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Supplementary information

Physiological blood–brain transport is impaired with age by a shift in transcytosis

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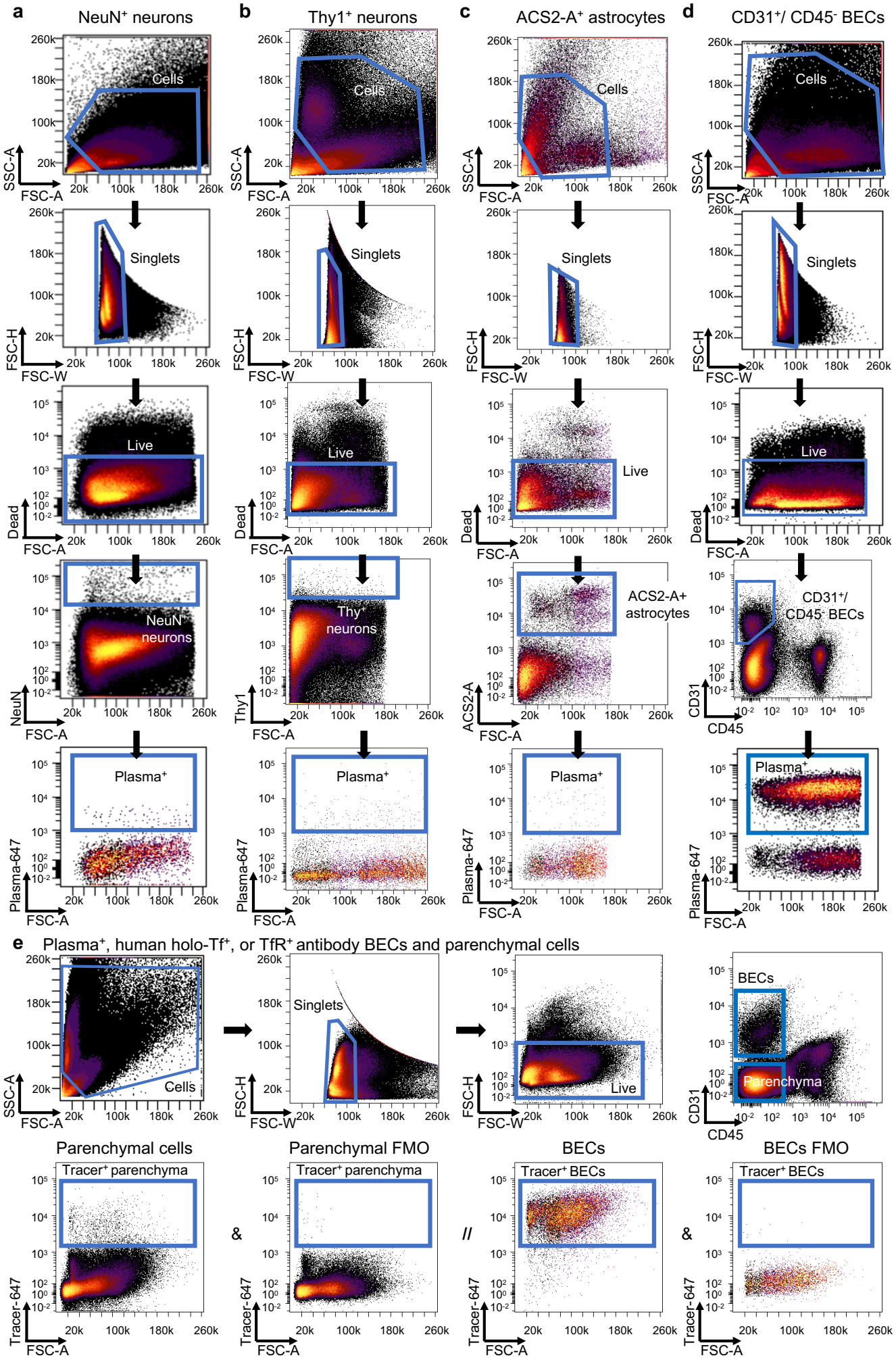
Supplemental Figure 1



Supplementary Table 1 Tracers injected or perfused to probe BBB permeability

WB: Western Blot, FM: fluorescence microscopy, LM: light microscopy, TEM: transmission electron microscopy, MRI: magnetic resonance imaging, PET: positron emission tomography, ELISA: enzyme-linked immunosorbent assay

Category	Injected substance	Wild-type/ healthy	Finding in wild-type control/ healthy	Model/ disease	Finding in model/ disease	Detection method
Macro-molecules	Horseradish peroxidase	-C57BL/6 mice[1,2] -C57BL/6J mice[3]	-No uptake into BECs[1]. -Increased uptake into BECs in old mice[2] -Low uptake into BECs[3]	-*Mfsd2a*^{D96A} mice -*Smpd1*$^{+/-}$ mice[2] -*Pdgfb*$^{ret/ret}$ mice[3]	-Uptake into BECs[1] -Reduced uptake in old tg mice compared to old WT mice[2] -Increased uptake into BECs[3]	-TEM, LM[1] -FM[2] -TEM[3]
	Bovine Serum Albumin	-C57BL/6J mice[3] -B6/SJL mice[4]	-No accumulation in brain parenchyma[3] -Passive transport or fluid phase endocytosis[4]	-*Pdgfb*$^{ret/ret}$, *R26P*$^{+/0}$, *R26P*$^{+/+}$ mice[3] -*APP-PS1* mice[4]	-Accumulation in brain parenchyma in *Pdgfb*$^{ret/ret}$ and less in *R26P*$^{+/0}$ but not in *R26P*$^{+/+}$ mice[3] -Decreased permeability compared to WT[4]	-FM[3] -Radiotracing[4]
	Exogenous IgG	C57BL/6J mice[3,5]	-Intact BBB with no difference between young and aged mice[5] -No accumulation in brain parenchyma[3]	-*PS2-APP* mice[5] -*Pdgfb*$^{ret/ret}$, *R26P*$^{+/0}$, *R26P*$^{+/+}$ mice[3]	-No difference in BBB permeability compared to WT[5] -Accumulation in brain parenchyma in *Pdgfb*$^{ret/ret}$ and less in *R26P*$^{+/0}$ but not in *R26P*$^{+/+}$ mice. Treatment with Imatinib rescues leaky phenotype[3]	-Radiotracing, WB[5] -FM[3]
	Human insulin	B6/SJL mice	Insulin undergoes receptor-mediated transcytosis	*APP-PS1* mice	Increased permeability	Radiotracing[4]
	Dextran (≤10 kDa)	C57BL/6 mice [1,2,5,6]	-Confined to vasculature[6] -Leakage into parenchyma in old mice[2] -Confined to vasculature[1] -Intact BBB with no difference between young and aged mice[5]	-*Mfsd2a*$^{-/-}$ mice[6] -*Smpd1*$^{+/-}$ mice[2] -*Mfsd2a*^{D96A} mice[1] *Mfsd2a*; *Cav1* single and double KO mice[1] -*PS2-APP* mice[5]	-Increased BBB permeability[6] -Leaky phenotype in old mice is rescued in Tg model[2] -Increased transcytosisis into parenchyma[1] -Increased permeability only in *Mfsd2a* KO. Phenotype is rescued in double KO -No difference in BBB permeability compared to WT[5]	-FM[6] -FM[2] -FM, LM[1] -Radiotracer[5]
	Dextran (>10 kDa)	-C57BL/6 mice[2,7,8] -C57BL/6J[3]	-Large dextrans are leaking less into old mice parenchyma compared to smaller dextrans - Almost no leakage for 2000 kDa dextran[2] -Confined to vasculature[8] -No accumulation in parenchyma[3]	-*Smpd1*$^{+/-}$ mice[2] -*Apoe*$^{-/-}$, *TR-APOE2, TR-APOE3, TR-APOE4, GFAP-APOE4, Apoe*$^{-/-}$ *Ppia*$^{-/-}$ mice[8] *Pdgfb*$^{ret/ret}$, *R26P*$^{+/0}$, *R26P*$^{+/+}$ mice[3] -Nγ1-KO mice[7]	-Leaky phenotype in old mice is rescued in Tg model[2] -Increased permeability in Apoe$^{-/-}$, *APOE4* mice which increases with age; Leaky BBB phenotype is rescued by *CypA* inhibition and in *Apoe*$^{-/-}$*Ppia*$^{-/-}$ mice[8] -Accumulation in brain parenchyma in *Pdgfb*$^{ret/ret}$ and less in *R26P*$^{+/0}$ but not in *R26P*$^{+/+}$ mice. Treatment with imatinib rescues leaky phenotype[3] -Increased permeability compared to control[7]	FM[2,3,7,8]
	IgG brain shuttles	-C57BL/6 mice[5,9,10] -Male Sprague Dawley Rats[11,12]	-Anti-TfR-BACE1, -Glut1 and -CD98hc Abs enter brain parenchyma[5,9] -Anti-TfR Ab extravasates into the brain[12] -Enhanced uptake of MTX into brain parenchyma using α-TfR Ab as shuttle[11] -Low-affinity α-TfR bispecific Ab enters the brain and experiences less lysosomal degradation[10]	*PS2-APP* mice[5]	No difference in BBB permeability compared to WT[5]	-FM, ELISA[9,10] -Radio-tracing[5,9–12] -WB[10]
	Mab86	C57BL/6 mice	Mab86 is confined within the vasculature	*Tau-PS2-APP-pdgfb*$^{ret/ret}$ mice	Increased accumulation of Mab86 in hippocampus	FM[13]
	Human Aβ1–40	B6/SJL mice	Aβ1-40 undergoes receptor-mediated transcytosis	*APP-PS1* mice	No difference for Aβ1– 40 compared to B6/SJL mice	Radiotracing[4]
	Albumin	C57BL/6 mice[5]	Present in brain lysates. No difference with age[5]	*PS2-APP* mice[5]	No difference with age and between *PS2-APP* to WT[5]	Radiotracing, WB[5]

Endogenous proteins	IgG	-C57BL/6 mice[5,7,8,13] -Human patients[14]	-Present in intracellular vesicles in BECs[13] -Present in brain lysates. No difference with age[5] -Restricted to vasculature and ventricles[7] -No IgG in parenchyma[8] IgG, IgA, IgM, alpha-2 macroglobulin in brain vasculature, neuropil and in neuronal cell bodies in normal aged brain[14]	-*Pdgfb*$^{ret/ret}$ mice[13] -*Tau-PS2-APP-pdgfb*$^{ret/ret}$ mice[13] -EAE mice[15] -Nγ1-KO mice[7] -*Apoe*$^{-/-}$, *TR-APOE3, TR-APOE4, TR-APOE4 Ppia*$^{-/-}$, *GFAP-APOE4, Apoe*$^{-/-}$*Ppia*$^{-/-}$[8]	-Less IgG positive vesicles in BECs[13] -Increased accumulation in parenchyma[13] -Increased permeability -Lack of astrocytic laminin induces BBB breakdown[7] -Increased leakage into the brain in *Apoe*$^{-/-}$ and *APOE4* mice and with age. Leaky phenotype is rescued by *CypA* inhibition and in *Ppia*$^{-/-}$ mice[8]	-FM[13] -WB[15] -FM, TEM[7] -FM, WB[8] -FM, TEM[14]
	Hemosiderin	C57BL/6 mice	No hemosiderin deposits in sagittal brain sections	*Apoe*$^{-/-}$, *GFAP-APOE3, GFAP-APOE4, TR-APOE3, TR-APOE4* mice	Leaky phenotype in *Apoe*$^{-/-}$, *APOE4* mice which increases with age. *CypA* inhibition can normalize hemosiderin levels	LM[8]
	Fibrin (-ogen)	C57BL/6 mice	Low levels in brain	*Apoe-/-, GFAP-APOE3, GFAP-APOE4* mice	Leaky phenotype in *Apoe*$^{-/-}$, GFAP-APOE4 mice; fibrin accumulates in neurons. CypA inhibition normalizes fibrin levels	FM[8]
	Thrombin	C57BL/6 mice	Low levels in brain	*Apoe*$^{-/-}$, *GFAP-APOE3, GFAP-APOE4* mice	Leaky phenotype in *Apoe*$^{-/-}$, GFAP-APOE4 mice; accumulates in neurons. *CypA* inhibition normalizes thrombin levels	FM, WB[8]
	Leptin	C57BL/6J mice	Accumulation in mediobasal hypothalami and ventricular system	Obesity	Accumulation predominantly in mediobasal hypothalami and ventricular system	FM[16]
Small molecules	Evans Blue	-Rat[17] C57BL/6 mice[7] -Male Sprague Dawley rat[18] -C57BL6/J mice[3]	-Leakage into eye tissue without astrocytes[17] -Confined to ventricles Almost no signal elsewhere[7] -No EB in brain parenchyma[18] -No accumulation in brain parenchyma[3]	-Injection of astrocytes into eye[17] -Nγ1-KO mice[7] -Induced seizure[18] -*Pdgfb*$^{ret/ret}$, *R26P*$^{+/0}$, *R26P*$^{+/+}$ mice[3]	-No leakage with astrocytes implanted[17] -Lack of astrocytic laminin induces BBB breakdown and pericyte differentiation[7] -Increased permeability[18] -Accumulation in brain parenchyma in *Pdgfb*$^{ret/ret}$ and less in *R26P*$^{+/0}$ but not in *R26P*$^{+/+}$ mice[3]	-LM[17] -FM, TEM[7] -LM[18] -LM, FM[3]
	Cadaverine	C57BL/6J mice[3,8]	No accumulation in brain parenchyma[19]	-*TR-APOE3 Ppia*$^{-/-}$ mice treated with siLrp1[8] -*Pdgfb*$^{ret/ret}$, *R26P*$^{+/0}$, *R26P*$^{+/+}$ mice[20]	-Leaky phenotype in siLrp1 treated *TR-APOE3* but not in *Ppia*$^{-/-}$ mice[8] -Accumulation in parenchyma and neurons in *Pdgfb*$^{ret/ret}$ and less in *R26P*$^{+/0}$ but not in *R26P*$^{+/+}$ mice -Treatment with imatinib rescues leaky phenotype[20]	FM[3,8]
	Sodium fluorescein	Sprague Dawley Rat	Confined to vasculature	-Induced seizure -Excessive glutamate concentrations	Increased permeability to sodium fluorescein	FM[18]
	Riboflavin	Albino rat	Can pass the BBB and uptake is dose-dependent	-	-	Lumiflavin fluorescence[21]
	Lyso-phosphatidyl-choline	NOD-SCID mice	Uptake into parenchyma	Xenograft models of brain metastases	Reduced permeability to lysophosphatidylcholine compared to WT	FM[22]
	Gadolinium	Human patients[23–25]	-Increased hippocampal permeability[24] -Does not enter the brain[23]	-MCI[24] -MCI, early AD[25] -AD[23]	-Increased hippocampal permeability[24] -Increased leakage in hippocampus in MCI and in grey and white matter in early AD[25] -No BBB breakdown in AD[23]	-MRI[24,25] -PET[23]
	Pyruvate	-Rat -Pig	Pyruvate (and lactate) confined to vasculature	-Metastasis model to the brain -Mannitol osmotic shock	-Lactate production corresponds with BBB breakdown in the disease -Increased permeability	MRI[26]
	Fluorodeoxyglucose (FDG)	Human patients	Glucose uptake into brain	Age-related cognitive decline and AD	Diminished glucose transport	PET[27]
	Rubidium chloride	C57BL/6 mice	Intact BBB, no difference between young and aged	*PS2-APP* mice	No difference in BBB permeability compared to WT	Radiotracing[5]

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