

A Journey From Gut to the Brain: Amyloid Proteins Hijack EVs To Travel From Gut to the Brain And Participate In Alzheimer's Pathology

Muhammad Usman Munir, Thomas P. Davis, Ibrahim Javed

Australian Institute for Bioengineering & Nanotechnology, The University of Queensland, Brisbane, Queensland 4072, Australia

Abstract

Alzheimer's disease (AD) is characterized by the accumulation and propagation of amyloid- β ($A\beta$) aggregates throughout the nervous system. Increasing evidence suggests that the gastrointestinal tract may represent an early site of $A\beta$ accumulation; however, the mechanisms by which gut-derived $A\beta$ reaches neuronal tissues remain poorly understood. In this study, we developed complementary transwell, co-culture, and microfluidic gut-brain models to investigate the transfer of $A\beta$ from intestinal epithelial cells to neurons. Human intestinal Caco-2 cells and neuronal SH-SY5Y cells were used to establish in vitro gut-brain interfaces. Fluorescently labelled $A\beta$ seeds were introduced into intestinal cells, and their transfer, intracellular trafficking, and neuronal uptake were assessed using confocal microscopy and quantitative fluorescence analysis. We further evaluated the effects of direct neuronal exposure and investigated the role of endocytic transport mechanisms. Our findings demonstrate efficient transfer of $A\beta$ from intestinal epithelial cells to neuronal cells, resulting in increased neuronal accumulation and cytotoxicity. These results support the hypothesis that gut-derived $A\beta$ can propagate across the gut-brain axis and provide mechanistic insights into potential pathways contributing to Alzheimer's disease progression.

Introduction

Alzheimer's disease (AD) is characterized by the accumulation and propagation of amyloid- β ($A\beta$) aggregates throughout the nervous system. Emerging evidence suggests that the gastrointestinal tract may represent an early site of $A\beta$ accumulation; however, the mechanisms by which gut-derived $A\beta$ reaches neuronal tissues remain poorly understood. Understanding these pathways is important for elucidating the role of the gut-brain axis in AD progression. Here, we investigated the cellular mechanisms underlying gut-to-brain $A\beta$ transfer using complementary in vitro and in vivo models.

Methods

Experimental Design

- Establishment of co-culture of Caco-2 intestinal epithelial and SH-SY5Y neuronal cells.
- Development of gut-brain transwell and microfluidic co-culture systems.
- Exposure of intestinal cells to fluorescently labelled $A\beta$ seeds.
- Assessment of $A\beta$ transfer, cytotoxicity and ROS assays
- Extracellular vesicles (EVs) were isolated and characterized by electron microscopy, particle size analysis, and EV marker staining.
- In-vivo zebrafish larvae study.

Results and Discussion

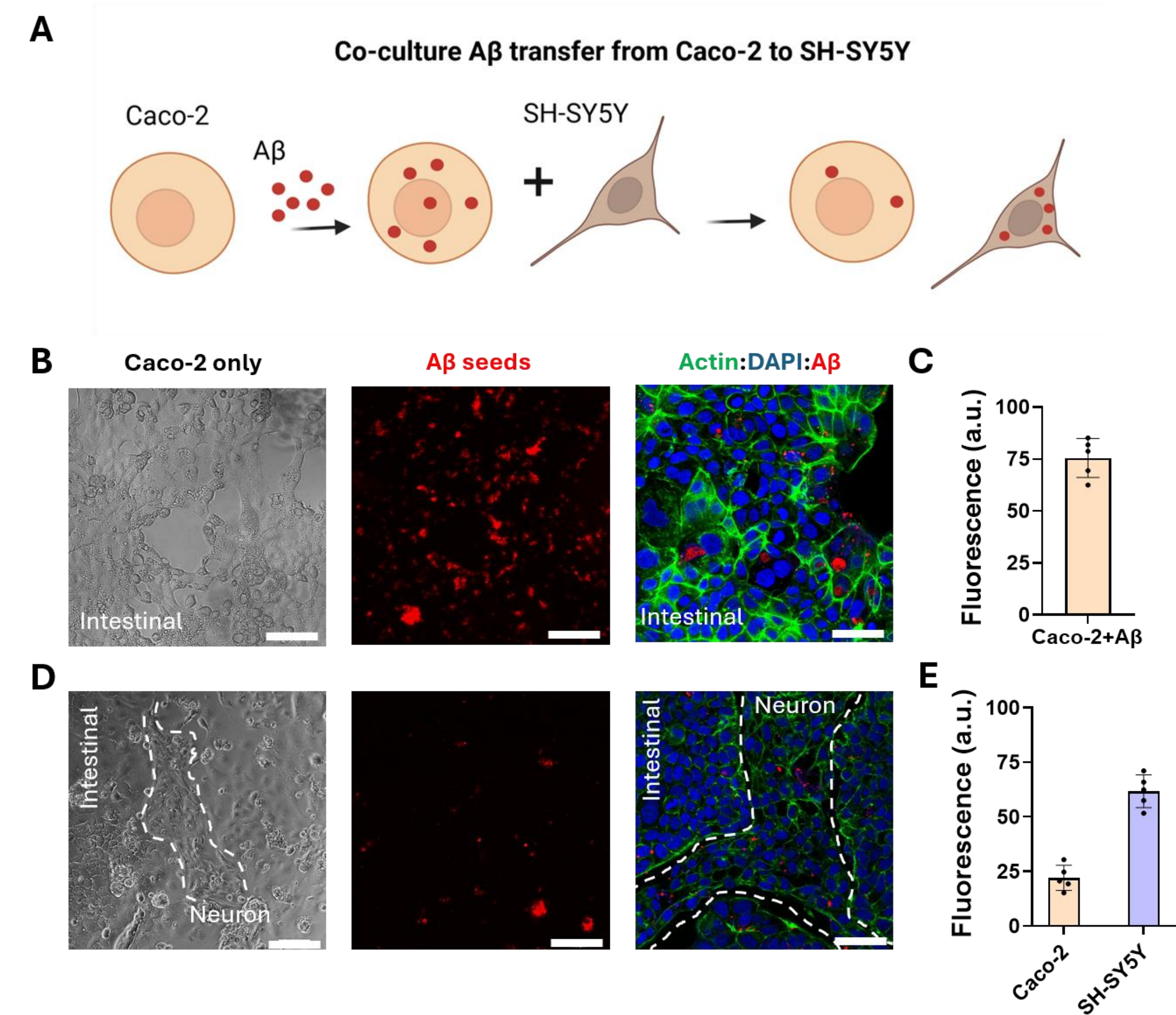


Figure 1. A) Schematic illustration depicting the experimental paradigm. B) Confocal images of Caco-2 cells exposed to AF555-A β seeds showing internalization of A β . C) Graphed quantification of AF555-A β fluorescence intensity in Caco-2 cells. D) Confocal images of Caco-2/SH-SY5Y co-culture system demonstrating the transfer of AF555-A β from Caco-2 to SH-SY5Y neurons. E) Quantification of AF555-A β fluorescence intensity in Caco-2 versus SH-SY5Y cells.

Results and Discussion

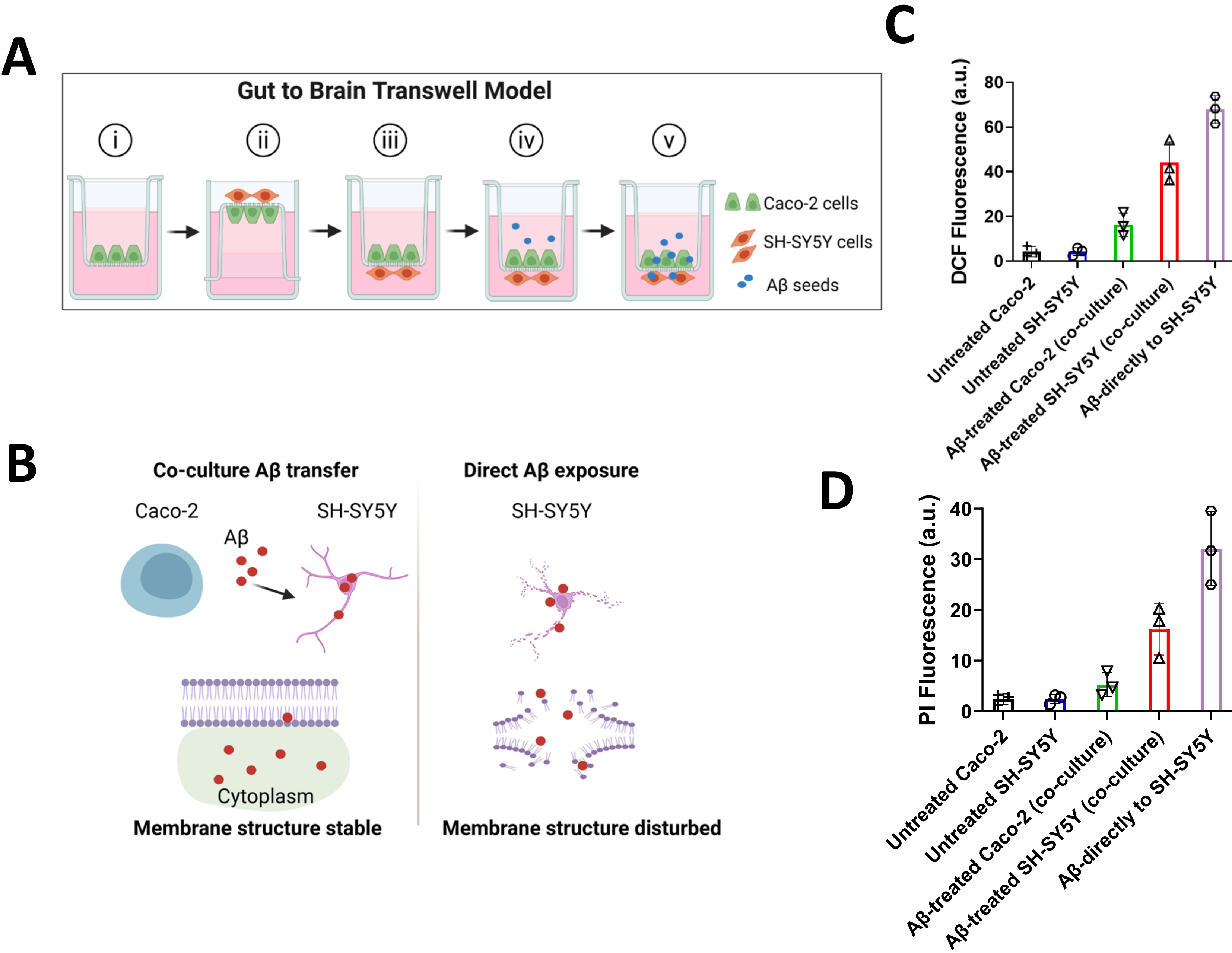


Figure 2. A) Development of an in vitro gut-brain transwell model. B) C, D) Cytotoxicity and ROS studies.

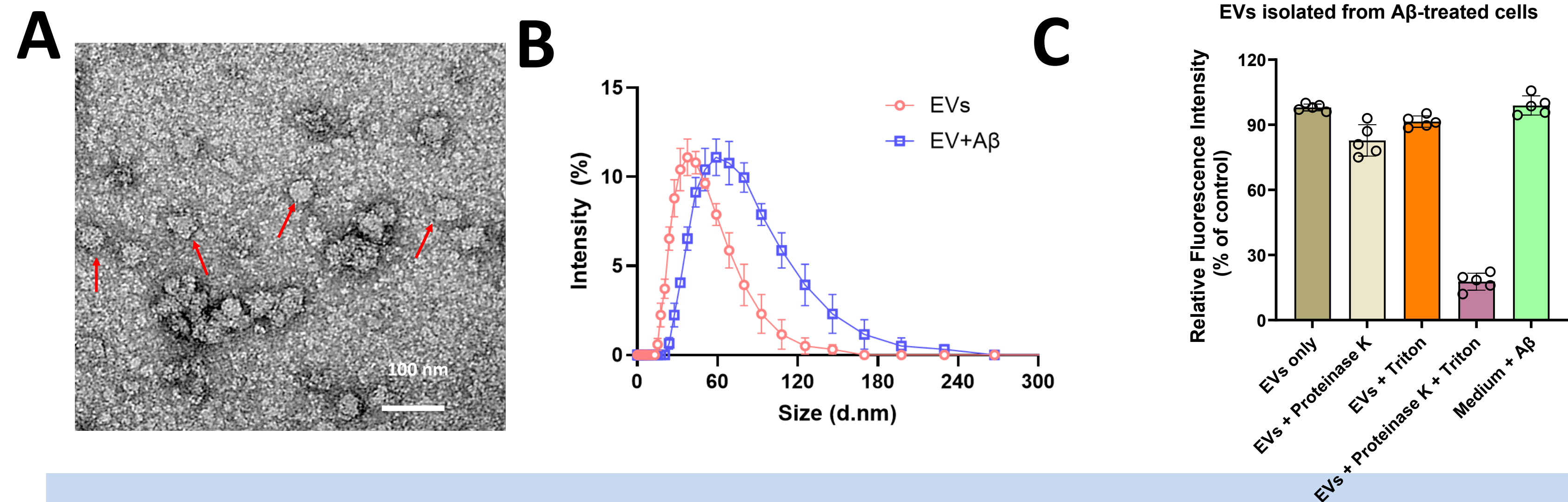


Figure 3. Characterisation of extracellular vesicles isolated from A β -treated intestinal cells.

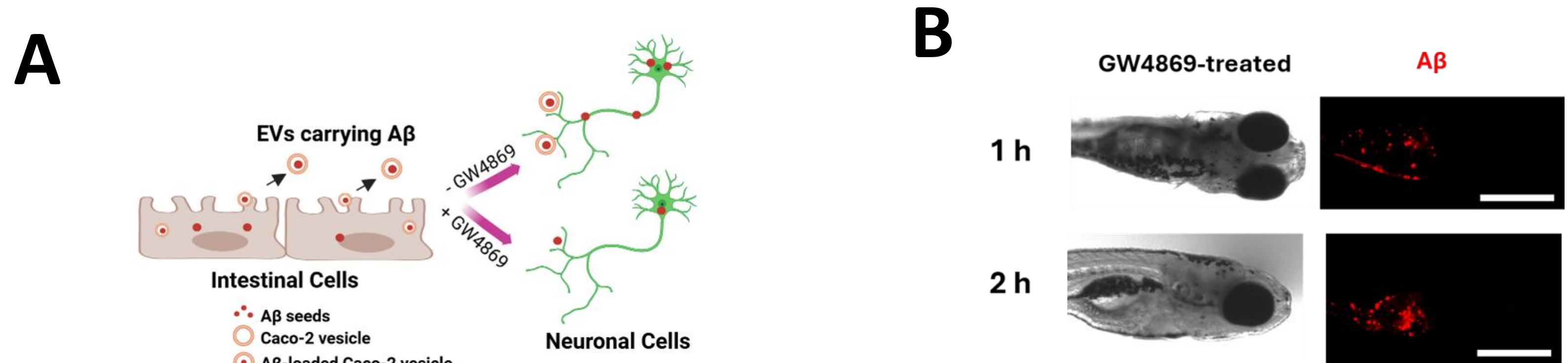


Figure 4. A) Schematic illustrating EV-mediated transfer of A β from intestinal epithelial cells to neuronal cells and inhibition of EV release using GW4869. B) Live images of zebrafish larvae pretreated with GW4869 by immersion before gut A β administration.

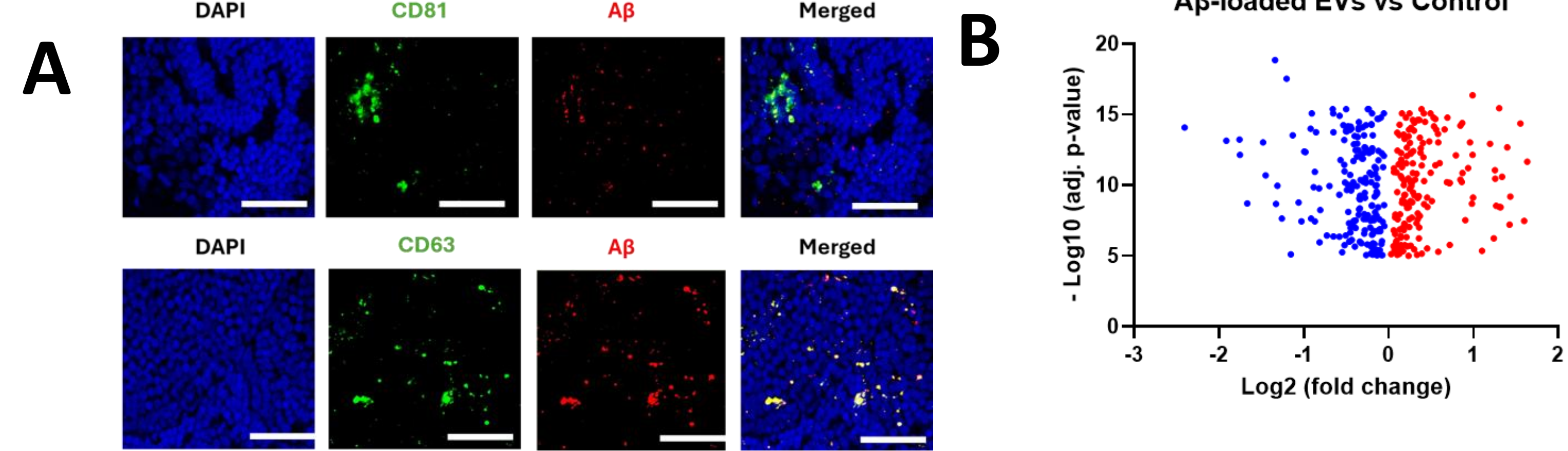


Figure 5. A) A β associates with extracellular vesicle markers. B) Proteomics comparison of A β -loaded EVs vs control

Conclusion

Gut-administered A β is transferred from intestinal epithelial cells to neuronal cells. Extracellular vesicles contribute to A β transport across the gut-brain interface. Neuronal uptake of gut-derived A β promotes intracellular accumulation and toxicity. These findings support the gut-brain axis as a potential pathway for Alzheimer's disease propagation.