SUPPLEMENTAL INFORMATIONS

Proof-of-Concept Study of Drug Brain Permeability Between *in Vivo* Human Brain and an *in Vitro* iPSCs-Human Blood-Brain Barrier Model.

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S1-Table 1. Rate constant for transfer from arterial plasma into brain obtained from compartimental modelling of PET data

Clinical PET radioligands	*K*1 (mL.cm^{-3}.min^{-1})	References
Befloxatone	0.20	unpublished data
Fluoro-A85380	0.11	33
Flumazenil	0.35	34
Raclopride	0.09	35
Loperamide	0.002	27
verapamil	0.04	36
Erlotinib	0.02	37
Buprenorphine	0.17	Unpublished data

S2-Table 2. Primer sequences used for semi-quantitative PCR analysis

Primer name	Primer sequence (5'>3')
OCT4_forward	AGCGAACCAGTATCGAGAAC
OCT4_reverse	TTACAGAACCACACTCGGAC
SOX2_forward	AGCTACAGC ATGATGCAGGA
SOX2_reverse	GGTCATGGAGTTGTACTGCA
REX1_forward	CAGTCCAGCAGGTGTTTGC
REX1_reverse	GCATTCTATGTAACAGTCTGAGA
NANOG_forward	TGAA CCTCAGCTACAAACAG
NANOG_reverse	TGGTGGTAGGAAGAGTAAAG
RPLPO_forward	CATTGCCCCATGTGAAGTC

S3-Table 3. References and working dilutions of antibodies used for immunofluorescence and flow cytometry experiments.

Targeted antigen	Antibody description	References / supplier	Final concentration for IF or Flow cytometry
ZO-1 (Clone ZMD.437)	Rabbit polyclonal anti-human	40-2300 / Thermo Fisher Scientific	5 µg/ml (IF)
Claudin-5 (Clone 4C3C2)	Mouse monoclonal anti-human	35 250 / ZYMED	10 µg/ml (IF)
Anti-mouse Alexa Fluor 488	Polyclonal Goat anti-mouse	A-11029 / Thermo Fisher Scientific	5 µg/ml (IF)
Anti-rabbit Alexa Fluor 594	Polyclonal Goat anti-rabbit	A-11037 / Thermo Fisher Scientific	5 µg/ml (IF)
SSEA4 (clone eBioMC-813-70)	Monoclonal Mouse anti-human	12-8843-42 / eBioscience	20 µg/ml (Flow cytometry)
PE Mouse IgG3 Isotype Control (Clone B10)	Mouse IgG3	12-4742-42 / eBioscience	20 µg/ml (Flow cytometry)
TRA1-60 PE (Clone TRA1-60)	Monoclonal Mouse anti-human	12-8863-42 / eBioscience	20 µg/ml (Flow cytometry)

S4-Table 4. Primer sequences used for RT-Q-PCR analysis

Primer name	Primer sequence (5'->3')
hCAV1_forward	AGTTGCTGCAAACCTGACC
hCAV1_reverse	CTCCTCCCCCATCTTCTTTC
hCAV2_forward	CAGTGCAGACAATATGGAAGAG
hCAV2_reverse	GGAAATGAACAGAACAGTGGTG
hβ-catenin_forward	TCTTACACCCACCATCCCAC
hβ-catenin_reverse	GCACGAACAAGCAACTGAAC
hEndothelin1-forward	CTCCTGCTCGTCCCTGATG
hEndothelin1-reverse	CGGTCTGTTGCCTTTGTGG
hTFRC_forward	TCCCTTCCTTCAATCACACTC
hTFRC_reverse	TCTTTCAGCACATTGCTCAC
hINSR-forward	TGTTCATCCTCTGATTCTCTG
hINSR-reverse	GCTTAGATGTTCCCAAAGTC
hbEGF-forward	TGACCACACAACCATCCTG
hbEGF-reverse	TCCACATCATAACCTCCTCTC
hPgp-forward	TGAATCTGGAGGAAGACATGAC
hPgp-reverse	CCAGGCACCAAAATGAAACC
hMRP1-forward	AATAGAAGTGTTGGGCTGAG
hMRP1-reverse	CGAGACACCTTAAAGAACAG
hBCRP1-forward	TGCCCAGGACTCAATGCAAC
hBCRP1-reverse	ACGTGATTCTTCCACAAGCCC
hLRP1-forward	TGCTACTGCAACAGCAGCTTTC
hLRP1-reverse	TGCCGTACACTGAGCACTCATC

hZO1-forward	TGATCATTCCAGGCACTCG
hZO1-reverse	CTCTTCATCTCTACTCCGGAGACT
hCD31-forward	GAGTATTACTGCACAGCCTTCA
hCD31-reverse	AACCACTGCAATAAGTCCTTTC
hGAPDH-forward	TCAAGAAGGTGGTGAAGCAGGC
hGAPDH-reverse	AGTGGGTGTCGCTGTTGAAGTC

References

33. Gallezot, JD. *et al.* In vivo imaging of human cerebral nicotinic acetylcholine receptors with 2-18F-fluoro-A-85380 and PET. *J Nucl Med.* **46**, 240-247 (2005).

34. Delforge, J. *et al.* Quantification of benzodiazepine receptors in human brain using PET, [^{11}C]flumazenil, and a single-experiment protocol. *J Cereb Blood Flow Metab*. **15**, 284-300 (1995).

35. Pappata, S. et al. n vivo detection of striatal dopamine release during reward: a PET study with [^{11}C]raclopride and a single dynamic scan approach. *Neuroimage* **16**, 1015-1027 (2002).

36. Bauer, M. *et al*. Approaching complete inhibition of P-glycoprotein at the human blood-brain barrier: an (R)-[^{11}C]verapamil PET study. *J Cereb Blood Flow Metab*. **35**, 743-746 (2015).

37. Bauer, M. *et al*. A Proof-of-Concept Study to Inhibit ABCG2- and ABCB1-Mediated Efflux Transport at the Human Blood-Brain Barrier. *J Nucl Med*. ***60***, 486-491 (2019).