

Comparative Evaluation of Neuron-Derived Extracellular Vesicle Markers Identifies GluR2 as the Most Effective Target for Immuno-Affinity Isolation

Maryam Touhidinia^{*1}, Ahmed M. Sidky^{1,2}, Tanzeem Butt¹, Richard Mantey¹, Roohollah Sobhani¹, N. Ahmad Aziz^{1,3,4}

¹ Population Health Sciences, German Center for Neurodegenerative Diseases (DZNE), Bonn, Germany

² Biochemistry Division, Chemistry Department, Faculty of Science, Minia University, Minia, 61519, Egypt.

³ Centre of Neurology, Clinic for Parkinson, Sleep and Movement Disorders, University Hospital Bonn, University of Bonn, Bonn, Germany

⁴ Institute for Medical Biometry, Informatics and Epidemiology (IMBIE), University of Bonn, Faculty of Medicine, Bonn, Germany

2416

INTRODUCTION

- Extracellular vesicles (EVs)** are nano-sized (30–1000 nm), lipid bilayer-enclosed particles secreted by most cell types, carrying proteins, lipids, and nucleic acids that reflect the physiological or pathological state of their cells of origin [1].
- Neuron-derived EVs (NDEVs)** provide a minimally invasive window into central nervous system activity and are a promising source of blood-based biomarkers for neurodegenerative diseases; however, their selective isolation and characterization remain technically challenging [2]. In particular, there is an urgent need for the identification and characterization of more specific surface markers for the isolation of NDEVs [3].
- Glutamate receptor 2 (GluR2)** is selectively expressed in neurons and therefore could be a more neuron-specific marker than other reported markers for the isolation of NDEVs from blood samples [4].

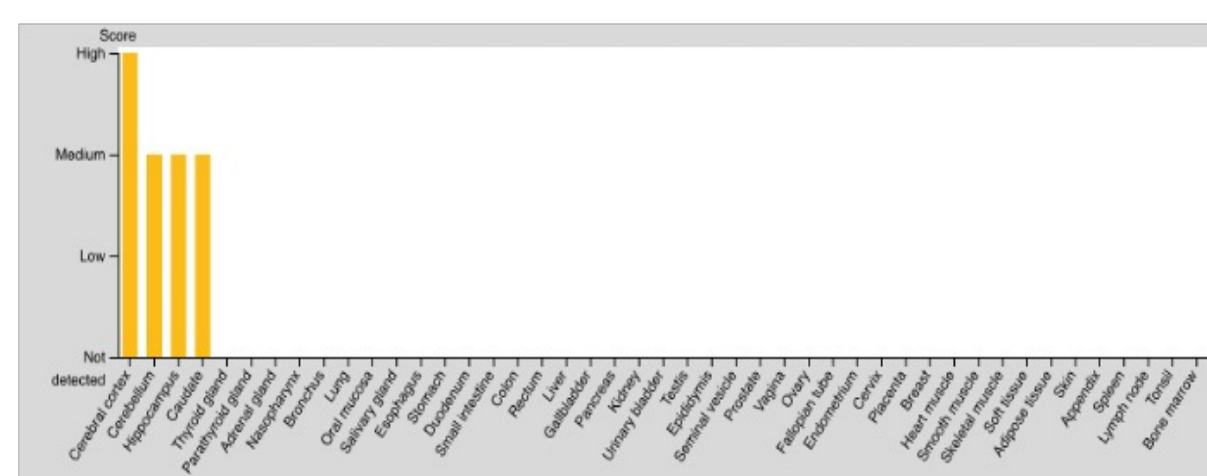


Figure 1. Tissue expression profile of GRIA2 (GluR2) obtained from the Human Protein Atlas, demonstrating enriched expression in the central nervous system.

METHODS

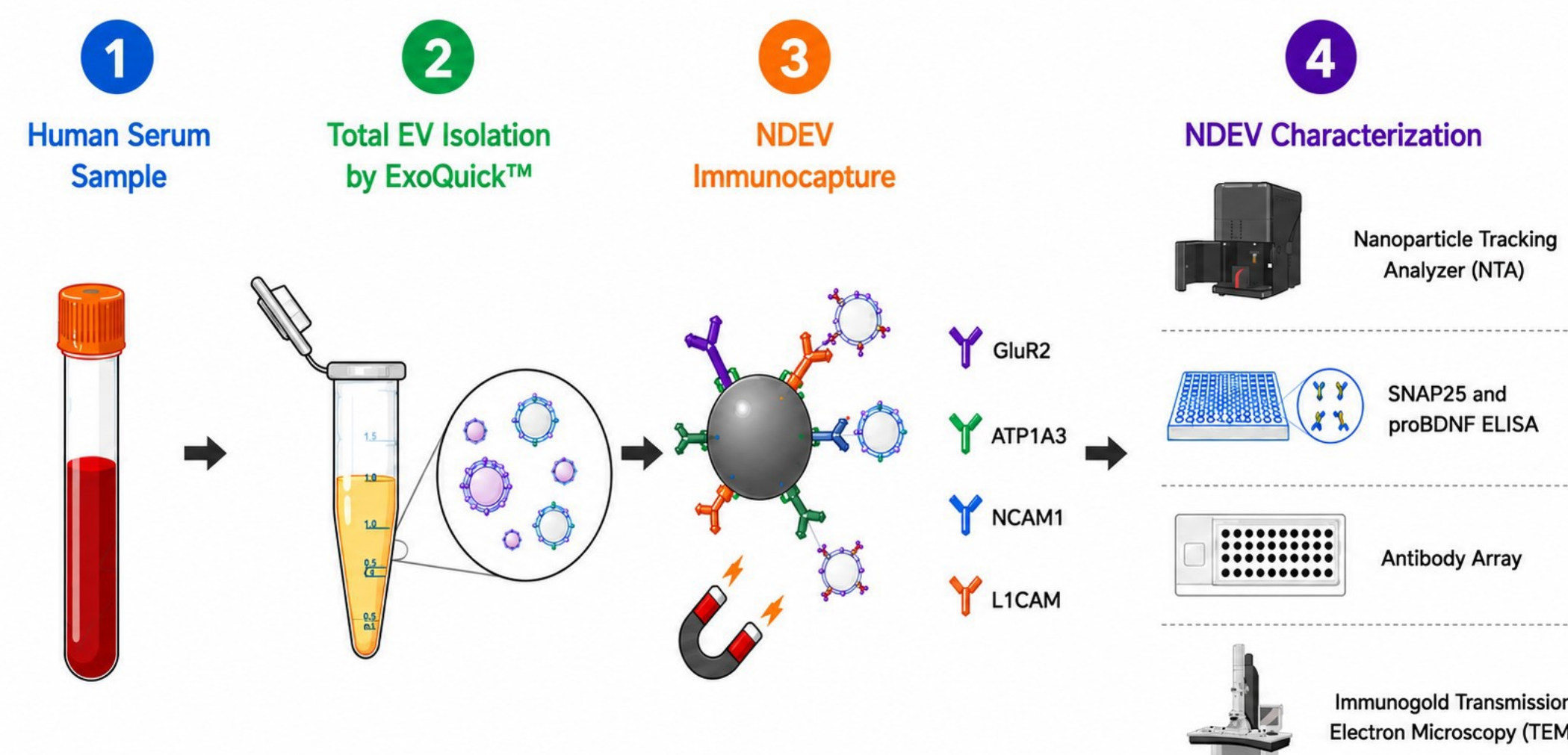


Figure 2. Workflow for immunocapture and characterization of NDEVs.

RESULTS

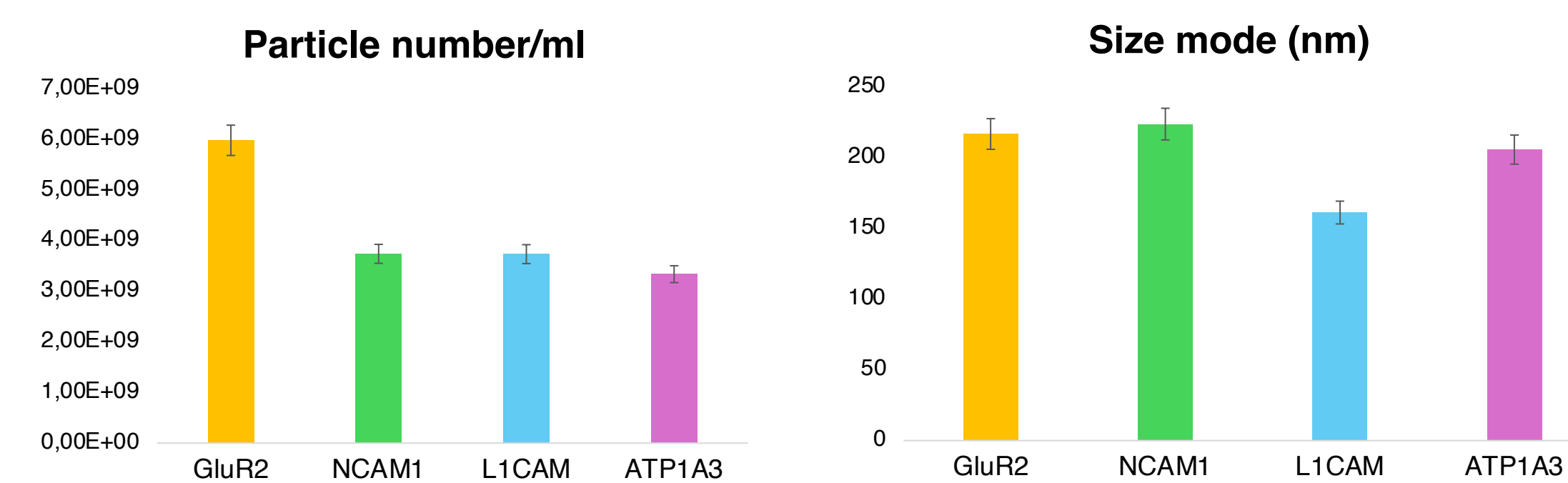
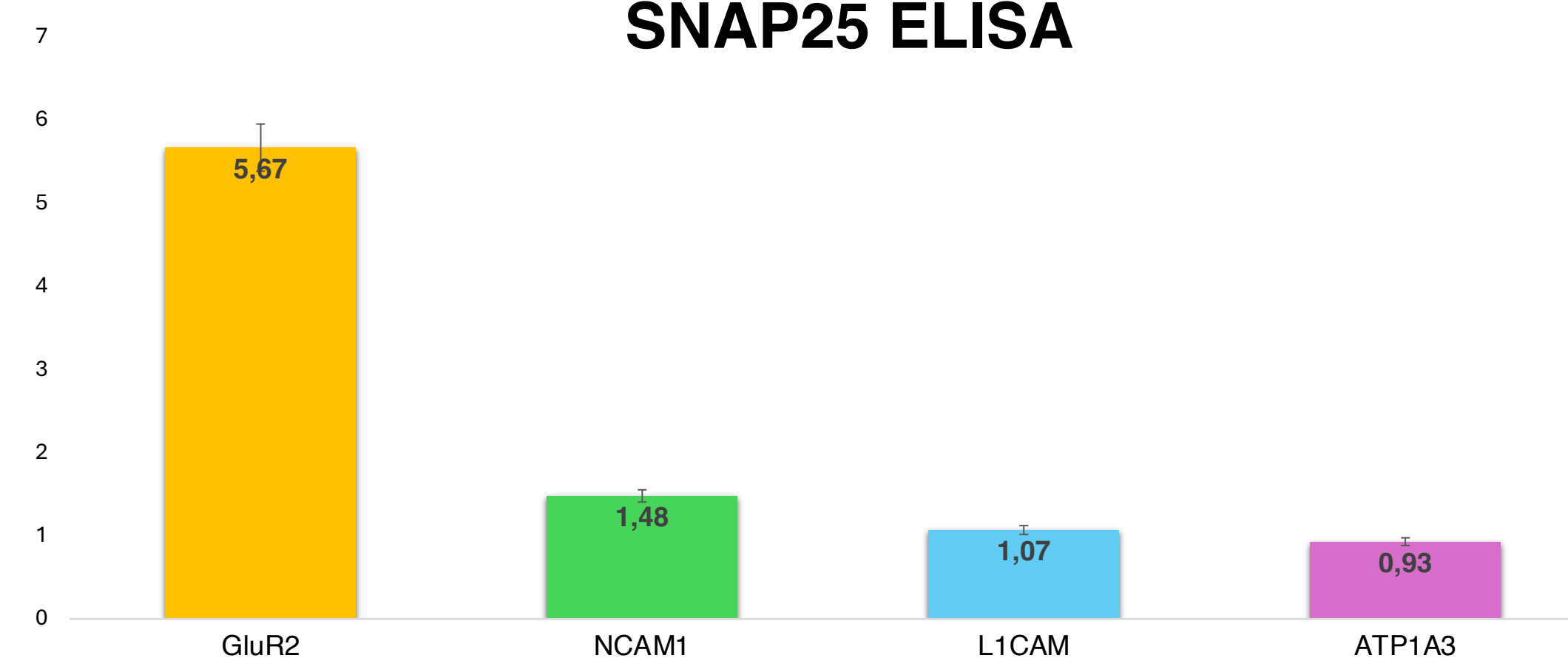


Figure 3. Nanotracker analysis-based characterization of NDEVs isolated using antibodies against GluR2, L1CAM, ATP1A3, and NCAM1. Left: particle concentration (particles/mL). Right: size mode (nm).

SNAP25 ELISA



proBDNF ELISA

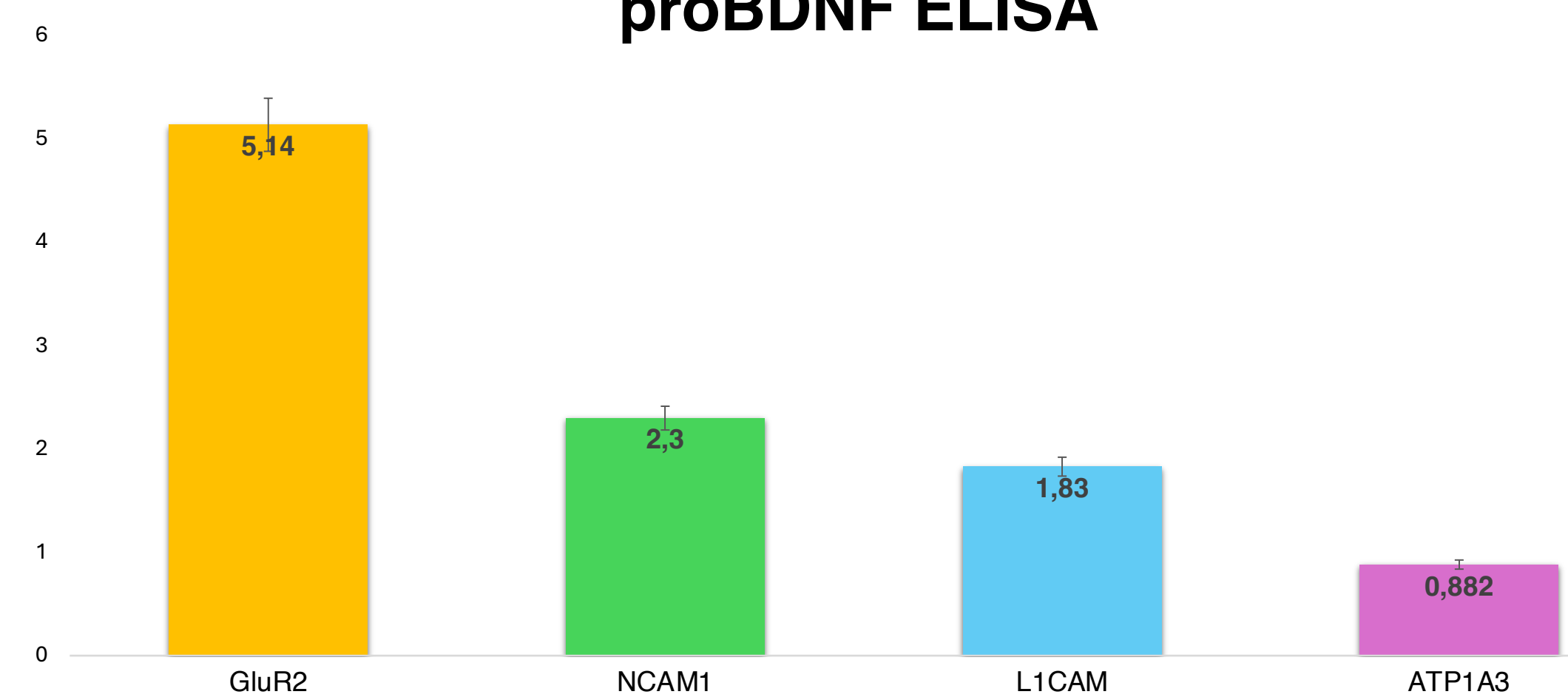


Figure 4. Fold enrichment of the neuron-specific intracellular markers SNAP25 and proBDNF relative to isotype IgG controls in NDEVs isolated using different neuronal surface markers.

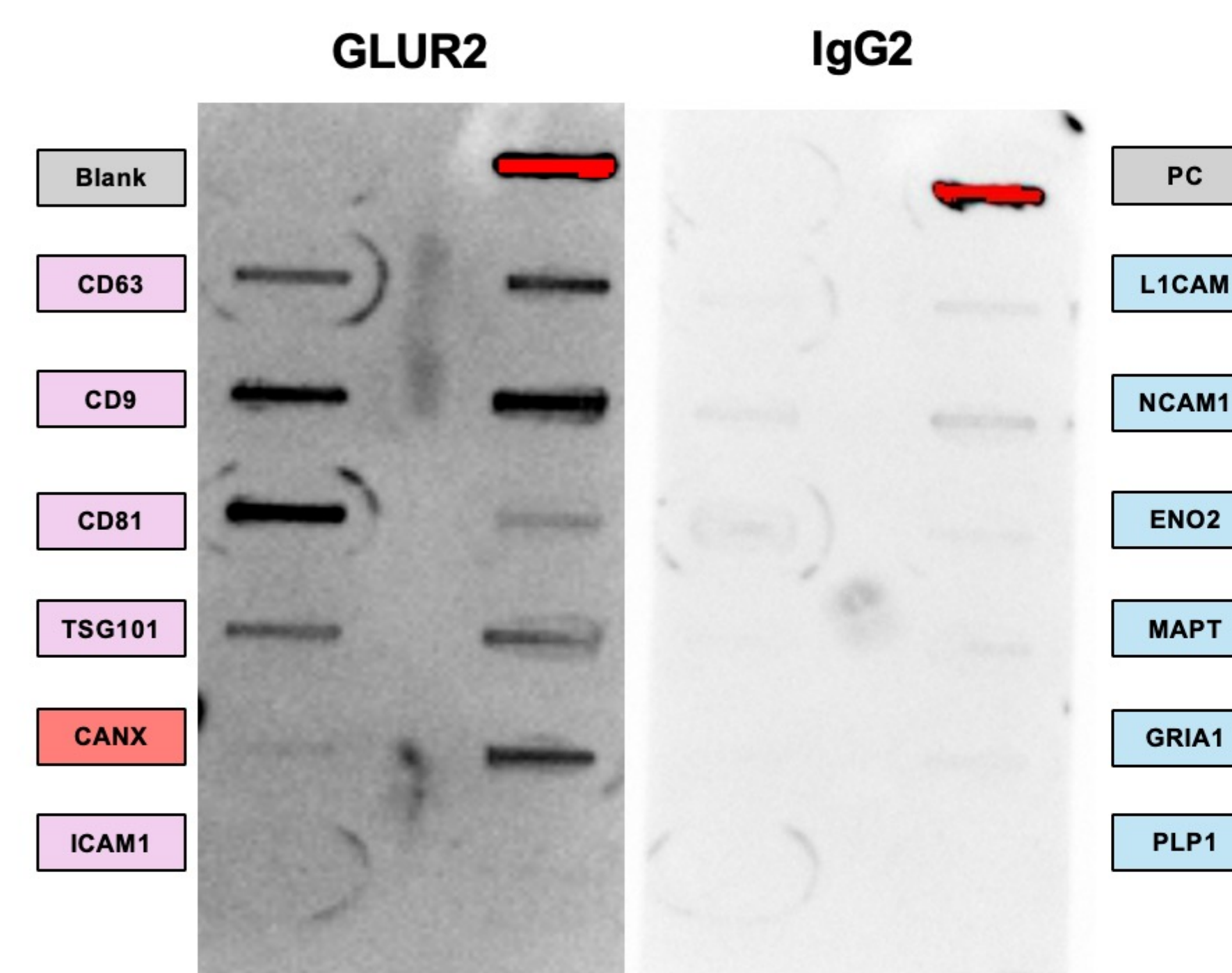


Figure 5. Antibody arrays of GluR2-immunocaptured NDEVs and IgG2 isotype control. Pink: EV markers, blue: neuronal markers, red: cellular debris marker, grey: blank and positive control (PC).

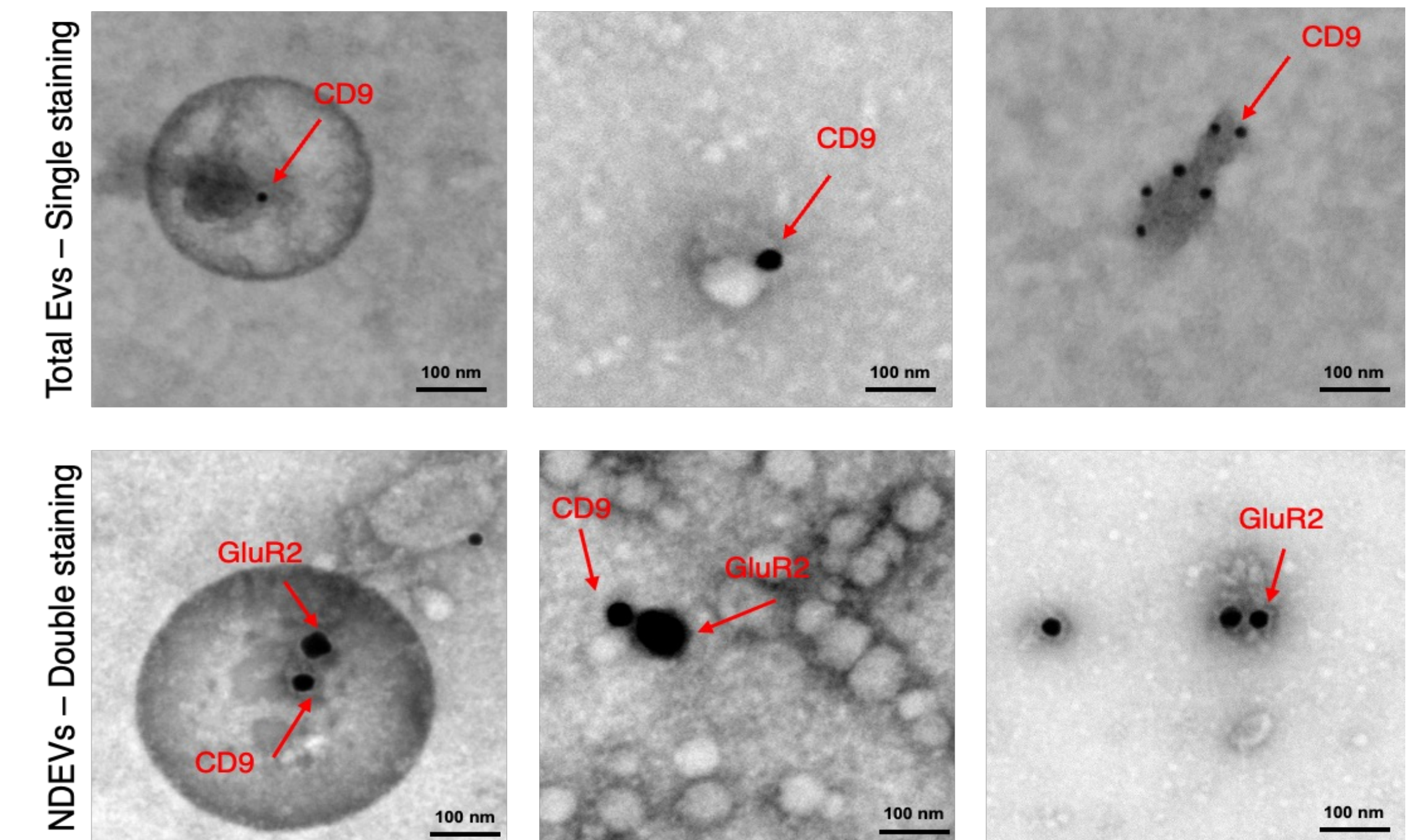


Figure 6. Immunogold Transmission Electron Microscopy (TEM) analysis of total EVs and GluR2-immunocaptured NDEVs. Upper panels show CD9-positive total EVs, while lower panels demonstrate GluR2-positive NDEVs and co-localization of CD9 and GluR2. Scale bars = 100 nm.

CONCLUSION

- Our results indicate that GluR2 is a more neuron-specific marker for the isolation of NDEVs from blood compared to other widely used neuronal surface markers, including NCAM1, L1CAM and ATP1A3.
- In addition, GluR2 also results in a comparatively higher yield of NDEVs from blood, likely due its high expression levels in neuronal tissue.

REFERENCES

- Théry C, Witwer KW, Aikawa E, et al. Minimal information for studies of extracellular vesicles 2018 (MISEV2018). *J Extracell Vesicles*. 2018;7(1):1535750.
- Mustapic M, Eitan E, Werner JK Jr, et al. Plasma extracellular vesicles enriched for neuronal origin: a potential window into brain pathologic processes. *Front Neurosci*. 2017;11:278.
- Norman M, Ter-Ovanesyan D, Trieu W, et al. ATP1A3 as a target for isolating neuron-specific extracellular vesicles from human brain and biofluids. *Mol Psychiatry*. 2021;26:1599–1614.
- Human Protein Atlas. GRIA2 (GluR2) gene expression profile. Available from: <https://www.proteinatlas.org>

ACKNOWLEDGEMENTS

Grants: European Research Council (#101041677), DZNE institutional funds. More information about our lab can be found here .



Contact: Maryam.Touhidinia@dzne.de