

Neuronal enriched plasma extracellular vesicles (CD61-ve) for Biomarkers of Alzheimer's Disease

Sithara Vivek

Assistant Professor

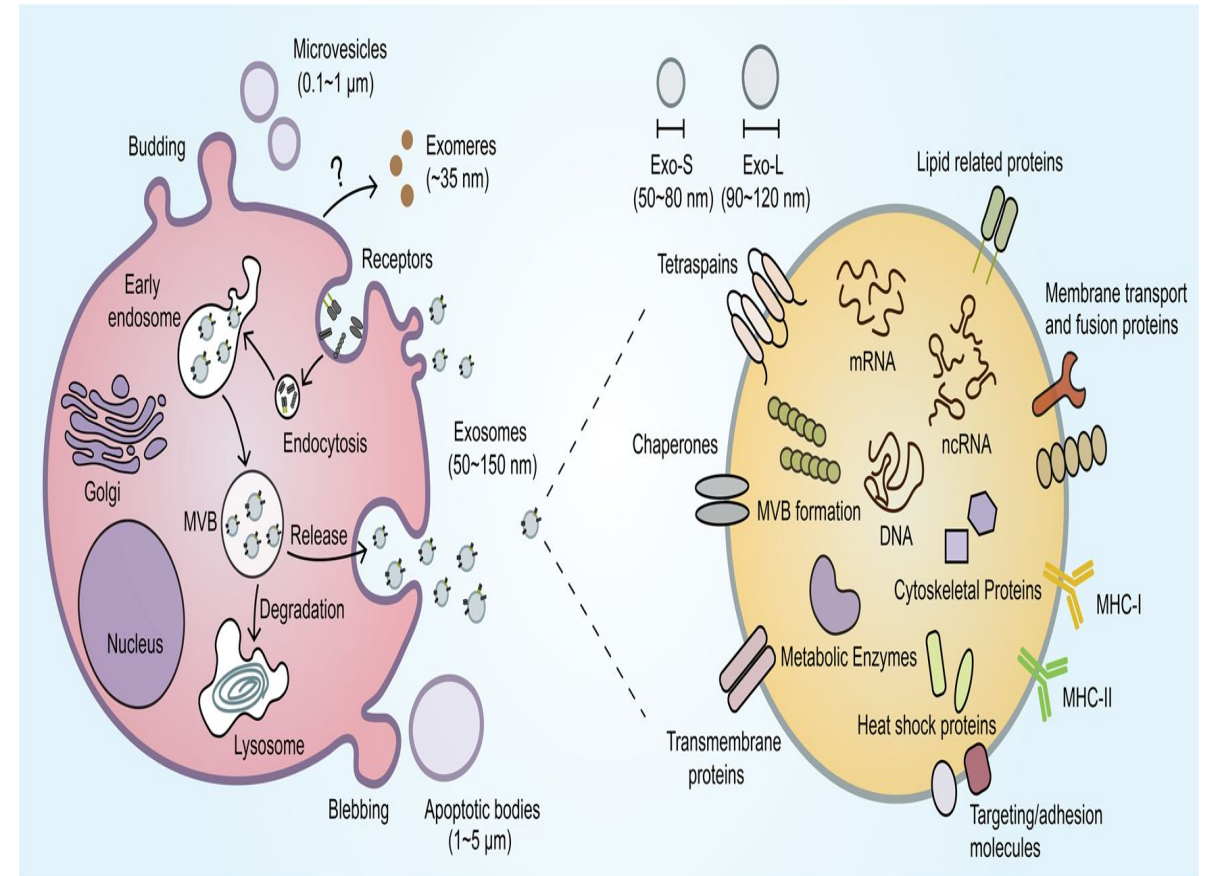
Department of Laboratory Medicine and Pathology

University of Minnesota – svivek@umn.edu



Extracellular vesicles (EVs)?

- Small membrane-bound vesicles secreted by cells into the extracellular space
- Size range = 30-150 nm
- Novel mediator of intercellular communication
- EVs carry bioactive molecules
- EVs as diagnostic biomarkers for diseases
- Neuronal derived EVs (NDEVs)

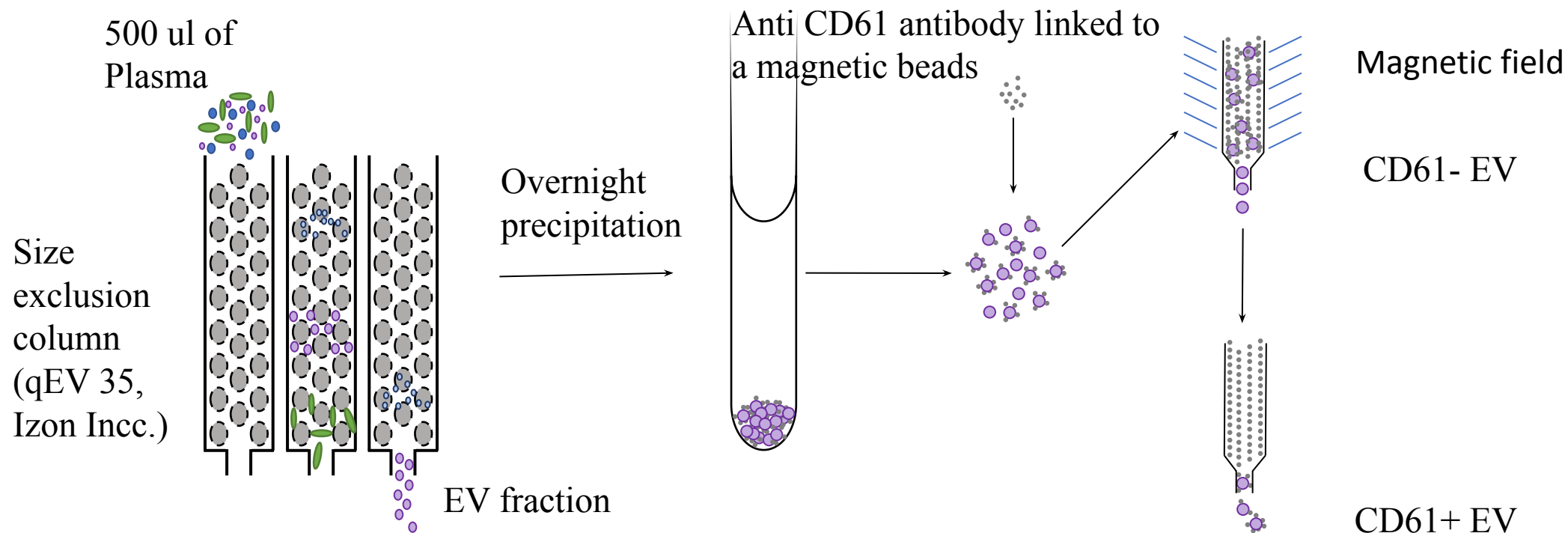


Eren E, Leoutsakos J-M, Troncoso J, Lyketsos CG, Oh ES, Kapogiannis D. *Cells*. 2022; 11(3):436.

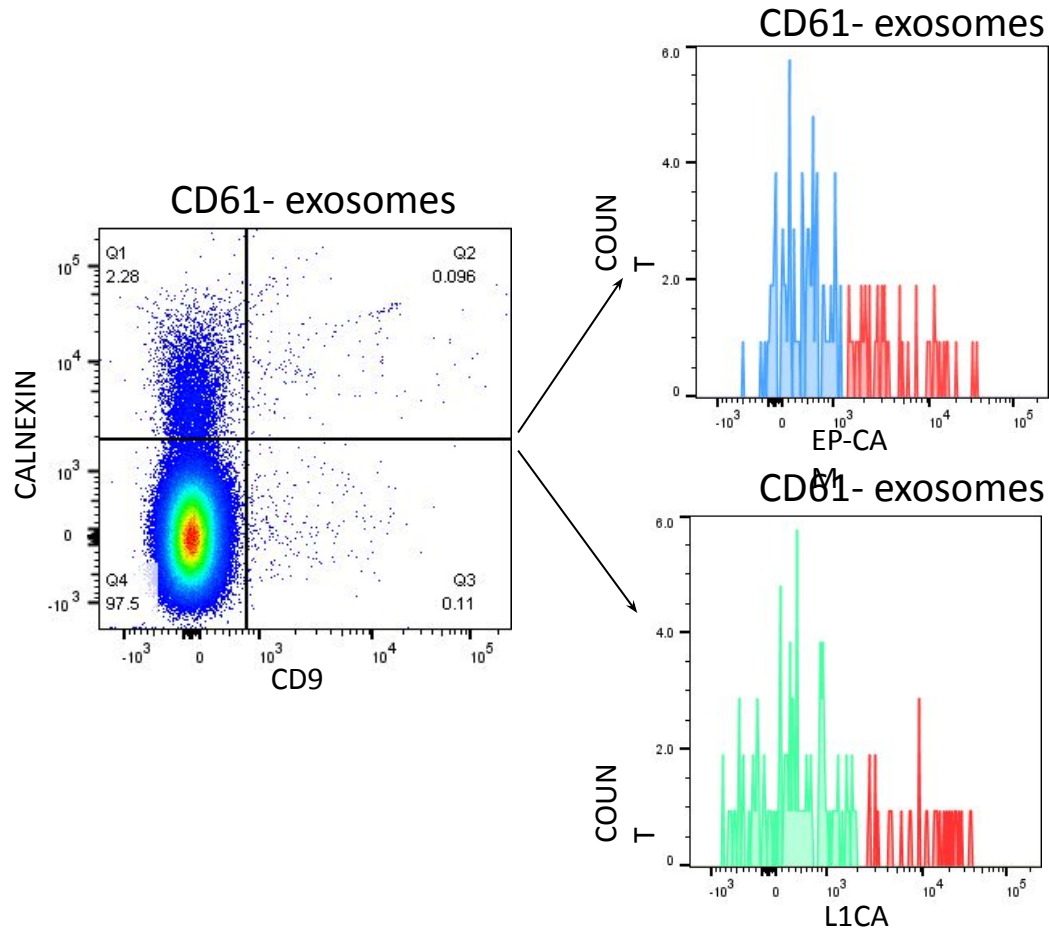
Zhou, X., Xie, F., Wang, L. *et al. Cell Mol Immunol* 17, 323–334 (2020)



Isolation and enrichment for CD61-ve EVs



Enrichment in the CD61-ve fraction



Pilot Project

Aim 1

- Determine the association between neuronal-enriched EV biomarkers and dementia.
- Exploratory Aim: Evaluate the differences in the association of neuronal enriched EV biomarkers and plasma-based biomarkers with dementia.

Study sample: 50 plasma samples collected from institutional bio-banking study of metastatic prostate cancer patients

Aim 2

Identify pre-analytical variables that may impact the stability of EVs.

1. Delayed Processing (0, 24, 48 and 72 hours)
2. Freeze-thaw cycles

Study sample: Three plasma samples collected from volunteers for the stability experiments



Quanterix Human Neurology 4-Plex E (N4PE) assay

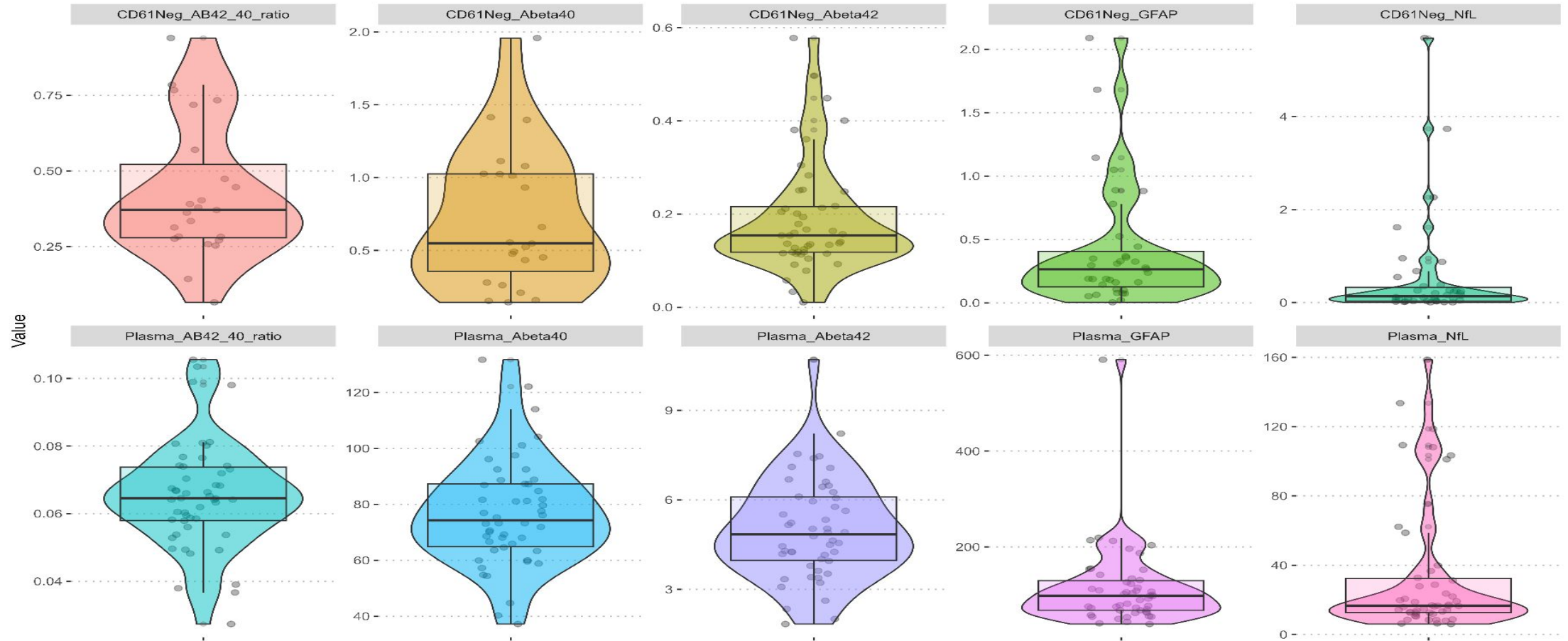
- Amyloid β 42
- Amyloid β 40
- Neurofilament Light Chain (NfL)
- Glial Fibrillary Acidic Protein (GFAP)



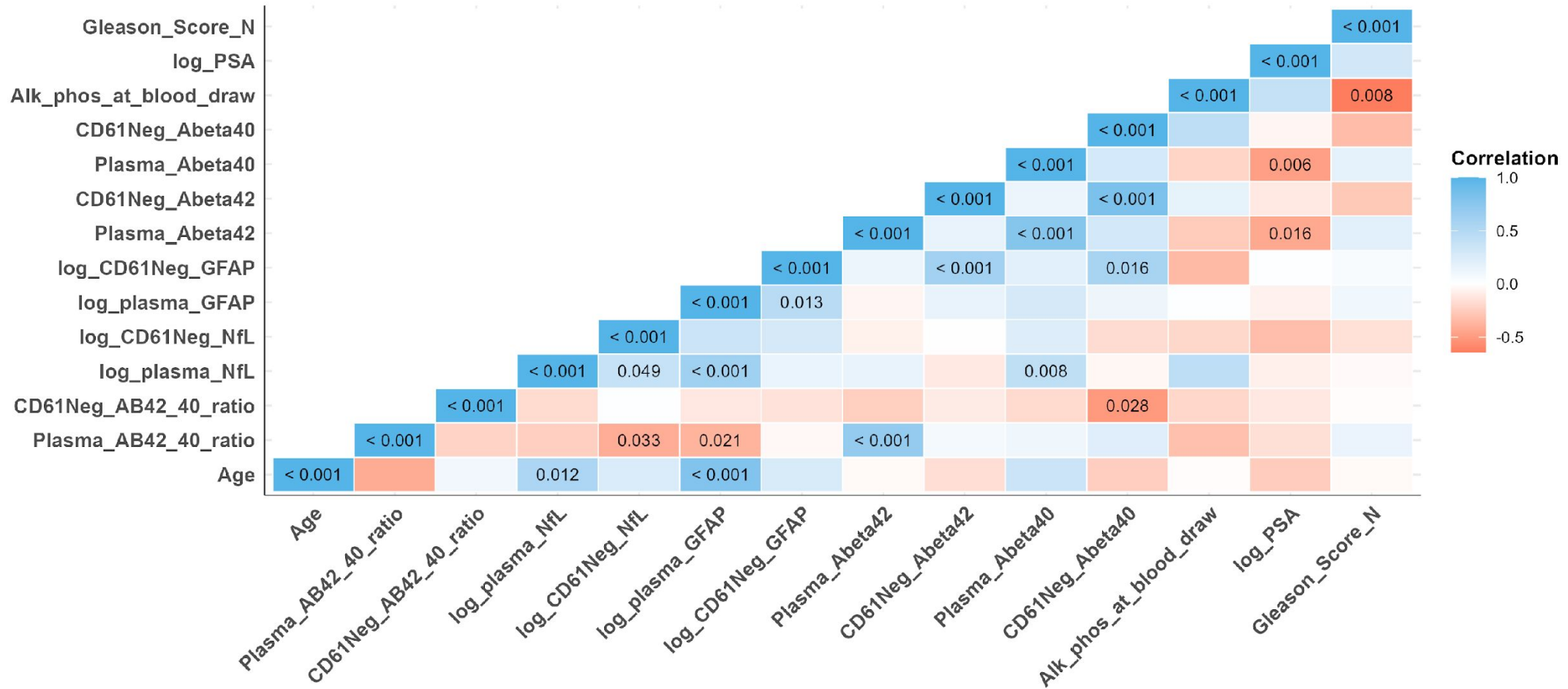
Simoa® Quanterix



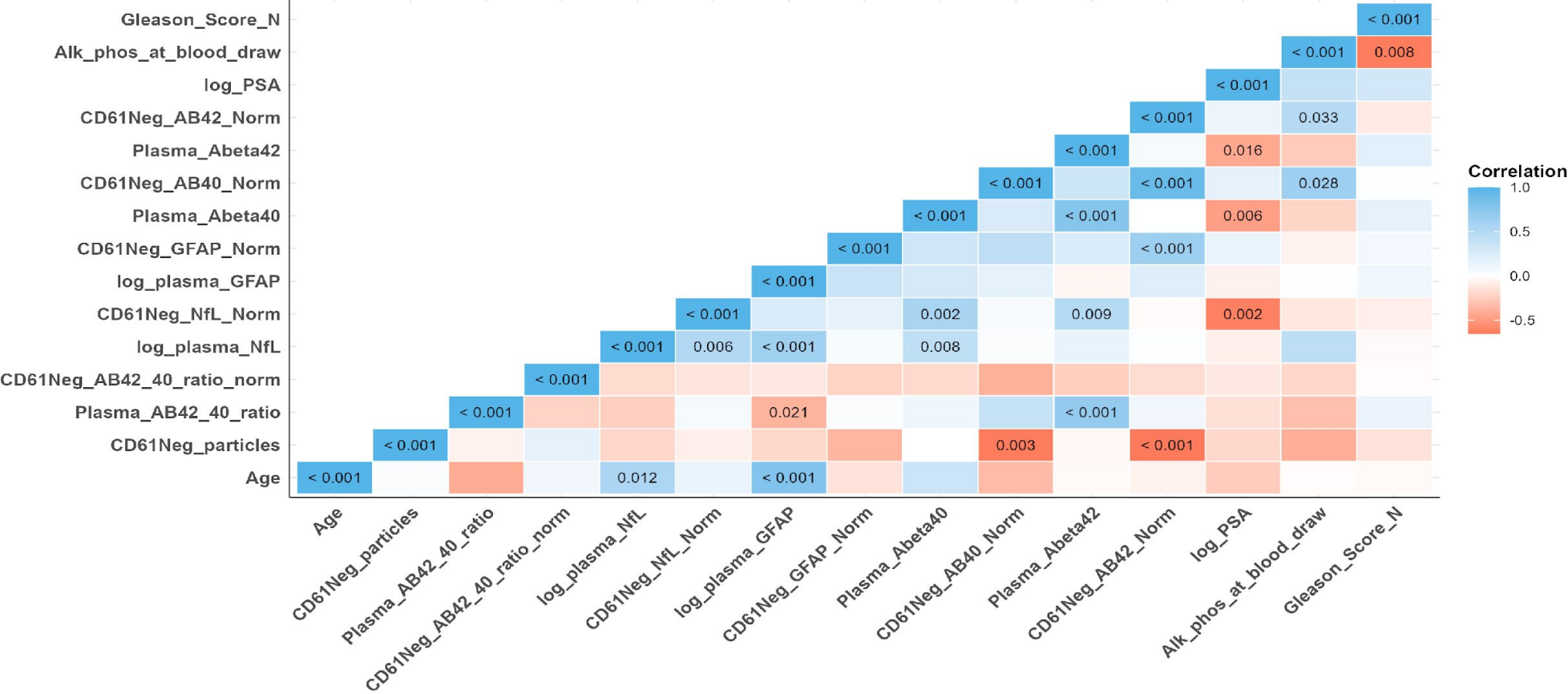
Plasma Vs CD61-ve EV biomarkers



AD biomarkers with prostate cancer clinical variables



AD biomarkers with prostate cancer clinical variables – Normalized for EV number



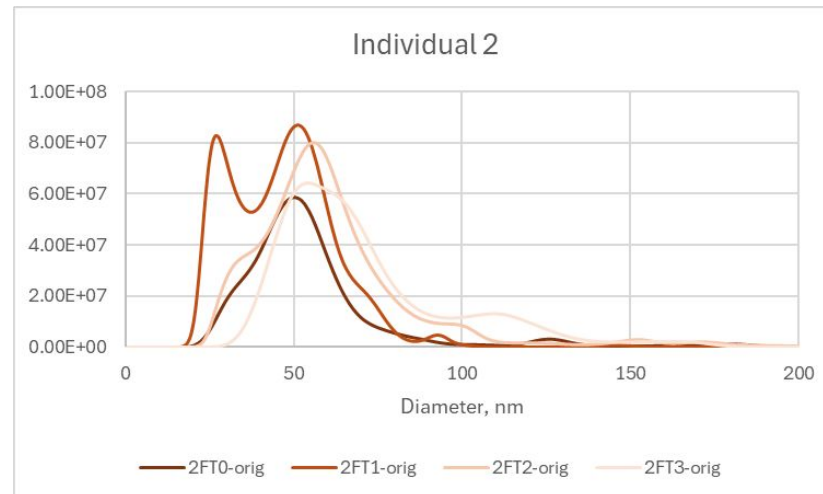
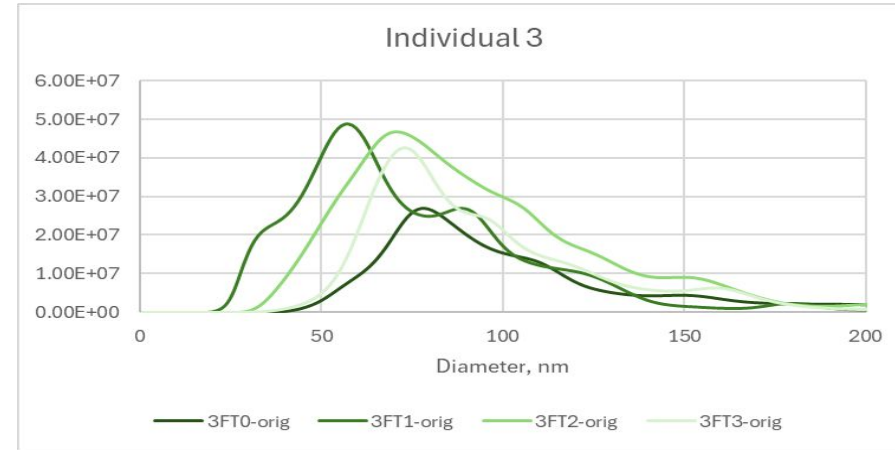
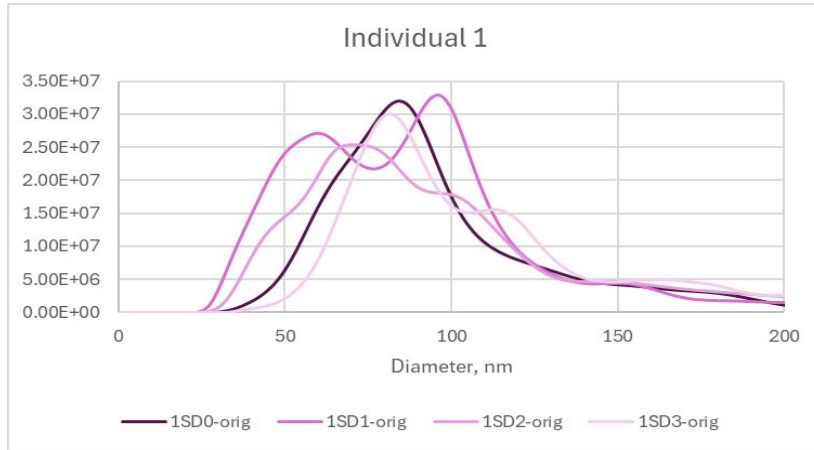
Utility in population studies – Ongoing work

Determine the impact of pre-analytical variables that may impact the stability of EVs

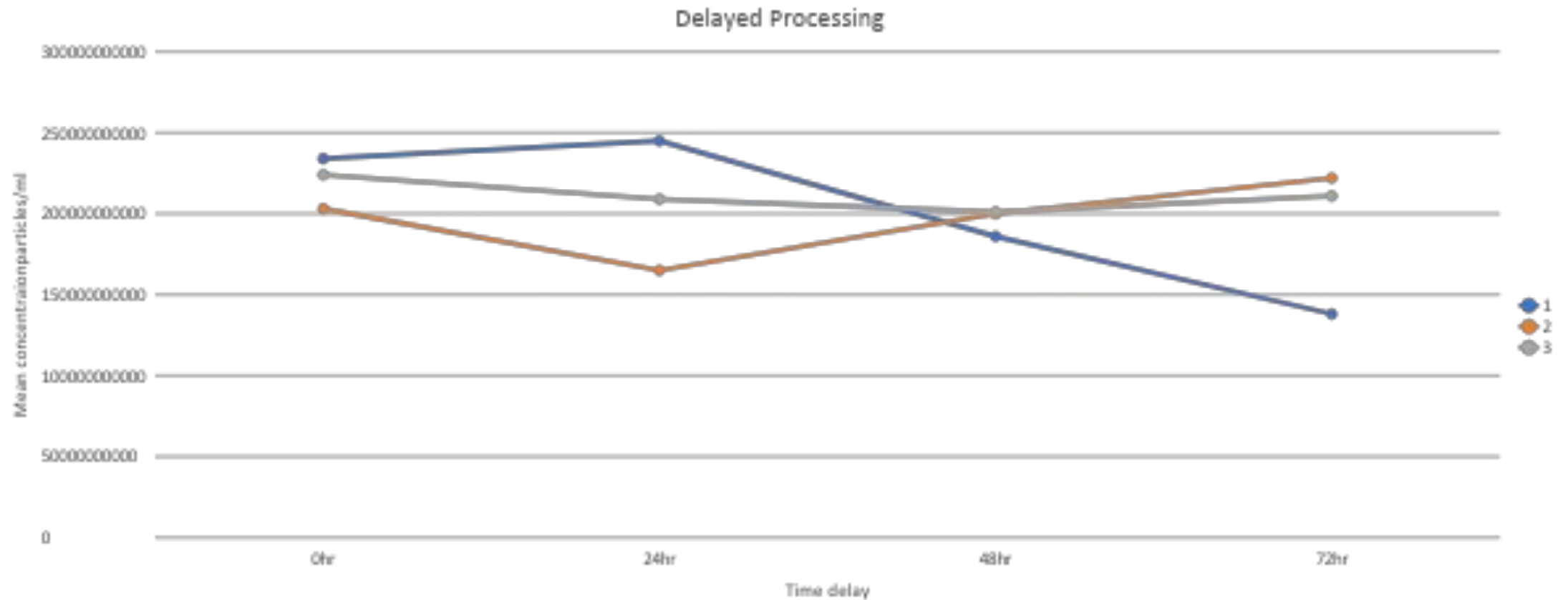
- Delayed Processing (0, 24, 48 and 72 hours)
- Freeze-thaw cycles for stability experiments



Processing delay



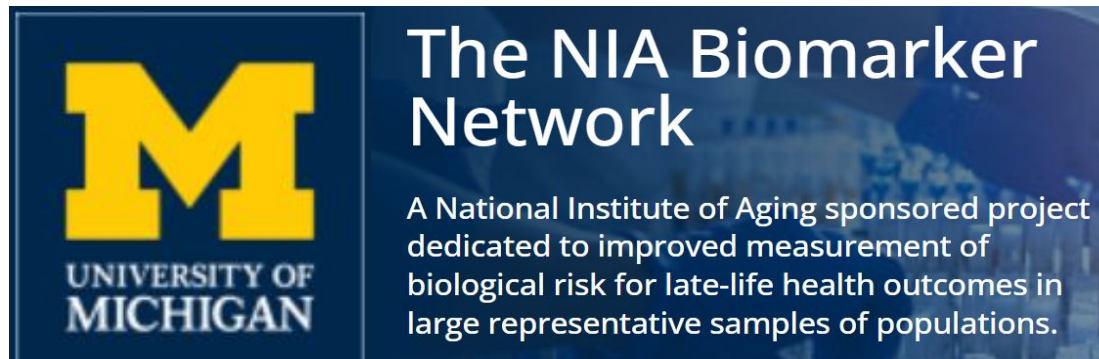
Effect of delayed processing CD61-ve EVs



Acknowledgements



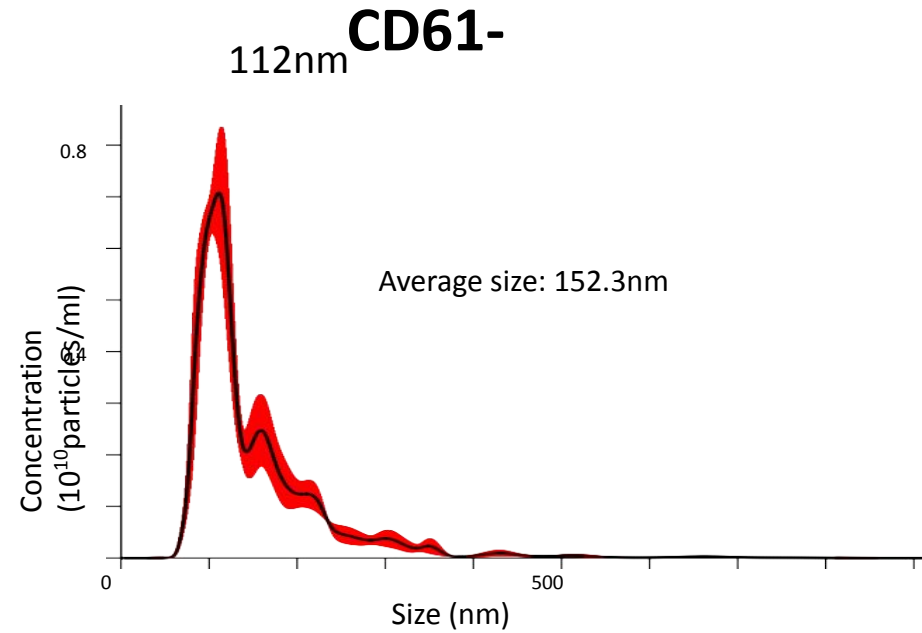
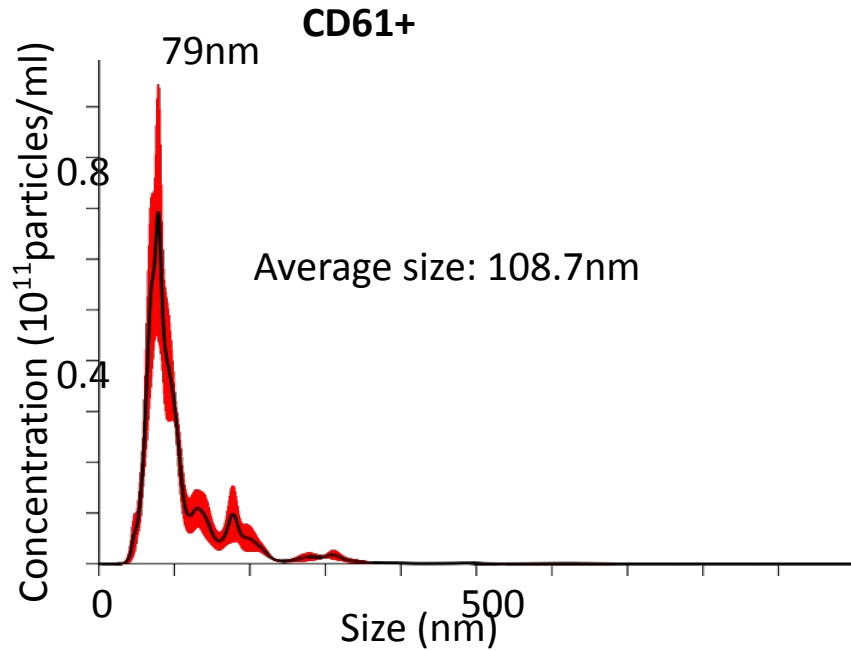
Dr. Bharat Thyagarajan
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Lucas Bolender
Jonathan Barnes



Dr. Jessica Faul
Dr. Colter Mitchell
Dr. Eileen Crimmins



NANOPARTICLE TRACKING ANALYSIS

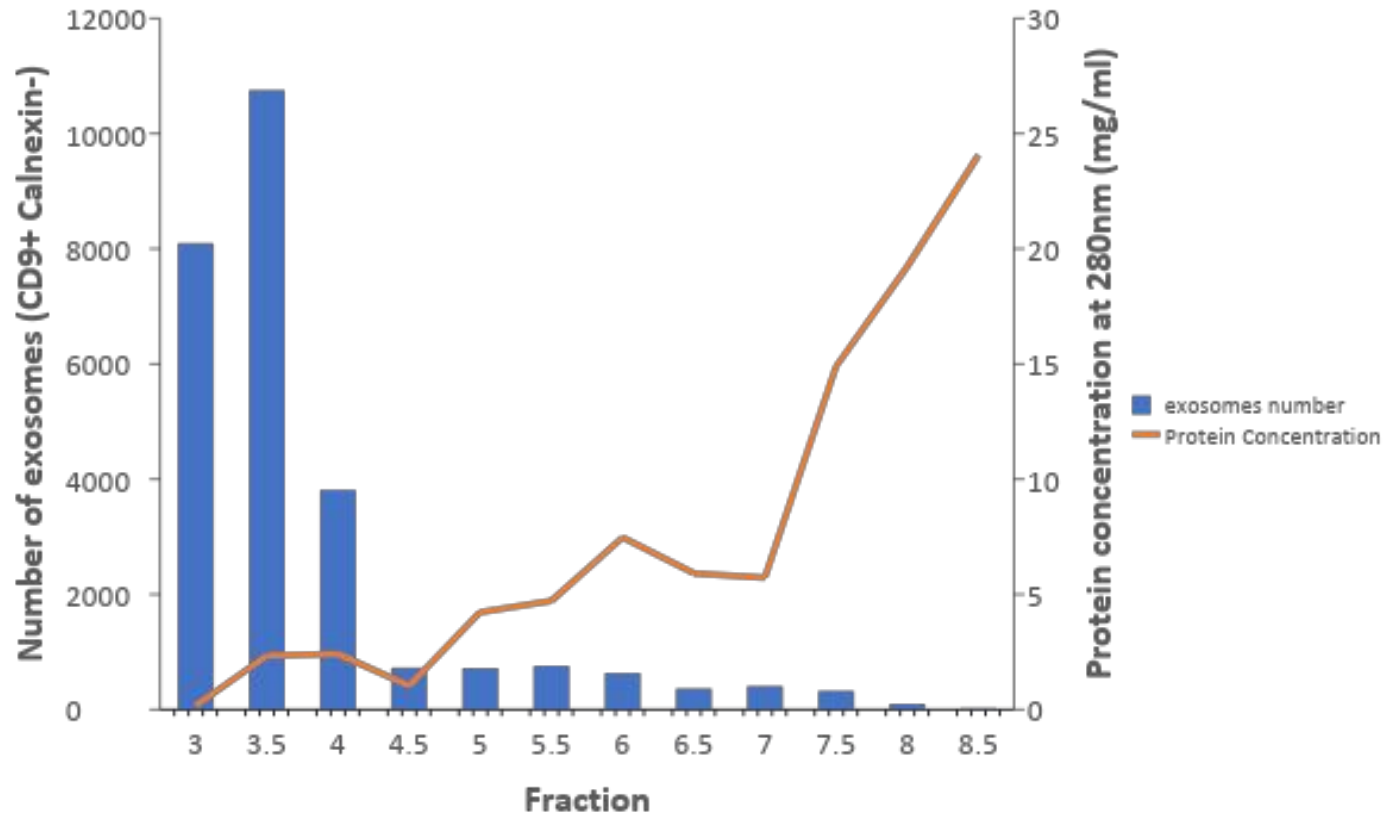


	Average CV of exosomes concentration between duplicates (n=4)	Average CV of exosomes size between duplicates (n=4)
CD61- MV	34%	8%
CD61+ MV	14%	9%



EXOSOME PURITY USING THE qEV COLUMN

AMOUNT OF EXOSOME PARTICLES AND PROTEIN IN FRACTIONS



- Fractions between 3 to 5 ml contain ~75% of CD9+ EVs and also have very low protein contamination
- We have performed 10 isolations using samples collected from different people with different anticoagulants to document that pure EV isolation with minimal protein contamination occurs between 3 and 5 mls

