

Supplementary materials for:

Hypoxia increases expression of blood-brain barrier transporters SLC2A1, SLC7A5 and TFRC, while maintaining barrier integrity, in brain capillary endothelial monolayers.

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Table S1. Overview of the primer pairs used in this study.

Gene symbol	Gene name	PCR efficiency	Product Size (bp)	Primer Sequence (5' to 3')
HPRT1	Hypoxanthine guanine phosphoribosyl transferase 1	2.03	144	F: CGTGGTGATTAGCGATGATG R: TTCATCACATCTCGAGCCAG
YWHAZ	Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein zeta	1.90	100	F: AGCTGGTACAGAAGGCCAAA R: TTGGATAATTCAGCTCCTTGC
SDHA	Succinate dehydrogenase complex	1.92	105	F: CTTCAAGGAGAGGGTTGACG R: TCAACGTAGGAGAGCGTGTG
CLDN5	Claudin-5	2.06	122	F: CAGAAGTACGAGCTGGGAGC R: TACTTCACCGGGAAGCTGAA
ABCB1	P-glycoprotein (Pgp)	1.99	127	F: CGGGACAGAAAGCTCAGTTC R: TAATGGCGCAAAATACACCA
SLC2A1	Glucose transporter 1 (GLUT-1)	1.92	139	F: TACCCCAAGAGGTGGCTATG R: CTGGTCTCAGGCAAGGAAAG
SLC7A5	L-type amino acid transporter 1 (LAT-1)	1.91	111	F: TAGCCAATCTGGATCCCAAG R: TCAAGTAATTCCATCCCCCA
FLT1	Vascular endothelial growth factor receptor 1 (VEGFR1)	1.91	292	F: CCTGCTTCCAAGAACAAAGC R: CTGTTGTCTCGCAGGTCAAA

Table S2. Primary and secondary antibodies used in immunocytochemistry and Western blot analysis.

Target protein	Clonality	Host	Dilution factor	Supplier and catalogue ID
Primary antibodies				
HIF-1 α	Monoclonal	Rabbit	1:1000	Cell Signaling (36169T)
von Willebrand factor	Polyclonal	Rabbit	1:250	Abcam (ab6994)
Claudin-5	Polyclonal	Rabbit	1:200	Abcam (ab15106)
Zonula occludens-1	Polyclonal	Rabbit	1:100	Abcam (ab59720)
Glut-1	Polyclonal	Rabbit	1:100	Thermo Scientific (pa1-1063)
LAT-1	Polyclonal	Rabbit	1:100	Bioss Antibodies (bs-10125R)
P-glycoprotein	Polyclonal	Rabbit	1:100	Abcam (ab129450)
For WB: P-glycoprotein	Polyclonal	Rabbit	1:500	Kindly provided by Lundbeck
Transferrin receptor	Monoclonal	Mouse	1:100	Thermo Scientific 13-6800
β -catenin (active)	Monoclonal	Rabbit	1:100	Cell Signaling (19807T)
β -actin	Monoclonal	Mouse	1:2500	Sigma-Aldrich (A5441)
Secondary antibodies conjugated with Alexa-488 or HRP				
Anti-rabbit	Polyclonal	Goat	1:7000	Molecular Probes (G-21234)
Anti-mouse	Polyclonal	Goat	1:4500	Invitrogen (62-6520)
Anti-rabbit	Polyclonal	Goat	1:200	Life Technologies (AV1008)

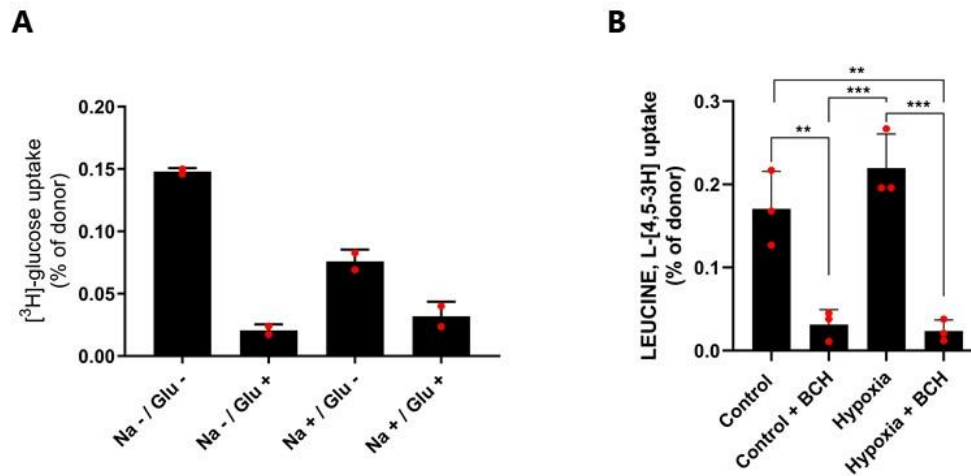


Figure S1. Activities of GLUT-1 and LAT-1 in bovine brain capillary endothelial cells (BCEC) differentiated under normoxic and hypoxic conditions. A) The luminal uptake of [³H]-glucose in the presence and absence of 2 mM cold glucose and sodium in cells cultured under hypoxic conditions. The uptake data are shown as mean ± SEM of three individual cell passages of duplicates (n=3, total N=9). B) The luminal uptake of L-[4,5-3H]-leucine into endothelial cells was examined in the presence and absence of 100 μM BCH. The uptake data are

shown as mean $\pm$ SEM of three individual cell passages of duplicates (n=3, total N=6). The donor concentrations of [3H]-glucose and L-[4,5-3H]-leucine were 20 and 9.5 nM, respectively.

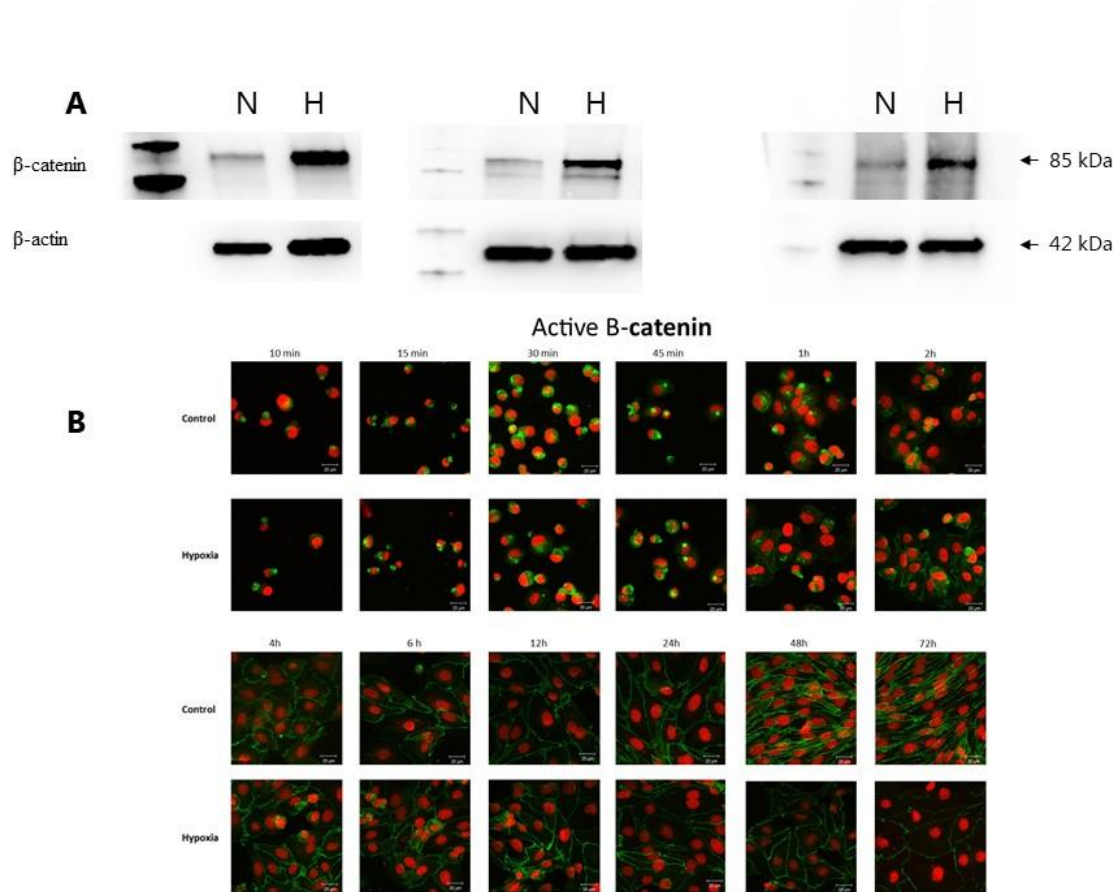


Figure S2. Expression of active β -catenin in BCECs cultured under normoxic and hypoxic conditions. A) Immunoblots of β -catenin in normoxic (N) and hypoxic (H) cells on the experimental day (n=3, total N=9). B) Immunocytochemical characterization of β -catenin in normoxic and hypoxic cells. Cells were stained after 10 min to 72 h upon seeding on permeable filter supports. All samples were counterstained with propidium iodide (red) to visualize cell

nuclei. Scale bars = 50 μm . Images are representative of three individual experiments in triplicate (n=3, total N=9).