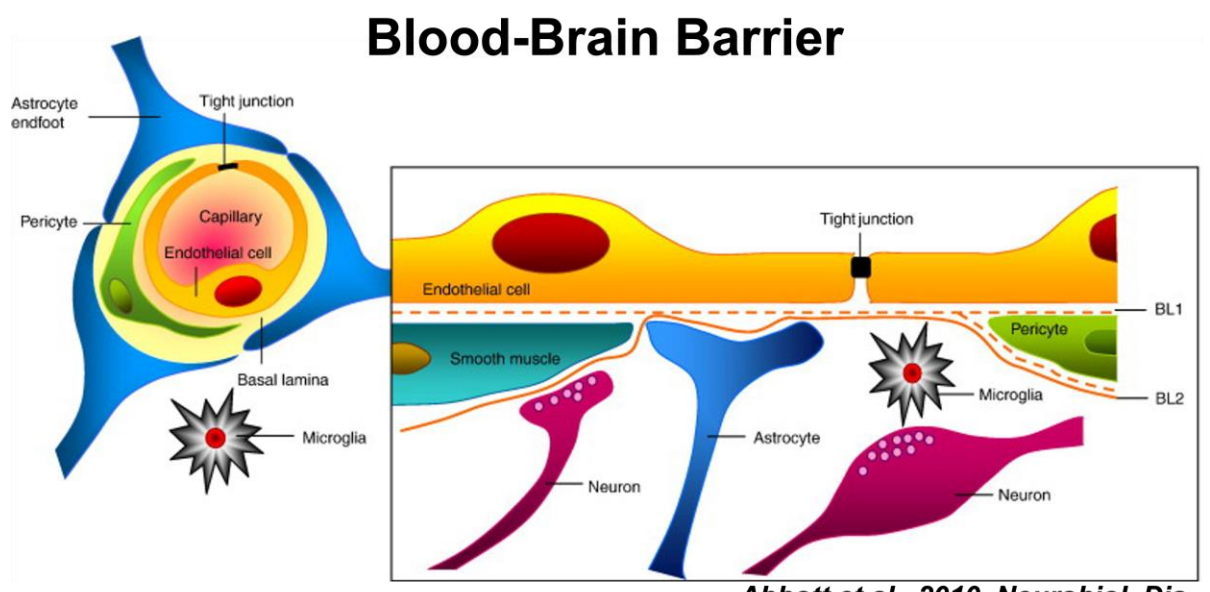


Modeling Neurovascular Communication in Alzheimer's Disease via Extracellular Vesicles in a 3D *In Vitro* Model of Blood-Brain Barrier

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INTRODUCTION



- Blood-brain barrier (BBB) dysfunction is the hallmark of Alzheimer's disease (AD).
- Central nervous system (CNS)-derived small extracellular vesicles (EVs) are established carriers of blood-based biomarkers for AD. However, they may also act as pathological mediators contributing to blood-brain barrier (BBB) injury, yet the mechanisms governing their trafficking across the BBB remain largely unexplored.

HYPOTHESIS

- EVs from AD individuals exhibit enhanced translocation across the BBB, thereby increasing BBB permeability and contributing to neurovascular dysfunction.

METHODS

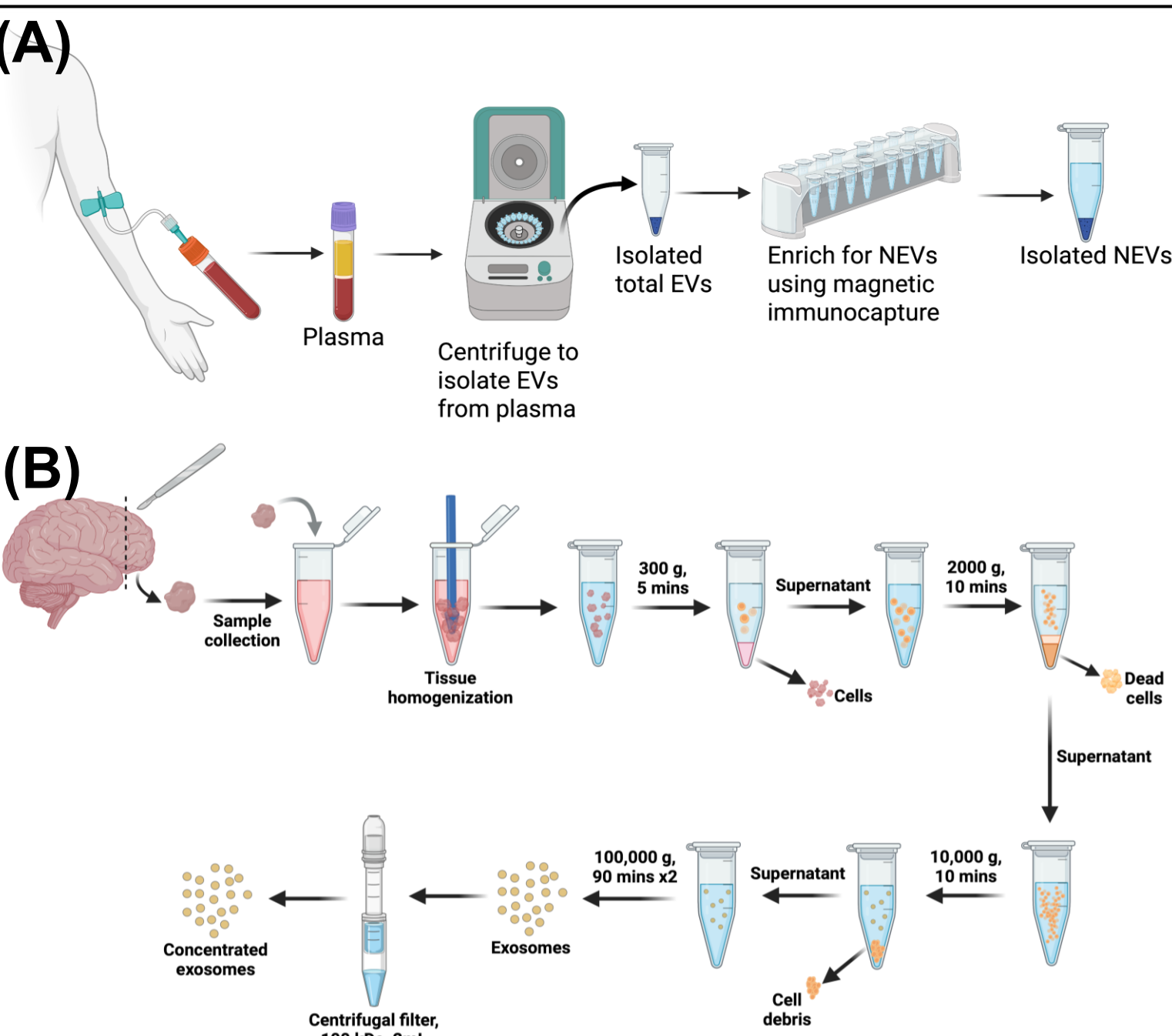


Figure 1: (A) Plasma EVs were isolated and Neuronal EVs (NEVs) were enriched using magnetic immunocapture targeting L1CAM. **(B)** Brain tissue-derived EVs (BDEVs) were isolated via differential ultracentrifugation.

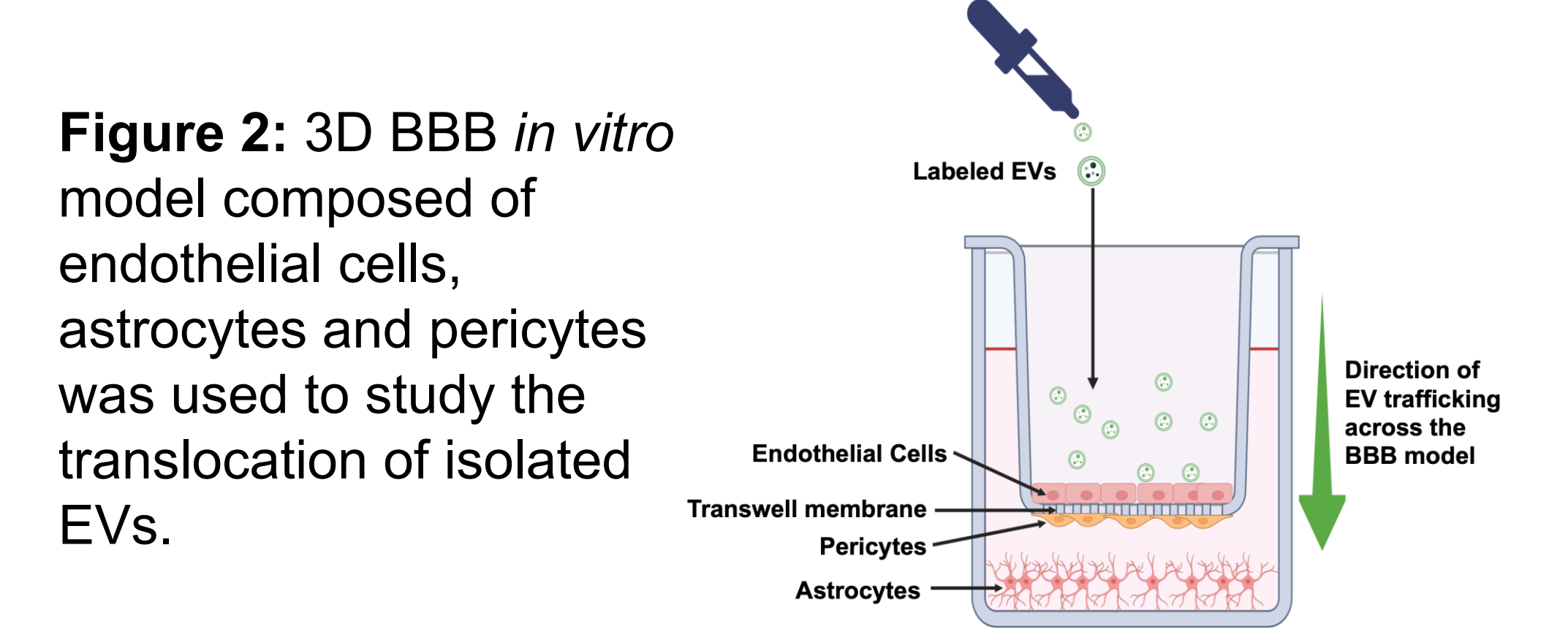


Figure 2: 3D BBB *in vitro* model composed of endothelial cells, astrocytes and pericytes was used to study the translocation of isolated EVs.

RESULTS

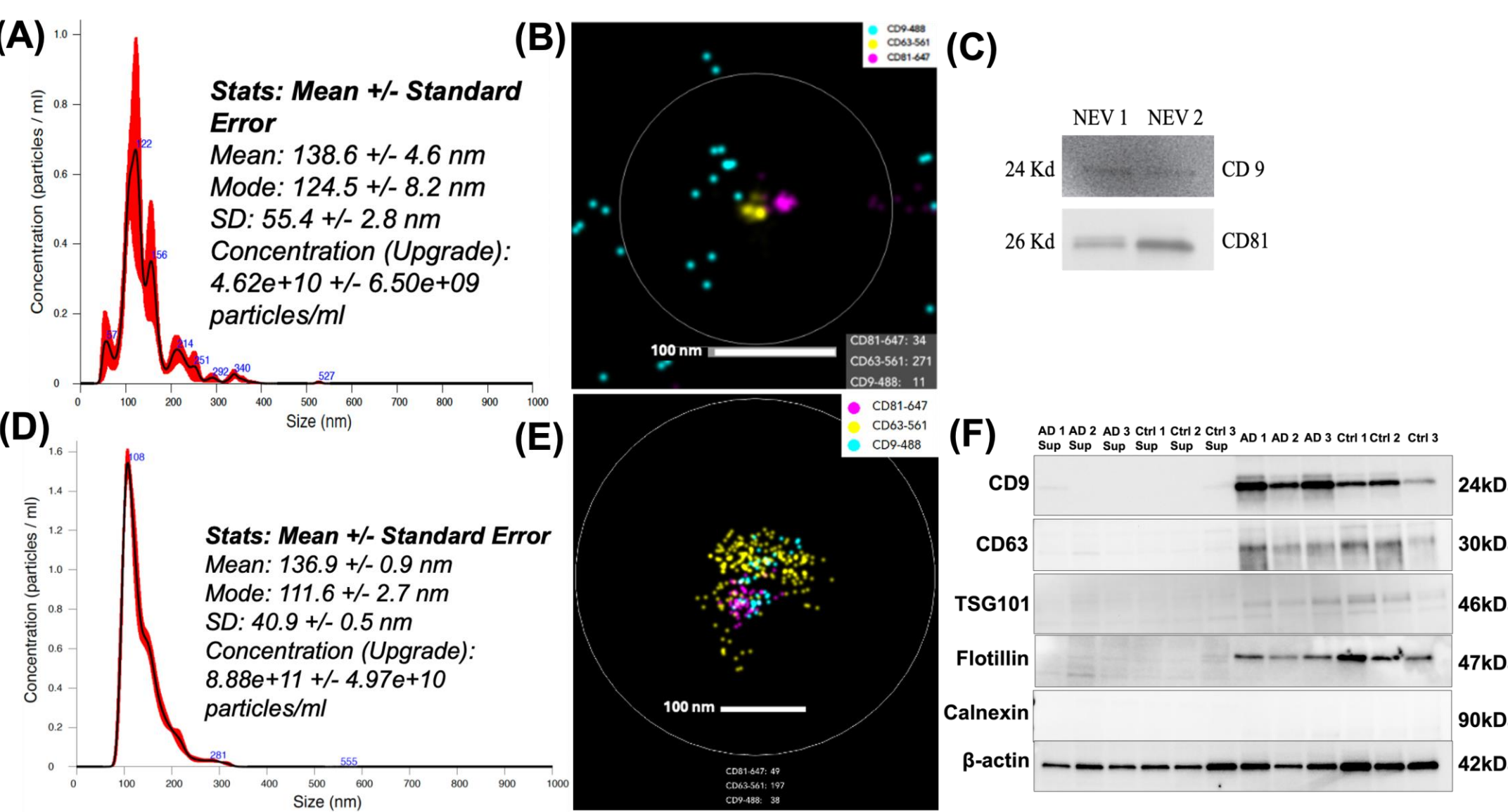


Figure 3: (A-C) Plasma NEVs and **(D-F)** BDEVs were characterized using **(A, D)** nanoparticle tracking analysis, **(B, E)** ONI super-resolution imaging, and **(C, F)** western blotting. Both EV populations showed expected small EV size profiles and expressed canonical EV markers, including CD9, CD63, CD81, and TSG101.

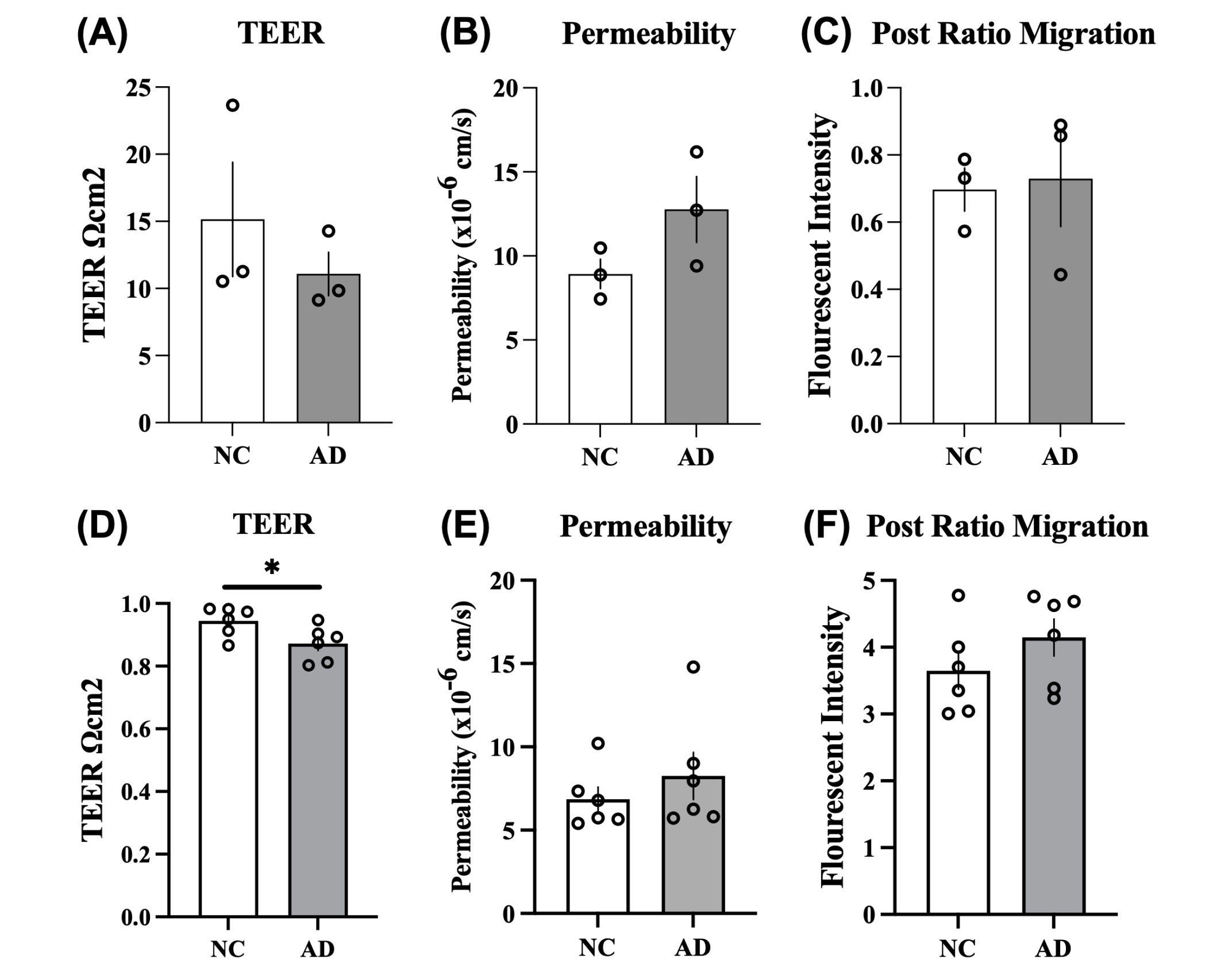


Figure 4: (A-C) Compared to plasma Non-AD Control-NEVs, AD-NEVs showed decreased transendothelial electrical resistance (TEER), increased BBB permeability and showed higher post-barrier migration as measured by fluorescence intensity. **(D-F)** BDEVs exhibit permeability trends similar to plasma NEVs. Data represent mean $\pm$ SEM; Plasma NEVs, n=3 per group, BDEV, n=6 per group, *p<0.05.

RESULTS

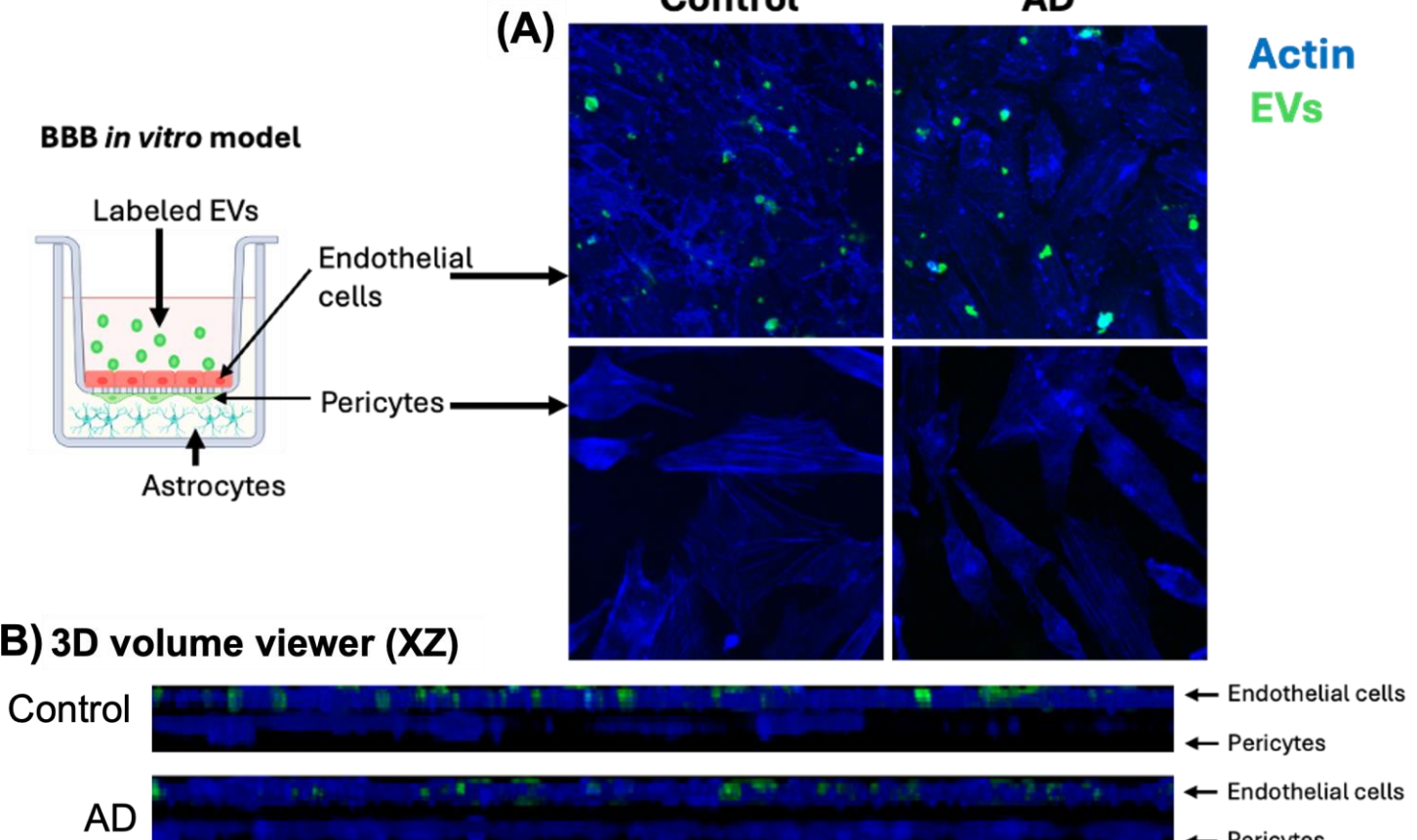


Figure 5: (A) Representative fluorescence images showing BDEV uptake by endothelial cells in 3D BBB *in vitro* model exposed to BDEVs. Actin, blue; EVs, green. **(B)** 3D volume viewer (XZ) images of basolateral chamber with pericytes not exposed to BDEVs, showing intact actin cytoskeletons but no BDEV signal. **(B)** Orthogonal projections (XZ) of images.

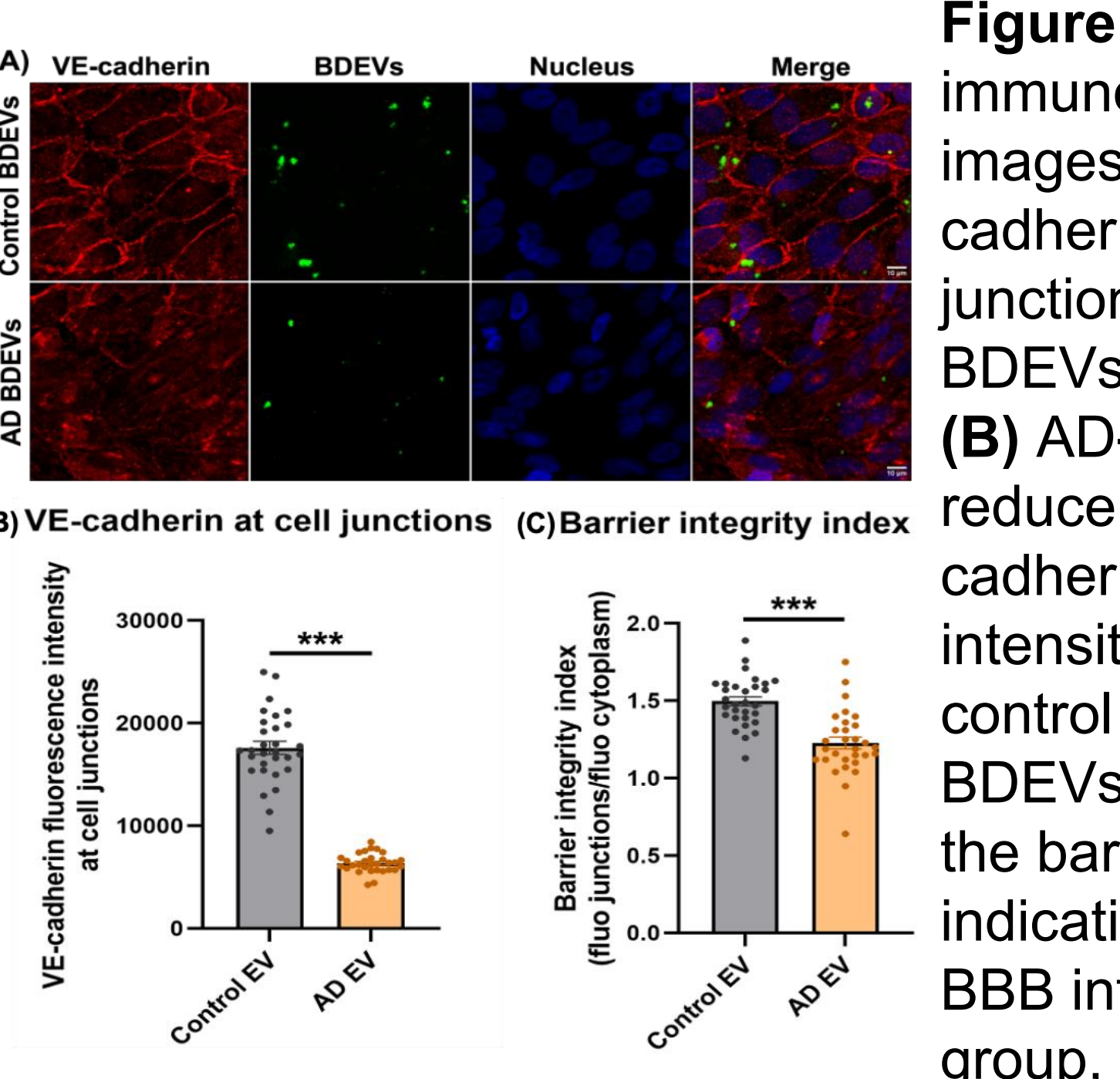


Figure 6: (A) 3D BBB immunofluorescence images showing VE-cadherin at endothelial junctions. VE-cadherin, red; BDEVs, green; nuclei, blue. **(B)** AD-BDEVs significantly reduce junctional VE-cadherin mean fluorescence intensity compared with control BDEVs. **(C)** AD-BDEVs significantly lower the barrier integrity index, indicating compromised BBB integrity. n=30 per group, ***p < 0.001.

CONCLUSIONS AND FUTURE DIRECTIONS

- Conclusion:** EVs from AD individuals readily cross the BBB and disrupt its integrity, suggesting a potential role for EVs in neurovascular dysfunction and as biomarkers or therapeutic targets in AD.
- Future Directions:** Determine whether NEVs exhibit enhanced bidirectional translocation across the BBB and disrupt BBB integrity, along with mechanistic pathways governing NEV trafficking across the BBB.

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