

# Profiling CNS-Derived, Salivary Extracellular Vesicles for Alzheimer's Disease Diagnosis and Clinical Monitoring

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## INTRODUCTION

The absence of curative therapies makes early, preclinical detection of Alzheimer's disease (AD) an urgent priority. While current neuroimaging and blood-based diagnostics are invasive, costly, and inaccessible to marginalized communities, saliva offers an inexpensive, non-invasive alternative.

Because salivary glands are highly vascularized, central nervous system (CNS)-derived extracellular vesicles (EVs) can enter salivary secretions, providing a critical window into brain pathology.

**Goal of study:** To evaluate the feasibility of utilizing saliva-derived EVs (sEVs) for early AD diagnosis and investigates the impact of storage temperature on biomarker stability within these cell-specific EVs to optimize clinical protocols.

## METHODS

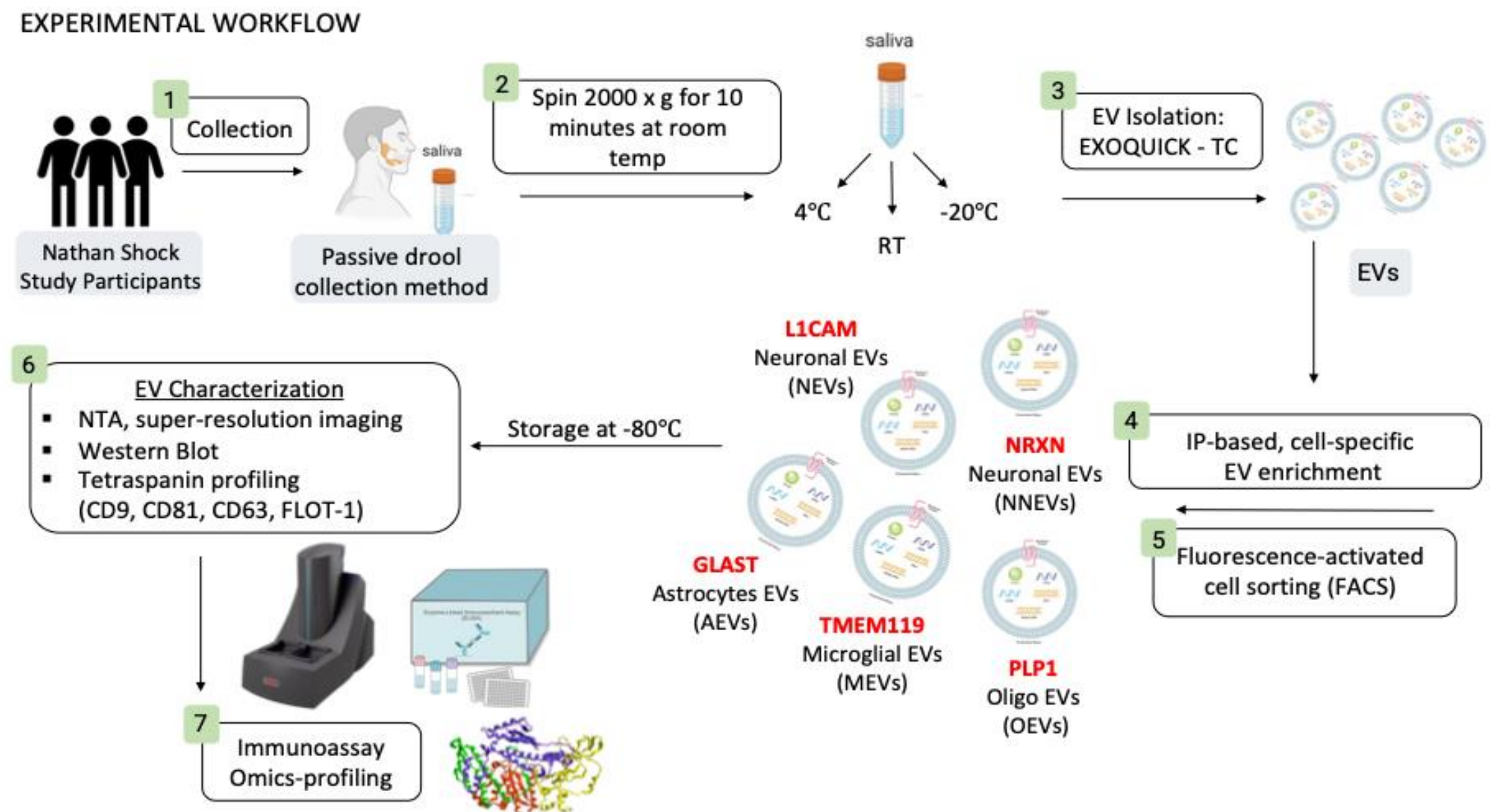


**Figure 1. Experimental workflow** Workflow illustrating passive drool saliva collection, EV isolation using ExoQuick-TC, CNS-specific EV enrichment, and downstream analysis of CNS-derived saliva EVs (sEVs) from 15 healthy older adults enrolled in the Nathan Shock Healthy Aging Study (mean age 71 years).

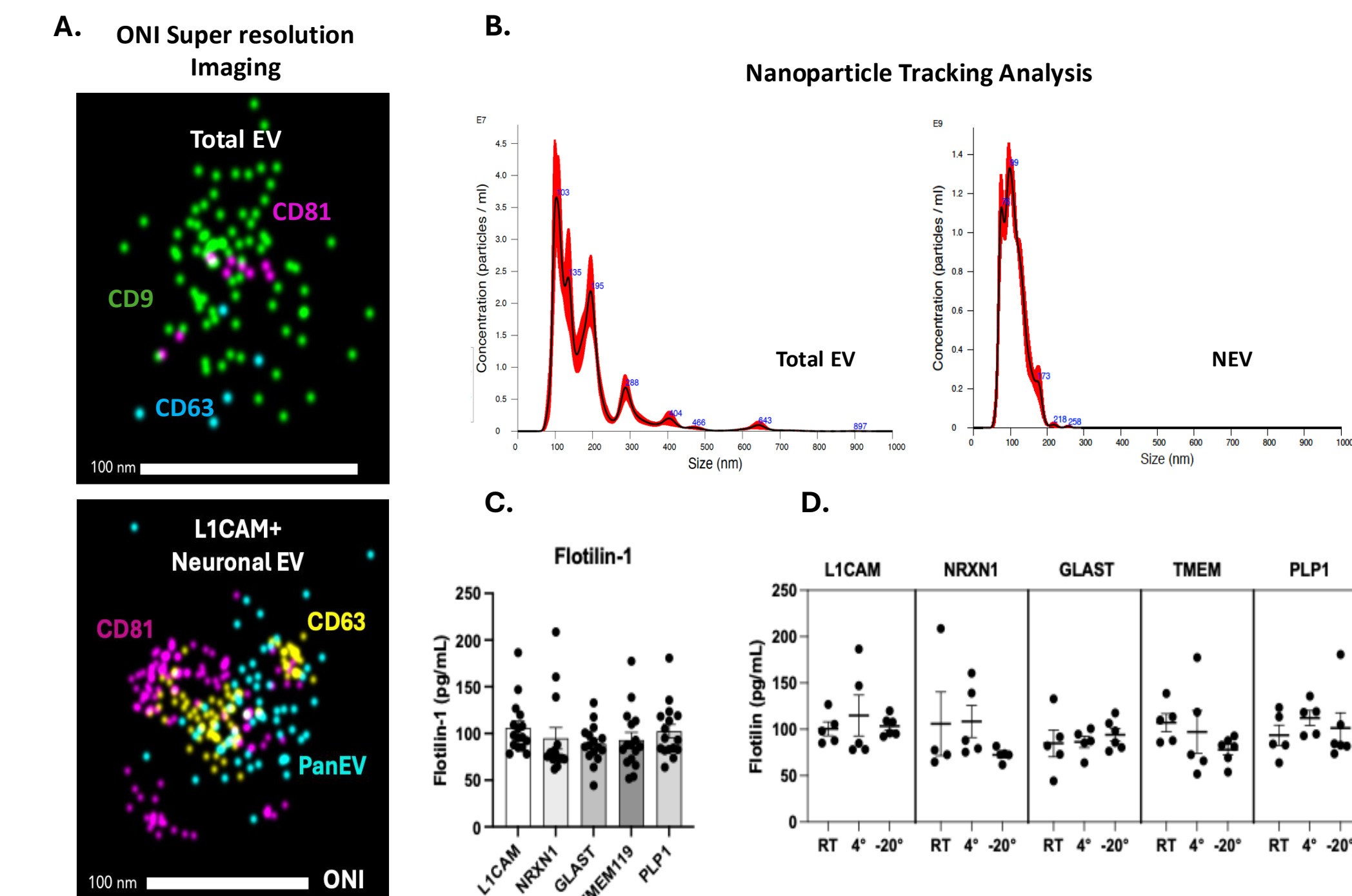


**Figure 2. Characterization of CNS-specific EVs.** A representative super-resolution microscopy image of a single total EV and neuronal EV (~100 nm) enriched for L1CAM (A). The image confirms the presence of canonical EV tetraspanin markers CD81 (purple), CD63 (yellow), and PanEV (blue). Nanoparticle tracking analysis was used to validate total EVs and neuronal EVs by confirming size and concentration (B). ELISA quantification of the EV membrane protein Flotillin in CNS-specific sEVs (C). Flotillin levels were not statistically different between CNS-specific EV subpopulations and remained stable across various storage conditions (D).

## RESULTS

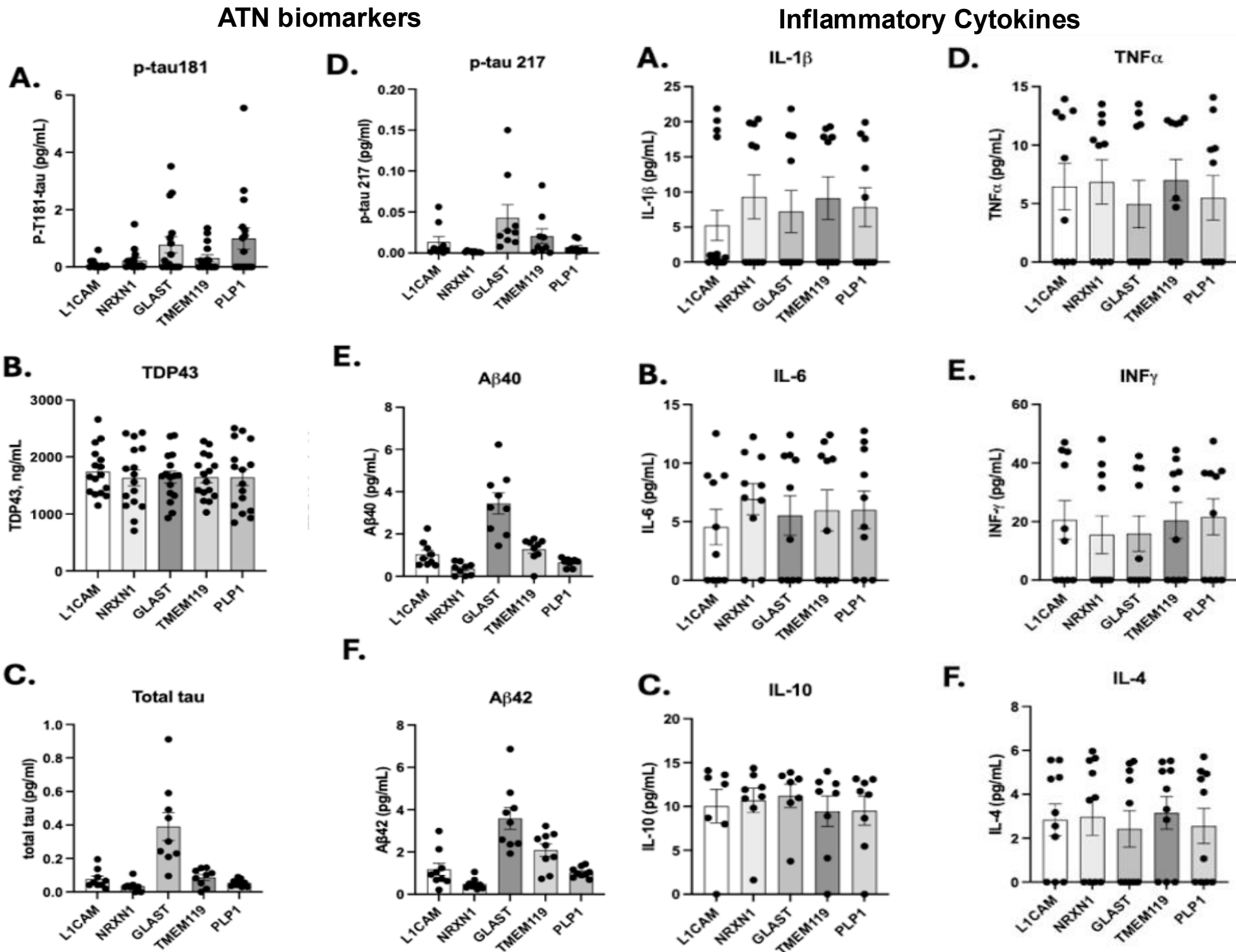


**Figure 3. Saliva EV Concentrations of ATN and Inflammatory Biomarkers.** Bioassays were used to determine how cellular origin affect concentrations of p-tau181, TDP43, total tau, p-tau217, Aβ40, Aβ42 and inflammatory cytokines in CNS-derived sEVs. GLAST-positive sEVs showed the highest levels of total tau (C), Aβ40 (E), and Aβ42 (F), suggesting astrocyte-associated enrichment of ATN biomarkers in saliva. p-tau217 (A) was most detectable in AEVs, while TDP-43 (B) was consistently present across all sEV fractions (B, D). Inflammatory cytokines exhibited variable expression across EV subpopulations (A-F).

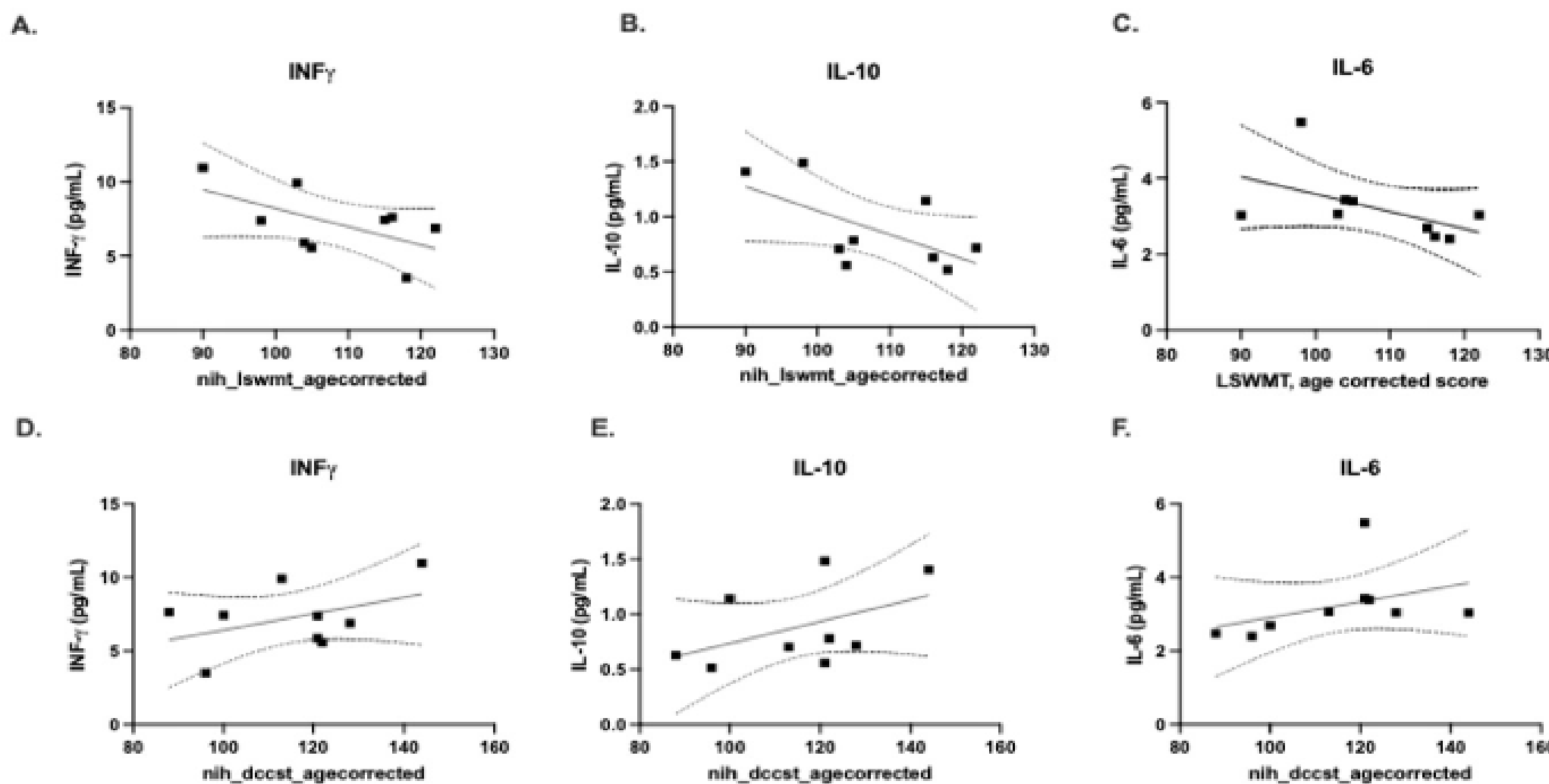
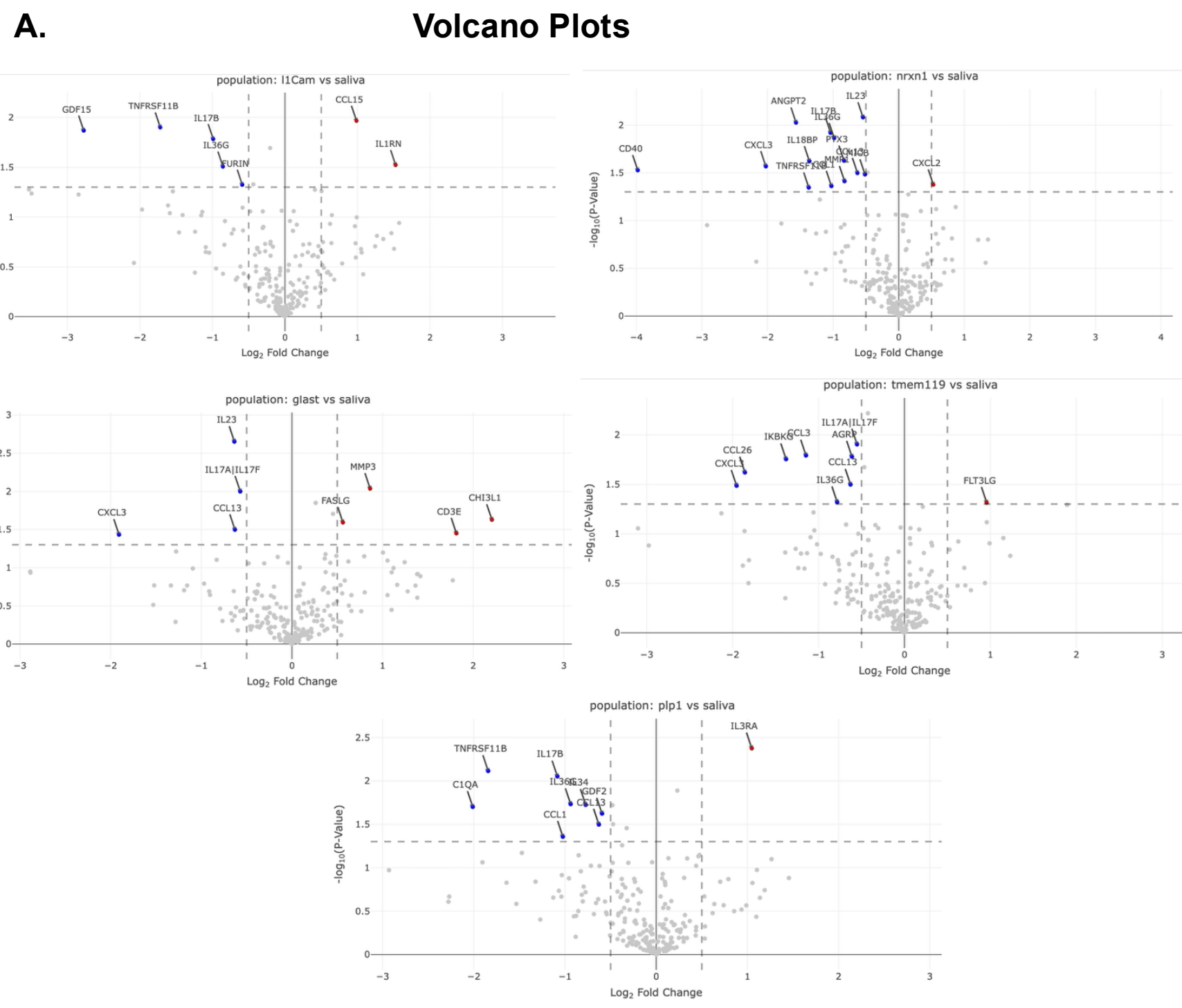


**Figure 4. Correlation between Salivary Inflammatory Cytokines and Cognitive Performance.** Higher concentrations of INF-γ, IL-10, and IL-6 were associated with lower List Sorting Working Memory Test (LSWMT) scores (A-C) and higher Dimensional Change Card Sort Test (DCCST) scores (D-F), suggesting that increased inflammatory cytokine levels are associated with poorer working memory but better cognitive flexibility.

| Covariate: Contrast              | # Sig | # Down | # Up | Coefficient | P-value |
|----------------------------------|-------|--------|------|-------------|---------|
| population: glast vs saliva      | 8     | 4      | 4    |             |         |
| population: l1cam vs saliva      | 7     | 5      | 2    |             |         |
| population: nrxn vs saliva       | 14    | 13     | 1    |             |         |
| population: plp1 vs saliva       | 9     | 8      | 1    |             |         |
| population: tmem119 vs saliva    | 9     | 8      | 1    |             |         |
| storage_temperature: rt vs x20C  | 4     | 3      | 1    |             |         |
| storage_temperature: x4C vs x20C | 2     | 1      | 1    |             |         |

**Figure 5. Application of the NULISaseq Inflammation Panel 250 to Saliva and CNS-Specific sEVs.** The NULISaseq Inflammation Panel 250 was used to profile 250 low-abundance inflammatory proteins in saliva and CNS-specific sEVs. In sEVs compared with matched whole saliva, 9 inflammatory targets were significantly upregulated and 38 were downregulated, indicating distinct protein enrichment profiles in CNS-specific sEVs.



| Cell Type (Marker)       | Cargo Protein | Role Category      | Clinical Implication of Upregulation in Saliva EVs                                                                                                               |
|--------------------------|---------------|--------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Neuronal (L1CAM / NRXN1) | CCL15         | Immune Evasion     | Indicates a "stealthy" neuronal state; suggests neurons are recruiting suppressive myeloid cells to prevent T-cell-mediated axonal damage.                       |
|                          | CXCL2         | Innate Alarm       | Acts as a specific neuronal distress signal; high levels in L1CAM+ EVs indicate acute synaptic stress or early Aβ excitotoxicity.                                |
| Astrocytic (GLAST)       | CHI3L1        | Reactive Gliosis   | The definitive A1 neurotoxic astrocyte signature; salivary levels correlate with amyloid plaque load and the transition from MCI to AD.                          |
|                          | MMP3          | Barrier Remodeling | Signals active blood-brain barrier (BBB) degradation; implies that the salivary gland is receiving "leaked" CNS proteins due to loss of neurovascular integrity. |
|                          | IL1RN         | Homeostatic Buffer | Represents a compensatory neuroprotective attempt; high levels indicate the astrocyte's effort to neutralize systemic IL-1 and prevent scarring.                 |
| Microglial (TMEM119)     | FLT3LG        | Proliferation      | Marks a shift toward Disease-Associated Microglia (DAM); suggests an expansion of the brain's resident immune cells in response to proteinopathy.                |
|                          | FASLG         | Cytotoxic Drive    | A "death signal" signature; implies microglia are in a pathologically aggressive state, actively triggering apoptosis in neighboring neurons.                    |
|                          | CD3E          | Adaptive Bridge    | Confirms neuro-immune crosstalk; presence in TMEM119+ EVs suggests microglia have engaged with infiltrating T-cells, a hallmark of chronic neuroinflammation.    |
| Oligodendrocytic (PLP1)  | IL3RA         | Myelin Support     | Indicates white matter metabolic stress; high levels in PLP1+ EVs correlate with myelin breakdown and oligodendrocyte precursor cell (OPC) dysfunction.          |

**Figure 6. CNS-Associated Inflammatory Signatures in sEVs Identified by NULISaseq.** Representative volcano plots of differentially expressed inflammatory proteins in CNS-specific sEVs vs. saliva (A). The NULISaseq Inflammation Panel revealed that differentially expressed proteins enriched for CNS-related pathways, supporting the neuronal, astrocytic, microglial, and oligodendrocytic origin of saliva EV subpopulations. These proteins are linked to neuroimmune signaling, cellular stress responses, blood-brain barrier remodeling, and neurodegenerative processes relevant to AD.

## KEY FINDINGS & FUTURE DIRECTIONS

This study demonstrates the successful isolation of CNS-derived sEVs from human saliva, establishing them as a viable platform for AD biomarker detection, cognitive monitoring, and neuroinflammatory profiling.

- Biomarker Partitioning:** ATN biomarkers were significantly higher in astrocyte-derived sEVs, whereas TDP-43 was most abundant in EV-depleted saliva.
- Inflammation & Cognition:** Distinct sEV inflammatory signatures identified via the NULISaseq Inflammation Panel 250 showed enrichment for CNS pathways matching their cellular origins. Furthermore, higher working memory performance correlated with lower levels of INF-γ, IL-10, and IL-6.

**Next Steps:** Future studies will validate the diagnostic utility of these sEV biomarkers in clinical cohorts comparing AD subjects against healthy controls and leverage multi-omics approaches to fully characterize the complex sEV cargo.