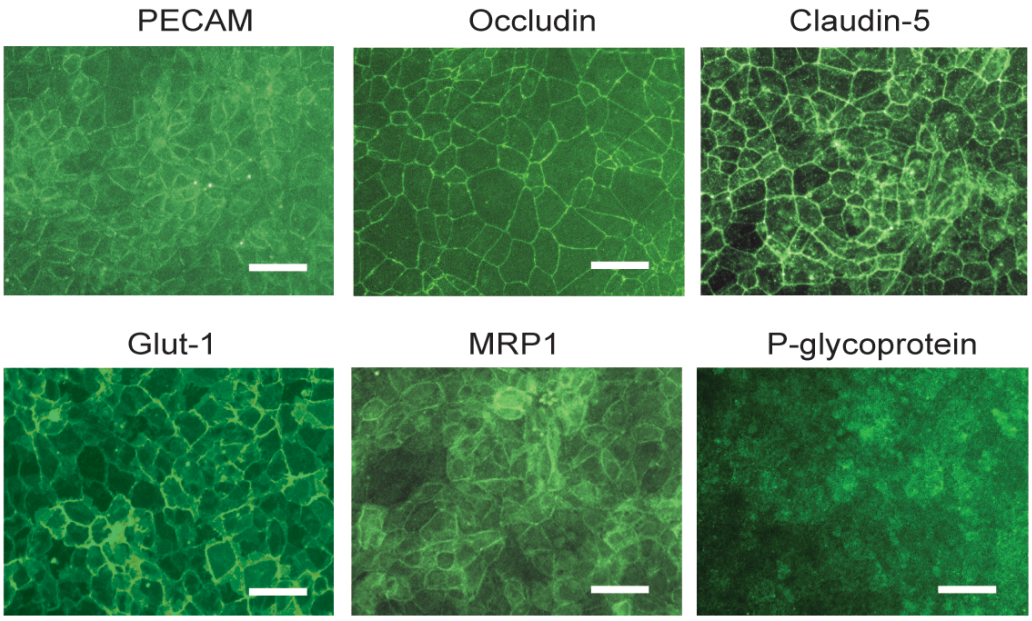
Antibody	Vendor, clone or product number	Dilution
PECAM-1	Thermo Scientific, RB-10333	1:25
Glut-1	Thermo Scientific, SPM498	1:100
VE-cadherin	Santa Cruz, BV9	1:25
Occludin	Invitrogen, OC-3F10	1:50
Claudin-5	Invitrogen, 4C3C2	1:200
ZO-1	Invitrogen, ZO1-1A12	1:100
P-gp	Thermo Scientific, F4	1:25
BCRP	Millipore, 5D3	1:50
MRP1	Millipore, QCRL1	1:25



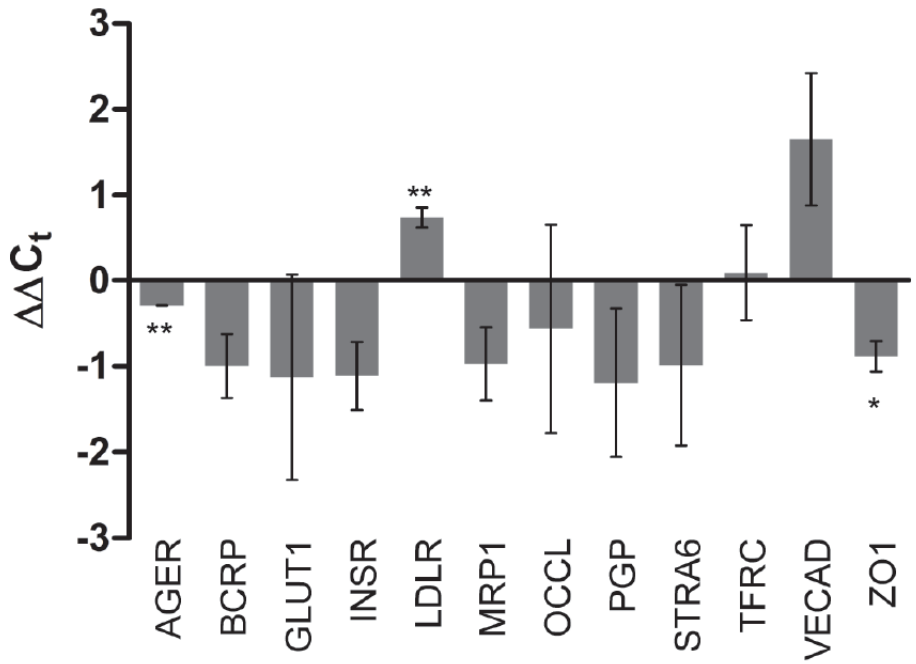
Supplementary Figure S1: Cell yield of cryopreserved iPSC-BMECs as a function of time. IMR90-4 iPSC-BMECs were cryopreserved at D8 of differentiation, and cells were thawed with or without 10 μM Y-27632. The number of attached cells was quantified at 6, 12, and 24 h post-thaw, normalized to the number of cells seeded and reported as percent attachment. Data represent the average ± standard deviation of triplicate wells from a single differentiation, and the experiment was replicated for an additional independent differentiation to confirm trends.



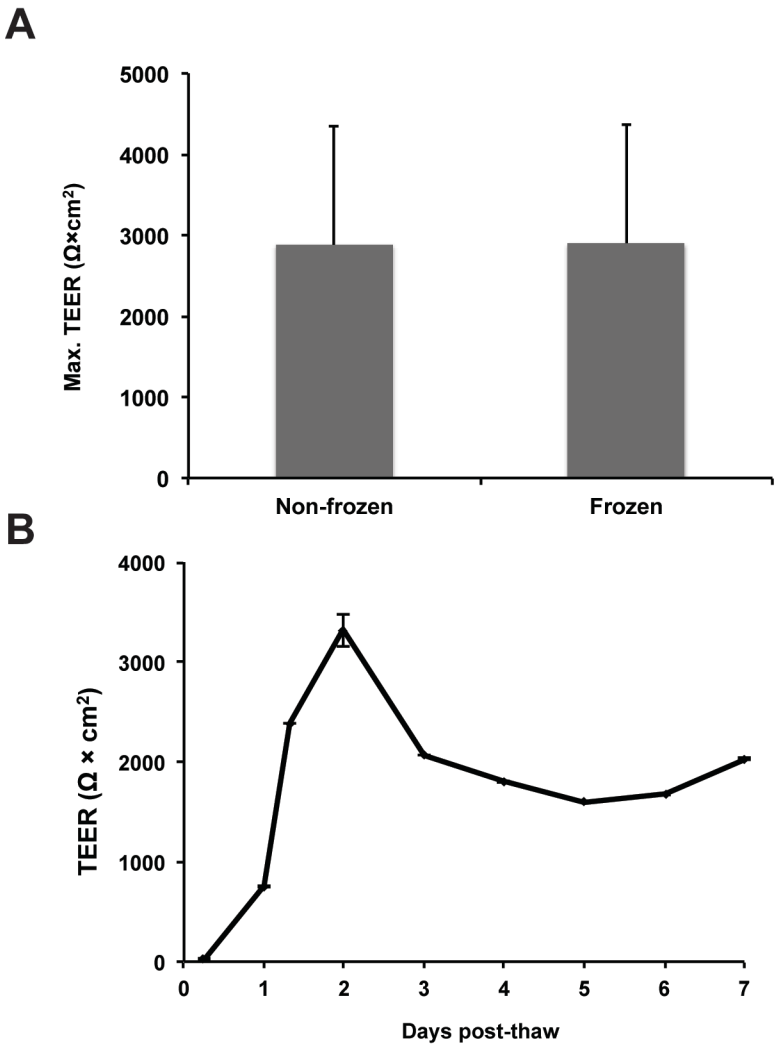
Supplementary Figure S2: Immunocytochemistry of cryopreserved IMR90-4 iPSC-BMECs. Immunocytochemistry of IMR90-4 iPSC-BMECs cryopreserved as a purified population. Cells were cryopreserved at D10 of differentiation, and immunocytochemistry was performed at D12 of differentiation, 2 days post-thaw. Scale bars, 50 μm.



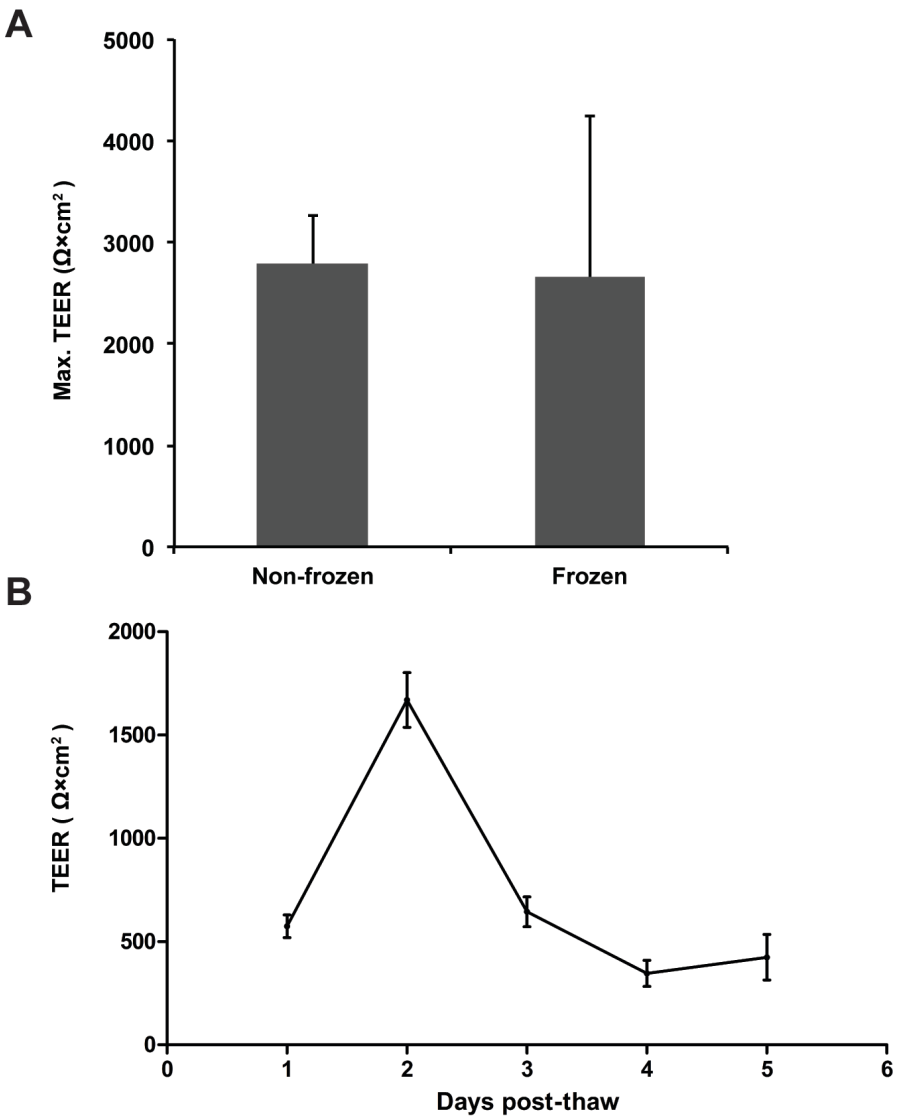
Supplementary Figure S3: Immunocytochemistry of CS03iCTRn2 iPSC-BMECs. CS03iCTRn2 iPSC-BMECs were cryopreserved on D8 of differentiation, and immunocytochemistry was performed on D10 of differentiation, 2 days post-thaw. Scale bars, 50 µm.



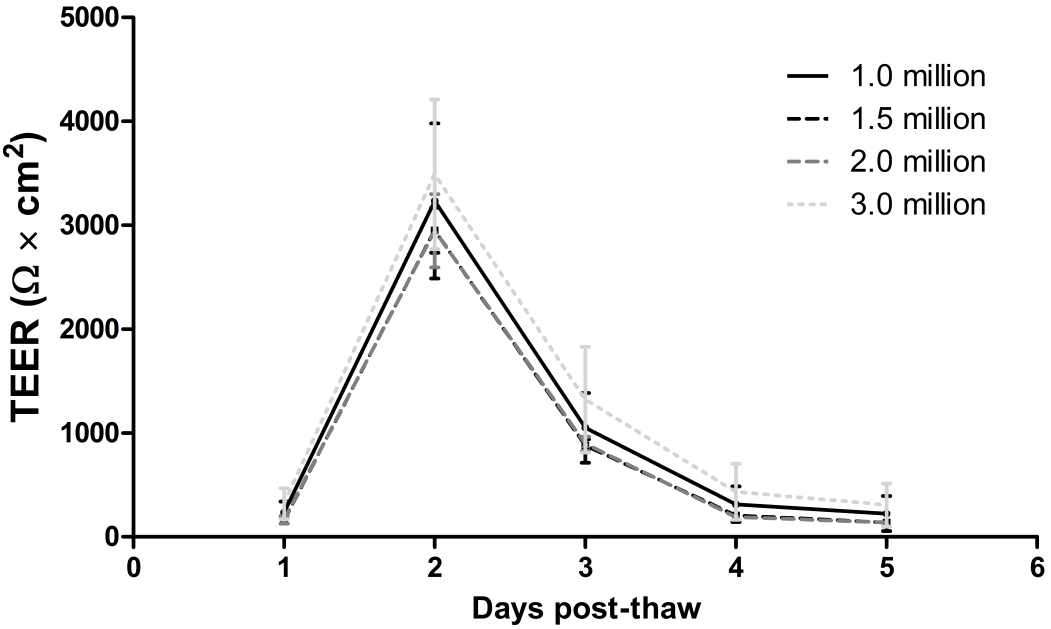
Supplementary Figure S4: Quantitative PCR of endothelial and BBB genes normalized to β-actin expression. IMR90-4 iPSC-BMECs were subcultured and/or cryopreserved at D8 of differentiation and RNA was collected on D10, 2 days post-thaw. Positive ΔΔCt values indicate increased expression in frozen iPSC-BMECs compared to non-frozen control. Frozen samples were paired to non-frozen samples from the same differentiation. Data represent the average ± standard deviation of three independent differentiations. Statistical significance was calculated via Student's unpaired t test (*$p<0.05$, **$p<0.01$).



Supplementary Figure S5: TEER of cryopreserved CS03iCTRn2 iPSC-BMECs. (A) Maximum TEER values of CS03iCTRn2 iPSC-BMECs cryopreserved at D8 of differentiation. Data represent the average ± standard deviation of eight independent differentiations. (B) Representative TEER profile of CS03iCTRn2 iPSC-BMECs cryopreserved at D8 of differentiation. Data represent the average ± standard deviation of triplicate Transwell filters from a single differentiation, and the experiment was replicated for three additional independent differentiations to confirm trends.



Supplementary Figure S6: TEER of profile of IMR90-4 iPSC-BMECs cryopreserved at D10. (A) Maximum TEER values of IMR90-4 iPSC-BMECs cryopreserved as a purified population at D10 of differentiation. Data represent the average ± standard deviation of three independent differentiations. (B) Representative TEER profile of IMR90-4 iPSC-BMECs cryopreserved at D10. Data represent the average ± standard deviation of triplicate Transwell filters from a single differentiation, and the experiment was replicated for two additional independent differentiations to confirm trends.



Supplementary Figure S7: Effect of filter seeding density on TEER profile of cryopreserved cells. IMR90-4 iPSC-BMECs were cryopreserved at D8 of differentiation and seeded onto Transwell filters at various densities. Data represent the average ± standard deviation of triplicate Transwell filters from a single differentiation, and experiments were replicated for an additional independent differentiation to confirm trends. The 1.5 million dataset is located under the 2.0 million dataset.