

Evidence-Based Selection of Neuronal Surface Markers Improves Extracellular Vesicle Isolation in Alzheimer's Disease

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Introduction

The identification of neuron-derived small extracellular vesicles (NDE) in peripheral circulation has revolutionized the non-invasive, brain-targeted diagnostics in Alzheimer's disease (AD) and related dementias (ADRD). NDE offer a promising 'liquid biopsy' approach to investigate molecular alterations in the brain, a tissue otherwise inaccessible in living individuals. Immune-isolation using neuron-specific surface markers, especially L1CAM (CD171), is the widely used method for isolating NDE from biofluids. However, growing evidence questions the neuronal or even the central nervous system specificity of these surface markers, raising the need for more reliable markers. In this study, we aimed to identify robust, disease-independent surface markers specific to neuronal EVs.

Methods

Small EVs ($\leq 200\text{nm}$) were isolated from three distinct brain regions of both healthy and AD donors (n=12 each) and subjected to mild trypsin digestion to extract surface proteins for mass spectrometry analysis. The initial analysis of mass spectrometry data identified BTBD17, GABRA1, GPM6A, and SNAP-25 as surface markers present on sEV from all brain regions, specific to neuronal cell types, and their expression was not affected by the disease status. These markers were used to isolate brain specific NDE from plasma of cognitively normal (CN, n=6) and mild cognitive impaired (MCI, n=5) individuals, and extensively characterized for isolation efficiency, purity, specificity, and their biological and diagnostic utility in AD.

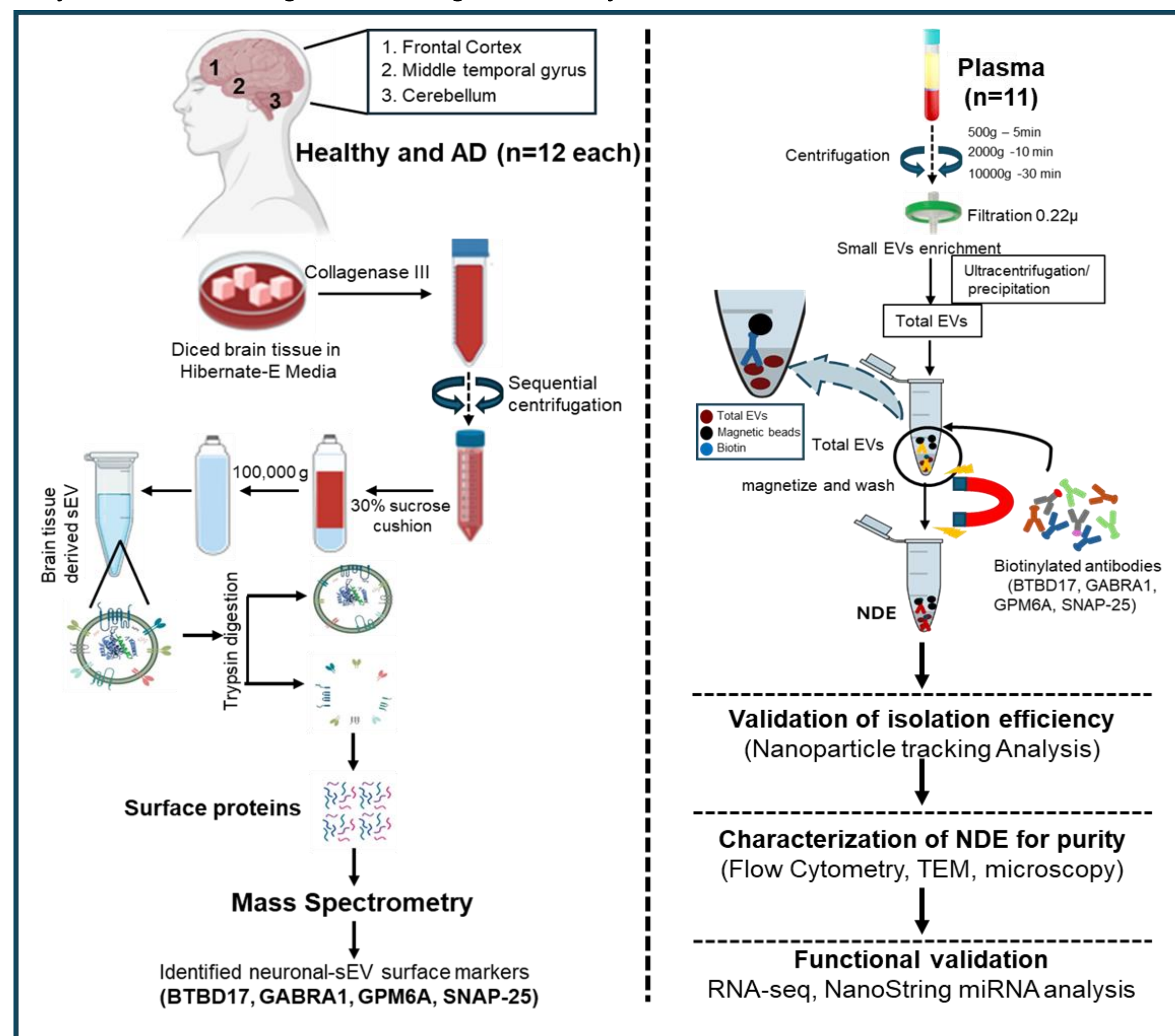


Figure 1. Workflow of identification of specific markers for neuronal sEV isolation.

Results

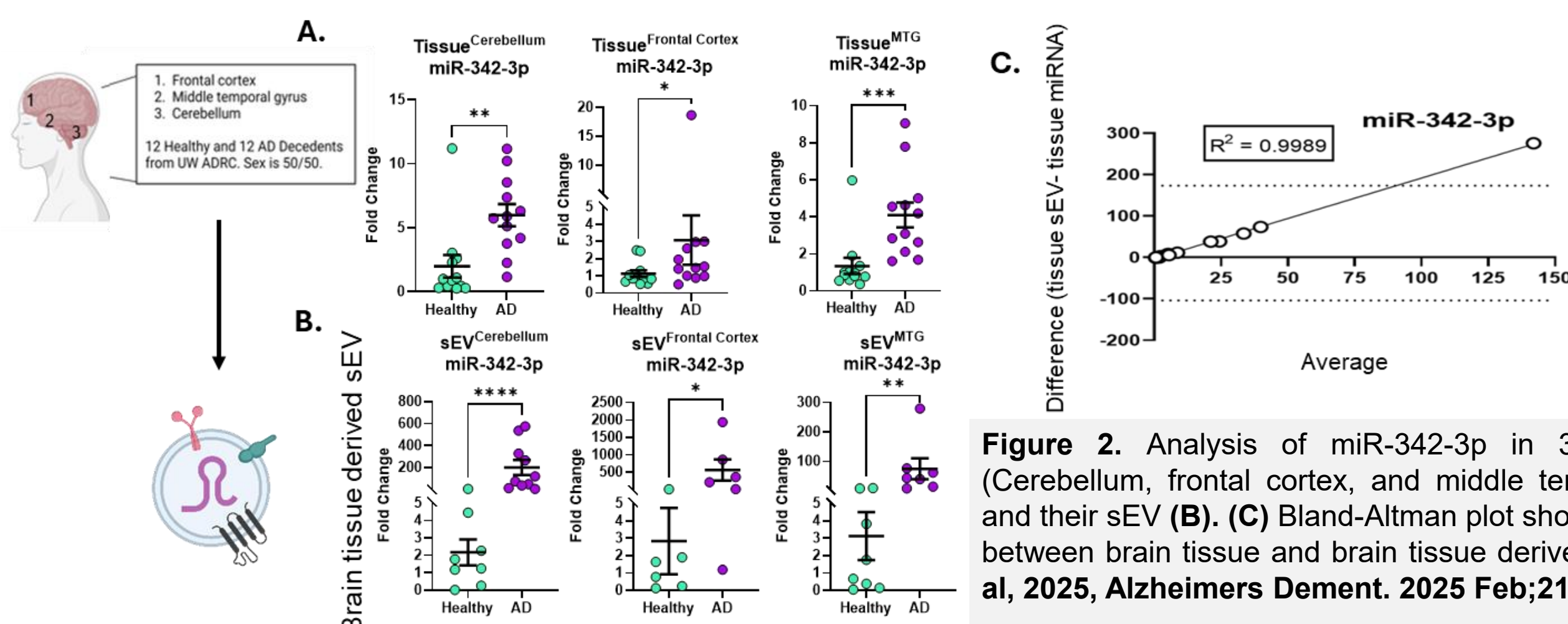


Figure 2. Analysis of miR-342-3p in 3 different brain regions (Cerebellum, frontal cortex, and middle temporal gyrus (MTG)) (A), and their sEV (B). (C) Bland-Altman plot showed the high concordance between brain tissue and brain tissue derived miR-342-3p. (Cleary et al, 2025, *Alzheimers Dement.* 2025 Feb;21(2):e14497).

Isolation and characterization of NDE using novel surface markers

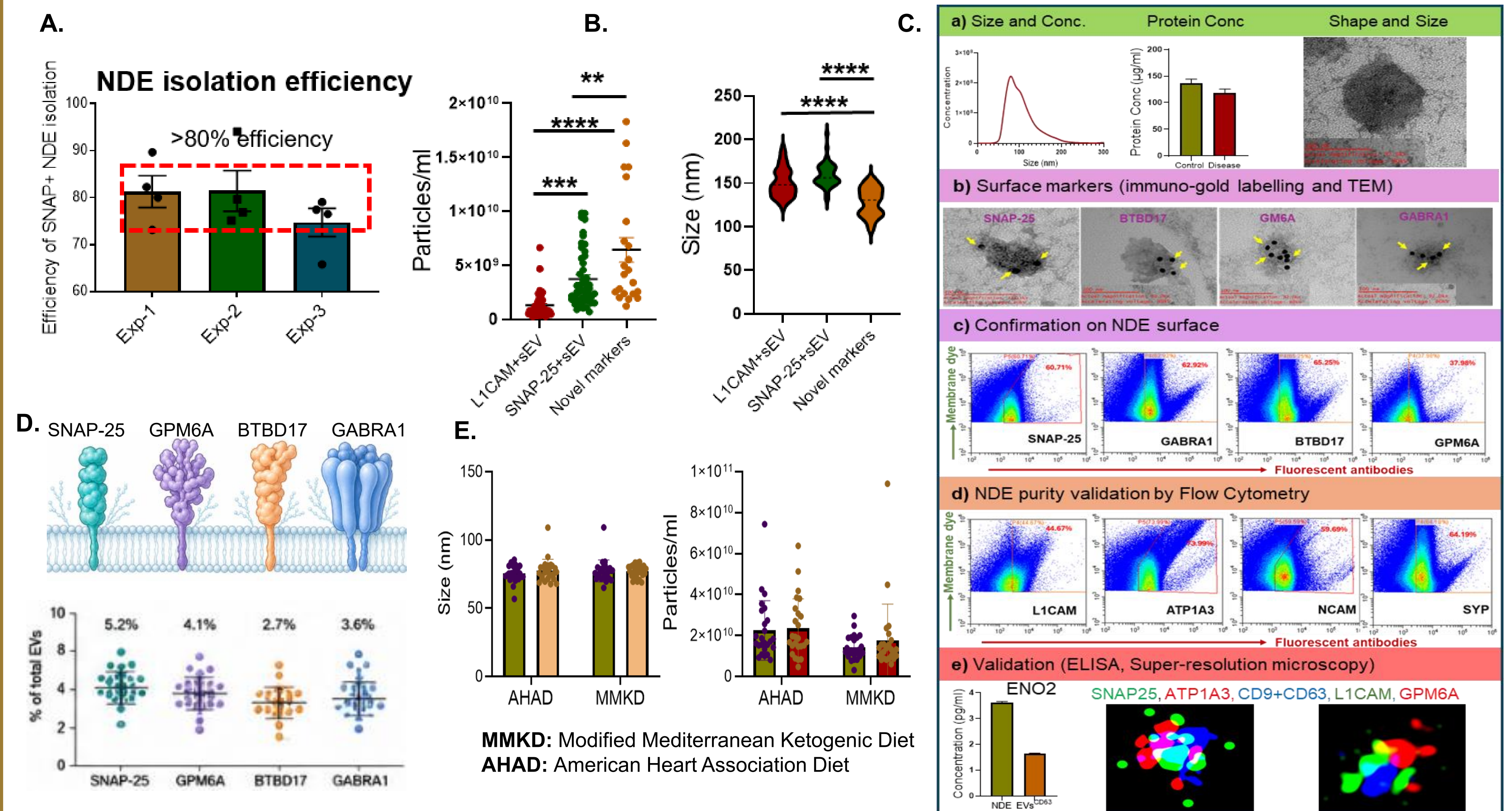


Figure 3. (A). Isolation efficiency and consistency of novel markers was tested in three independent experiments. (B) Concentration and size of NDE isolated from plasma sEV using established (L1CAM and SNAP-25 (n=48)) as well as our 4 shortlisted markers (n=22) were compared by nanoparticle tracking analysis (NTA). (C). Comprehensive characterization of isolated NDE was performed by NTA, super-resolution microscopy, ELISA for neuron specific enolase 2, immuno-gold labeling coupled with transmission electron microscopy, and nano-flow cytometry. (D) Levels of novel NDE isolation markers in TE were tested using nano-flow cytometry. (E) NDE, isolated from Brain Energy for Amyloid Transformation in Alzheimer's Disease (BEAT-AD) cohort (n=146) were analyzed for size and concentration using nanoparticle tracking analysis.

RNA-seq analysis of NDE isolated from cognitively normal (CN) and MCI individuals

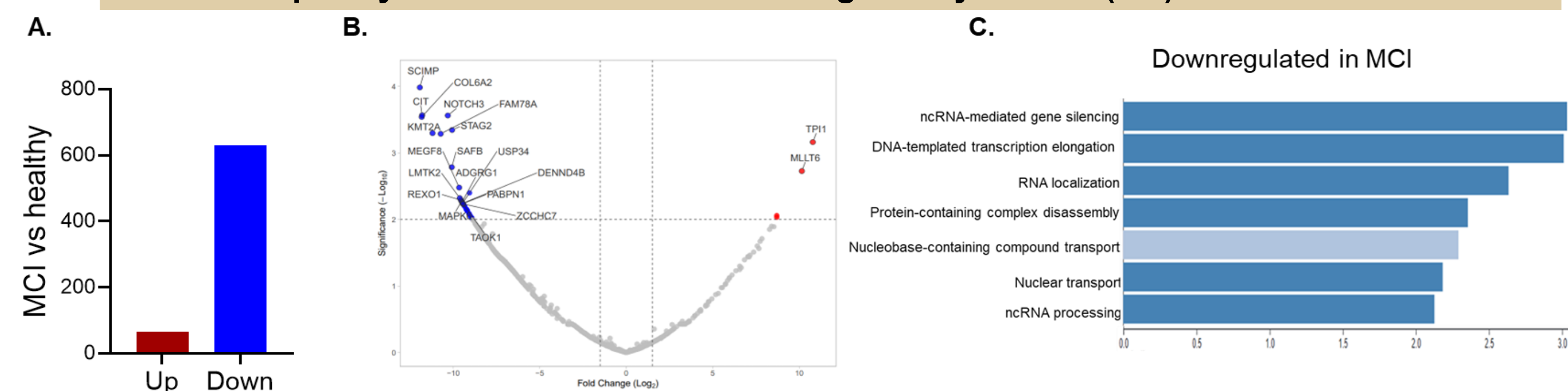


Figure 4. Total RNA was isolated from NDE for the capture-based whole transcriptome sequencing analysis. (A-B) RNA-seq data was analyzed to identify the differentially expressed genes between cognitively normal and MCI individuals. Volcano plot present top 20 significantly altered genes. (C) Downregulated genes were analyzed for associated molecular function.

miRNA cargos analysis of NDE isolated from cognitively normal (CN) and MCI individuals

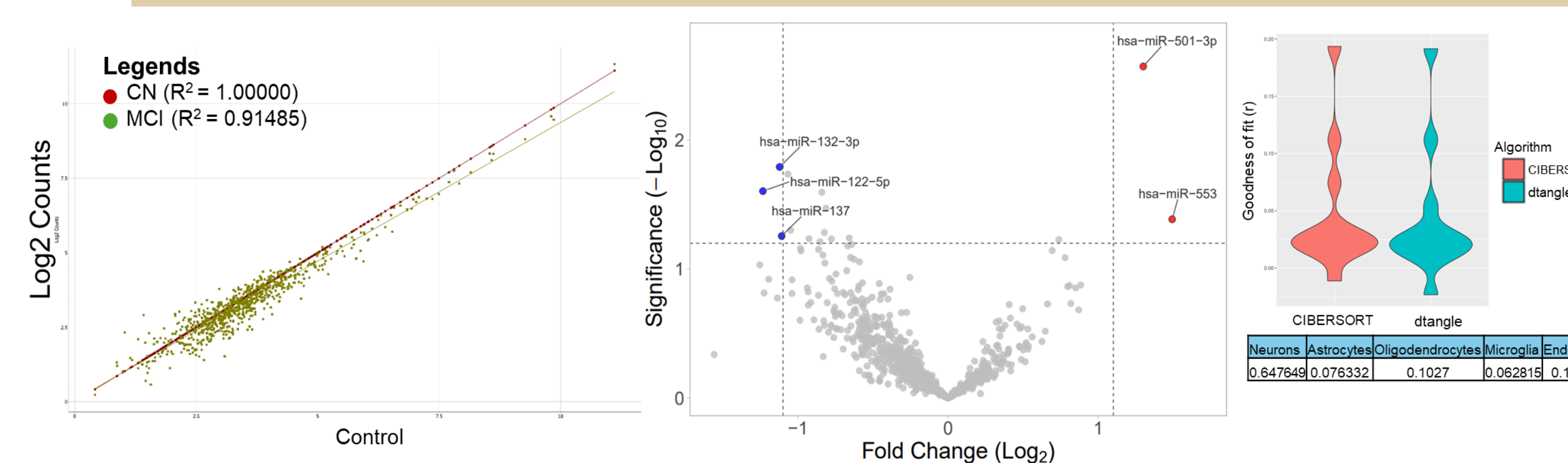


Figure 5. miRNAs were analyzed in the NDE isolated from CN (n=6) and mild cognitive impairment individuals (n=5) using NCounter analysis. Left panel (A) shows that overall miRNAs expression reduced in MCI individuals. (B) Significantly upregulated and downregulated miRNAs (2-fold change) with p value=0.05 are highlighted (right panel). (C) The RNA-seq data was first analyzed by BrainDeconvShiny online platform which uses CIBERSORT and dtangle as comparison matrix to identify brain cell type specificity.

Conclusion

We have identified novel surface markers that enable the selective isolation of NDE from human plasma. Utilizing these markers, we achieved highly pure NDE populations, which were rigorously characterized for purity, neuronal specificity, and isolation efficiency. Our preliminary analyses reveal that these NDE harbor distinct molecular signatures, underscoring their potential as a rich source of novel biomarkers for Alzheimer's disease. These findings lay the groundwork for future studies aimed at leveraging NDE for early diagnosis, disease monitoring, and therapeutic development in neurodegenerative disorders.