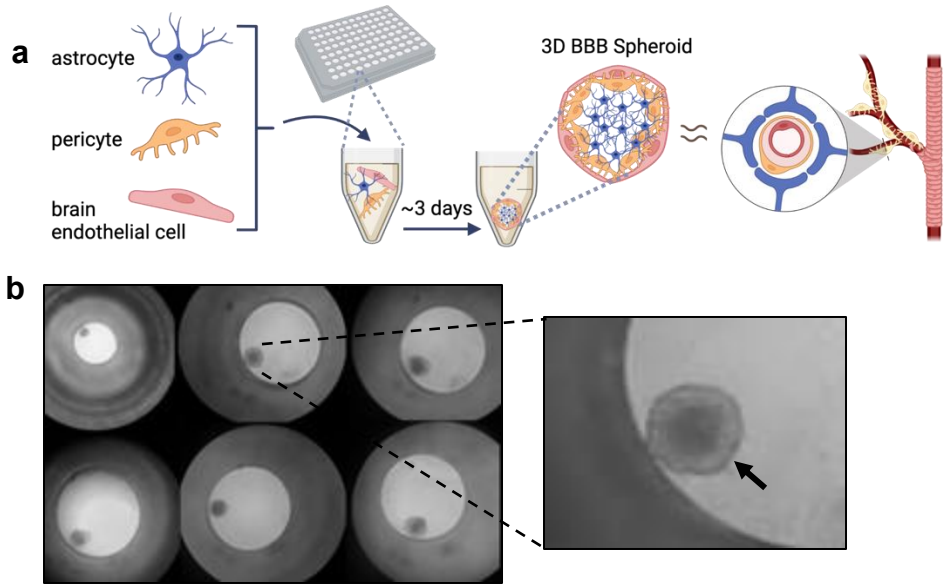
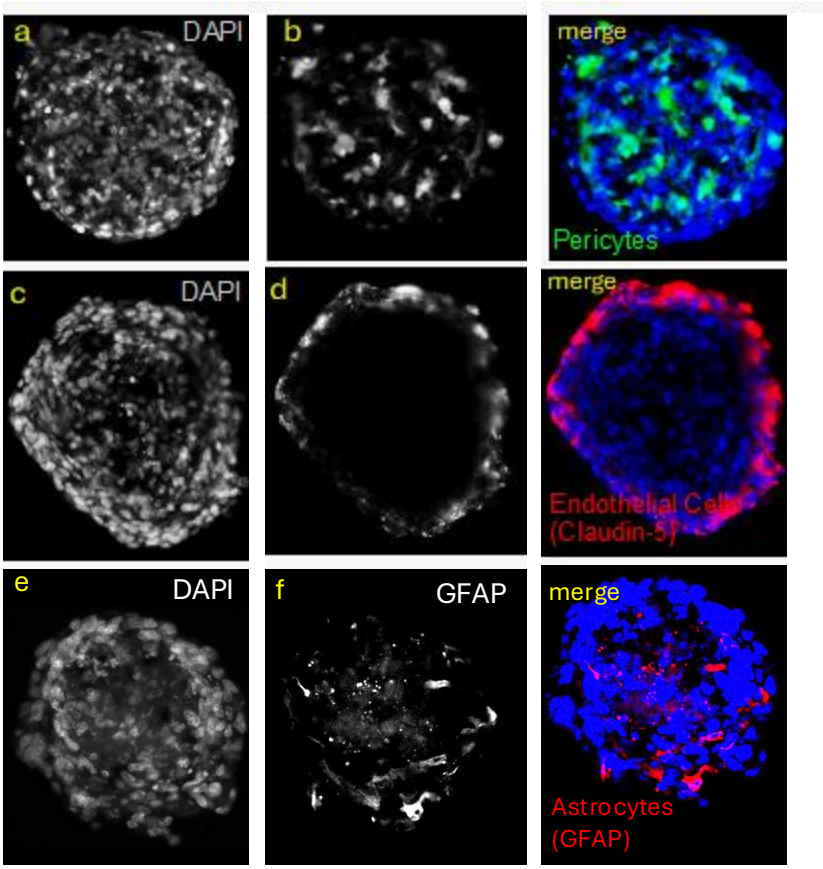
Supplementary Fig. 1



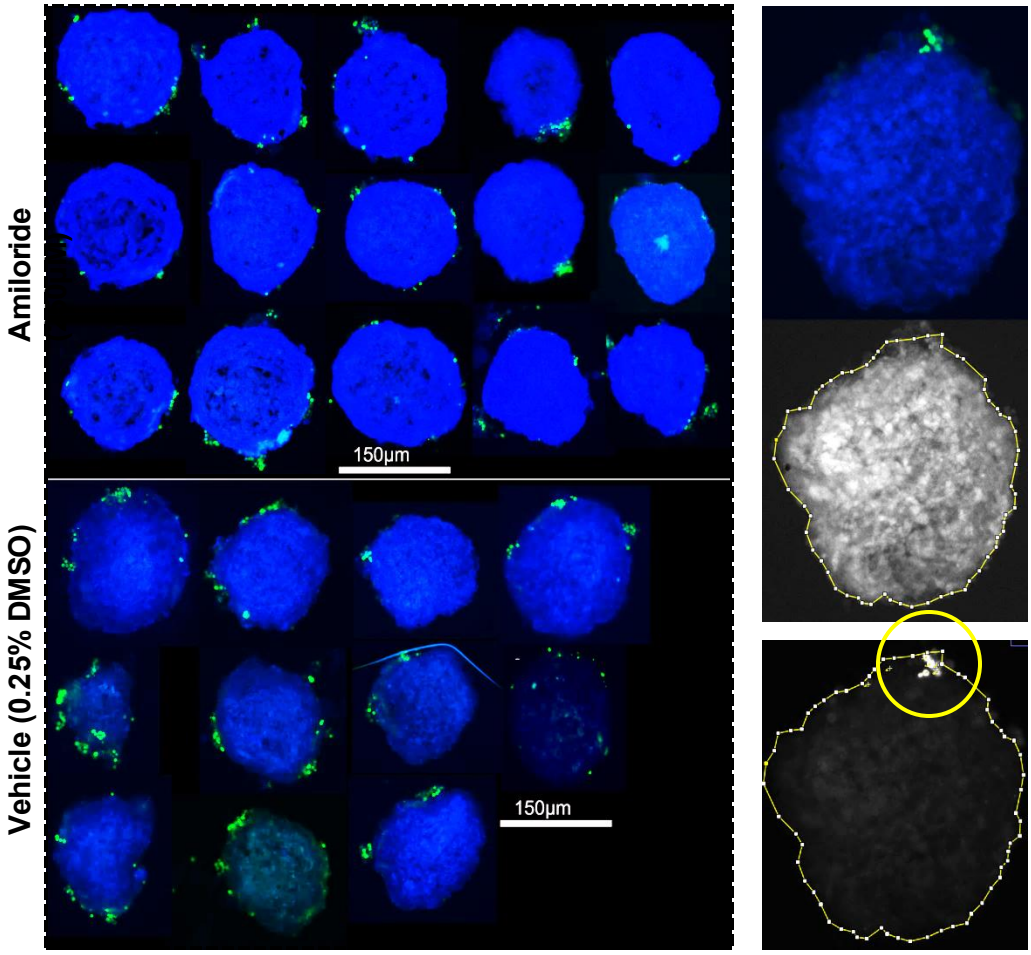
Supplementary Fig. 1. Schematics and images of 3D BBB organoid formation. **a** Organoids were formed from either primary human BMECs (passage < 6) or immortalized HCMEC/D3 cells (iBMECs, passage < 35), human primary pericytes (passage 4), and human astrocytes (passage 4). Primary cells were purchased from ScienCell (Carlsbad, California, USA). The organoids were formed by combining the three cell types in a 1:1:1 ratio, each at a concentration of $1x10^4$ cells/mL. The combined cells were grown in 100 µL media in a 96-well plate. **b** Brightfield view of organoids formed at the bottom of flat-bottom 96-well plates with a transparent non-stick polymer coating. Organoids were relatively homogenous in size and shape.

Supplementary Fig. 2



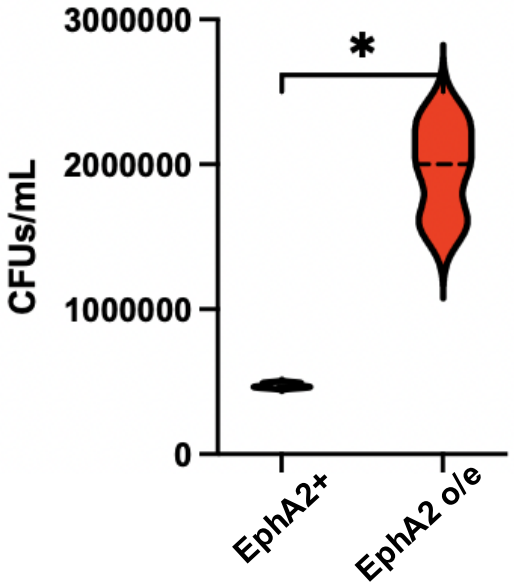
Supplementary Fig. 2. Organization of neurovascular cell types in human BBB organoids. First column **a**, **c**, **e** shows DAPI counterstaining. Second column **b**, **d**, **f** shows cell-type specific staining. Third column contains merged images from column 1 and column 2. Top row: pericytes were labeled in culture with CMFDA (CellTracker Green, Invitrogen) and combined with endothelial cells and astrocytes to form organoids. **a** DAPI, **b** pericytes, merged image to the right. Middle row: Organoids were probed with **d** rabbit anti-Claudin-5 (1:250) and goat - anti-rabbit TexasRed, merged image (middle row, right) with DAPI **c**. Bottom row: Astrocytes were visualized with GFAP (Invitrogen MA5-12023, 1:2000) **f** with DAPI counterstaining **e**, with merged images to the right. All images were acquired from 15 µm thick cryosections of PFA-fixed sections made from 1-week old organoids.

Supplementary Fig. 3



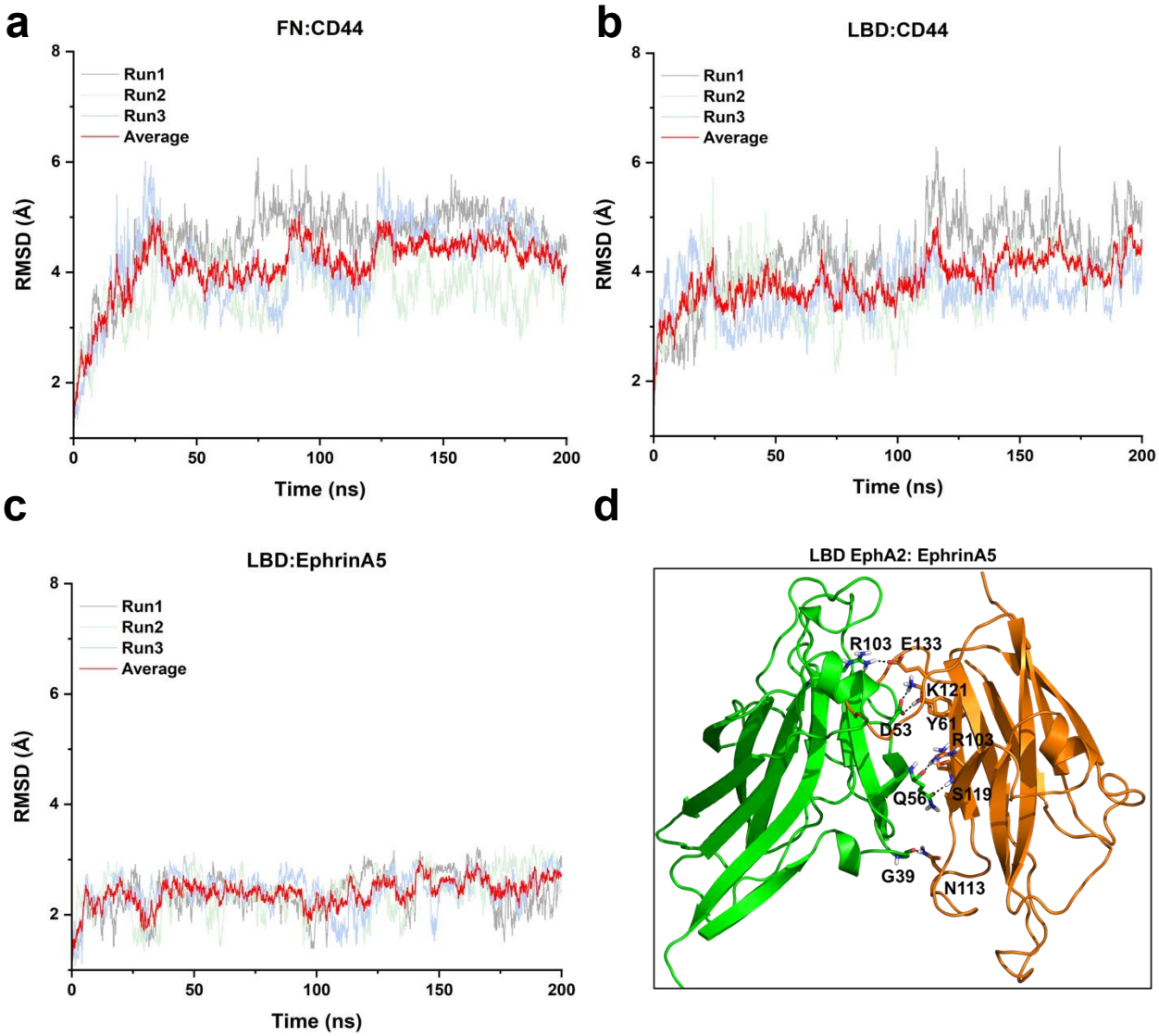
Supplementary Fig. 3. Organoid-based assays to examine the impact of Amiloride treatment on *Cn* BBB-internalization/crossing. Representative images showing organoids (blue, DAPI) incubated for 24 h with *Cn* (green, CFSE) in media containing either 250 µM amiloride (AML) (top-left) or an equivalent concentration of vehicle control (bottom left). Representative images from the analysis pipeline, with the "input" merged image at top right, border demarked around organoids in the DAPI channel right middle panel, and this border applied in the CFSE channel to quantify crossing/internalization of fungal cells (bottom right panel, yellow circle).

Supplementary Fig. 4



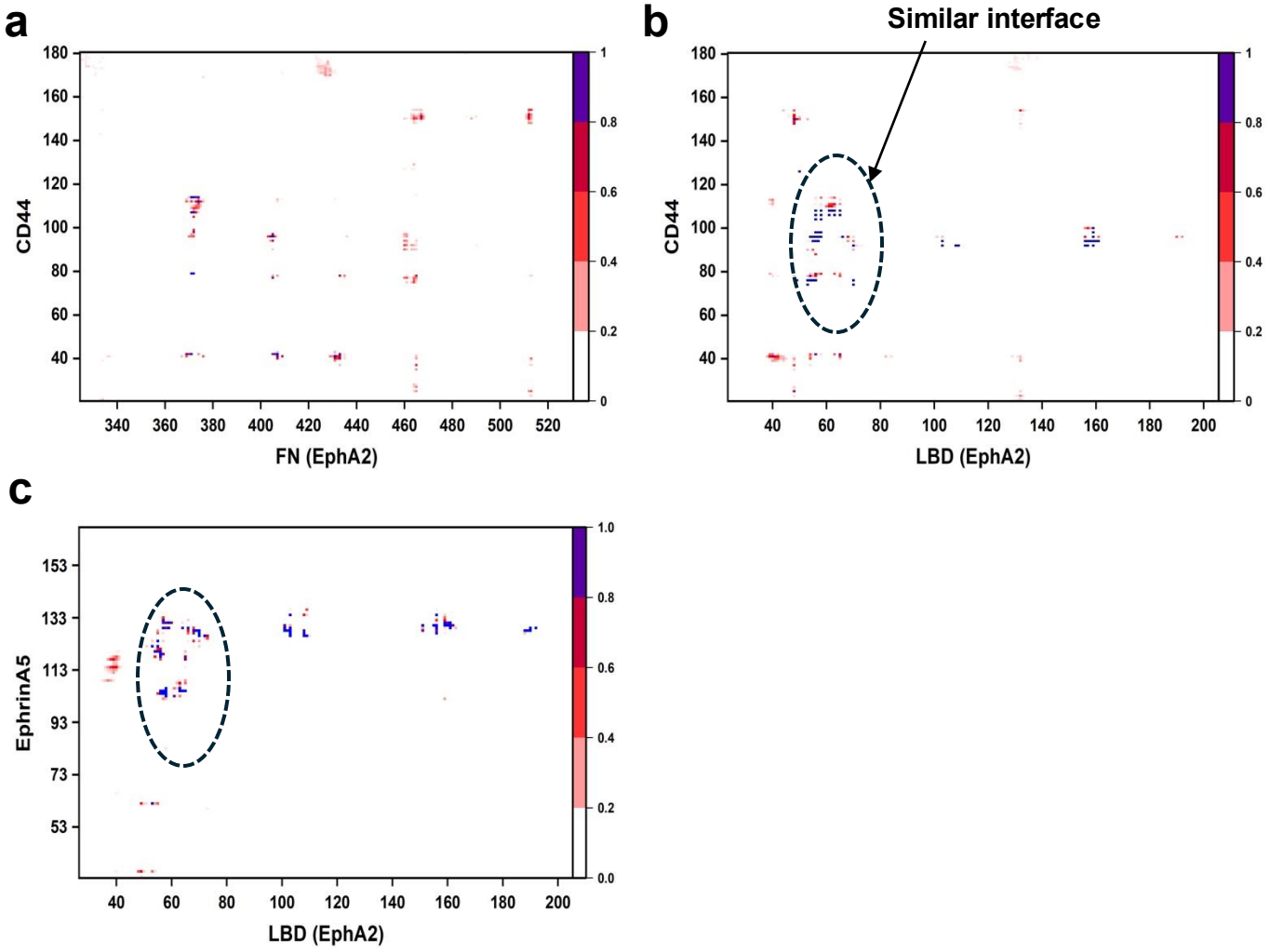
Supplementary Fig. 4. Overexpression of EphA2 in brain endothelial cells increases migration of *Cn* across the brain endothelium in an *in vitro* transwell model of the BBB. Transcytosis assay shows a significant increase in the movement of *Cn* across endothelial cells when brain endothelial cells (iBMECs) overexpress EphA2. EphA2+ iBMECs (wild type) and iBMECs transduced with EphA2 *via* lentivirus were exposed to *Cn* (MOI of 10) that was added to the apical side of the transwell. Following 12 h exposure, fungal cells were collected from the abluminal side of each transwell (well below transwell) and plated onto agar plates for CFU (colony forming units) determination. Each data point represents the fungal count from an individual well (n=3). Statistical analysis was done using a two-tailed, unpaired t-test with Welch correction (p = 0.0178, t= 7.358, df= 2.008)

Supplementary Fig. 5



Supplementary Fig. 5. Structural stability and binding conformations of EphA2 complexes. **a–c** Backbone RMSD profiles of the FN: CD44, LBD: CD44, and LBD: ephrinA5 complexes over the course of molecular dynamics simulations. Data from all replica trajectories as well as averaged RMSD values are shown. **d** Representative binding conformation of the LBD: ephrinA5 complex obtained from clustering of all replica trajectories. EphA2 is depicted in green and ephrinA5 in orange cartoon representation. For clarity, only the major interfacial hydrogen bonds are displayed.

Supplementary Fig. 6



Supplementary Fig. 6. Intermolecular contact interfaces of EphA2 complexes. (**a–c**) Contact maps of the FN: CD44, LBD: CD44, and LBD: ephrinA5 complexes obtained from molecular dynamics simulations. Contacts were calculated using a 5 Å cutoff. The color scale (white to red to blue) represents the fractional occupancy of each contact, ranging from 0 (no contact) to 1 (persistent contact). Data from all replica trajectories were included in the analysis. Notably, CD44 and ephrinA5 engage overlapping interface regions within the EphA2 LBD.

Supplementary Table 1 - Antibodies used in the current study.

Name	Type	Company	Catalog #	Use
mouse anti-CD44	primary	abcam	ab254530	PLA
rabbit anti-EphA2	primary	abcam	ab185156	PLA
mouse anti-Cdc42	primary	cytoskeleton	ACD04	WB
rabbit anti-EphA2	primary	Cell signaling	D4A2	WB
goat anti-mouse IgG H&L HRP	secondary	abcam	ab6789	WB
goat anti-rabbit IgG H&L HRP	secondary	abcam	ab6721	WB
rabbit anti-Claudin5	primary	abcam	ab15106	IF
goat anti-rabbit Cy5	secondary	abcam	ab6564	IF
mouse anti-GFAP	primary	Invitrogen	MA5-12023	IF
goat anti-rabbit TexasRed	secondary	abcam	ab6719	IF
goat anti-mouse Cy5	secondary	abcam	ab6563	IF