Supplementary Methods:

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BBB model development:

We strictly followed our previous methods for the BBB development procedure and used the same reagents with same catalogue numbers(Malik et al. 2024; Malik et al. 2023b). Briefly, human primary brain cells procured from ScienCell were sub-cultured in relevant fibronectin coated flasks. After confluency the cells were harvested by trypsinization, counted, and used for seeding on bovine Collagen I, fibronectin and poly-L-Lysine coated transwell inserts. TEER was measured after the transwell insert BBB development for confirm the tight junction formation. On the 2nd day of steady state TEER value measurement, permeability assay was performed before commencement of experiment with drugs. More detailed information can be found below or in our previous work(Malik et al. 2024; Malik et al. 2023b).

Cells and culture system: Human brain primary cells, namely, astrocytes (#1800), pericytes (#1200), human brain microvascular endothelial cells (HBMECs) (#1000), Human neurons (#1520) and growth supplements, were purchased from ScienCell. The ScienCell catalogs were: astrocyte media (AM) #1801, astrocyte growth supplement (AGS) #1852, pericyte media (PM) #1201, pericyte growth supplement (PGS) #1252, endothelial cell medium (ECM) #1001, endothelial growth supplement (ECGS) #1052, neuronal media (NM) #1521, neuronal growth supplement (NGS) #1562, Fetal bovine serum (FBS) #0010 and penicillin/streptomycin solution (P/S) #0503. Frozen cell vials were revived and cultured following the manufacturer's instructions. As per requirements, cells were grown in 25 cm^2, 75 cm^2, or 150 cm^2 culture flasks (TPP #90076). All the culturing flasks and plates were pre-coated with Bovine fibronectin at 2 µg/mL (ScienCell

#8248). Flasks with approximately 90% cell confluency were harvested by trypsinization (0.25% trypsin, Lonza #CC-5012) and washed in Dulbecco's phosphate-buffered saline ([DPBS], Dulbeccos #1960454). Cell counting was performed by mixing 10 µL of cell suspension with 10 µL of trypan blue and pipetting 10 µL from the mix to read in the cell counter (Invitrogen Countess).

Transwell plates and coating**:** Coating of plates followed our previously described methods(Malik et al. 2023b). Briefly, polystyrene transwell plates were coated with 50 µg/mL bovine collagen I in phosphate-buffered saline (PBS) overnight at 4°C. The next day, Fibronectin (3 µg/mL) in PBS was added to the collagen I coated dried transwell plate and incubated at 37°C for 3 hrs. Poly-L-Lysine (Sigma, #P4707) was added to the double-coated plates and incubated for 10 minutes at room temperature (RT) and then dried.

Cell seeding on transwell membrane 1). Astrocytes: Our earlier protocol was adopted for cell seeding with astrocytes seeding on the basolateral side of the transwell membrane and incubated at 37°C for 3 hrs. Excess media was removed from the transwell membrane and kept in their original position, followed by media addition to grow for 48 hrs.

2). Pericytes: Similarly, after 48 hrs, excess media was removed from the top of the transwell containing the growing astrocytes, and pericytes were seeded on the basolateral side of the transwell membrane and incubated for 3 hrs. Excess media was removed from the membrane. Astrocyte and pericyte media (1:1) were added to the wells and incubated for monolayer formation of astrocytes and pericytes.

3). HBMECs and Neurons: Approximately seven days after the seeding of astrocytes, HBMECs in endothelial media were seeded on the apical side of the transwell membrane, incubated for 6 hrs, followed by the addition of 400 µL of endothelial cells. The same day, human neurons were seeded on a Poly-L-Lysine-coated coverslip in a separate 12-well plate.

Combining cells: On day 11, all cells were combined. The transwell insert with cells in the apical and basolateral regions were carefully transferred to the 12-well plate containing neurons at the bottom of the well. The apical side of the transwell was topped with ECM, while the basolateral part was filled with 1:1:2 of AM, PM, and neuronal media. Transepithelial electrical resistance TEER was measured approximately three days after the combination of the cells using an epithelial volt/Ohm meter ($EVOM^2$). TEER value was checked daily for three more days.

Permeability test of BBB and BCSFB:

FITC-Dextran permeability assay: After the TEER measurement on day six, the barrier functionality was checked by performing a permeability test using Fluorescein Isothiocyanate-Dextran (FITC-dextran) (#FD4-100MG, 4 kDa) from Sigma Aldrich. The assay was performed according to earlier studies(Fu et al. 2021) and the manufacturer's instructions (equation below). In brief, 0.1 mg/mL of the FITC-dextran was prepared in the endothelial media, and 200 µL of mix was added to the apical compartment of each insert. Samples were collected from the basal compartment immediately before adding the FITC-dextran to the apical compartment for time "0" (initial). After that, samples were collected at 10, 20, and 30 minutes from the apical and basal regions of the transwell inserts. All the experimental and control samples were collected in an

ELISA plate, and the optical density (O.D.) was measured for the FITC intensity in a Fluorescence-based 96-well plate reader at 488 nm excitation, 525 nm emission.

The apparent permeability for the respective models was calculated using the formula below.

Pa= (Cb/Caxt) x (volume/area)

Pa= apparent permeability

Cb (Basal concentration) = O.D of FITC-Dextran in the basal compartment of the BBB models

Ca (Apical concentration) = O.D of Initial FITC-Dextran from the apical compartment

t= Time of sampling.

ARVs and AV (antiviral) experimentation:

ARV formulations**:** The following AVs were weighed and dissolved in 1 mL of dimethyl sulfoxide (DMSO) as a stock with different concentrations in mg/mL: Dolutegravir (DTG, 1.75 mg/mL), emtricitabine (FTC, 2.0 mg/mL), efavirenz (EFV, 2.5 mg/mL), elvitegravir (EVG, 2.0 mg/mL), tenofovir disoproxil fumarate (TDF, 1.0 mg/mL), raltegravir (RAL, 2.0 mg/mL), atazanavir (ATV, 4.0 mg/mL), lamivudine (3TC, 1.25 mg/mL), nirmatrelvir (NMR, 4.42 mg/mL) and ritonavir (RTV, 2.0 mg/mL).

Addition of AVs to the BBB and BCSFB**:** From the stock solution of each drug, the known clinical plasma C_{max} value in ng/mL for DTG-3500 ng/mL, FTC-2000 ng/mL, EFV-2500 ng/mL, EVG-1500 ng/mL, TDF-450 ng/mL, RAL-1500 ng/mL, ATV-4500 ng/mL, 3TC-3500 ng/mL, NMR-

2100 ng/mL, and RTV-1000 ng/mL(Dyavar et al. 2019) was prepared in 10 mL of endothelial media and mixed via vortexing. Media was pipetted out from the apical region of the BBB transwell plate, and 500 µL of the different AV mixed media was added to the respective apical region of the insert well. RTV was considered as a boosting agent and not an AV.

Reagents and standard procedures for protein analysis:

Antibodies and reagents: Antibodies against human permeability glycoprotein ([P-gp], #12683s) multidrug resistance protein-1 ([MRP1], #72202s) and 4 ([MRP4], #12857s), Breast cancer resistance protein (BCRP], #4477s), nuclear factor kappa-light chain-enhancer ([NF-Kß], #8242s), beta-actin (#5125s), and horseradish peroxidase (HRP)-conjugated anti-rabbit (#7074s) were purchased from Cell Signaling. Any kD™ Mini-PROTEAN® TGX™ (#4569035), 10x sodium dodecyl sulfate (SDS) buffer (#1610732), 10x tris-buffered saline ([TBS], #1706435), 10x transfer buffer (#1610734), 4x sample buffer (#1610734), and filter membrane (#16120214) from Bio-Rad. Other protein analysis reagent and materials were, Dithiothreitol (DTT), polyvinylidene fluoride (PVDF) membrane (Millipore, #IPVH09120), enhanced chemiluminescence (ECL) western blotting substrate (Invitrogen, #WP20005), bovine serum albumin ([BSA], MP Biologicals, #180561), Tween 20 (Sigma, #92287), and non-fat milk powder was purchased from a commercial retail grocery store.

Cell lysis procedure**:** After incubation, media was collected, and cells were washed once with DPBS and incubated with 10 mL of fresh DPBS at 37^0C for 15 minutes. The DPBS was discarded, and 1 mL of pre-warmed 0.25% Trypsin was directly added to the flask and incubated at 37^0C for 1 minute, followed by immediate dilution with 20 mL of DPBS. Cells in DPBS were collected in

a 50 mL tube and centrifuged at 1500 rpm for 10 minutes. The cell pellets were lysed with 200 µL of 1x lysis buffer (Cell Signaling, #9803) and hard centrifuged at 14000 rpm for 10 minutes. The supernatant was collected in a fresh tube for protein estimation and analysis. Only astrocytes were chosen for transporter expression, given the limited information available on the transporter expression of these cells in the available literature.

References:

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