NEURODEX

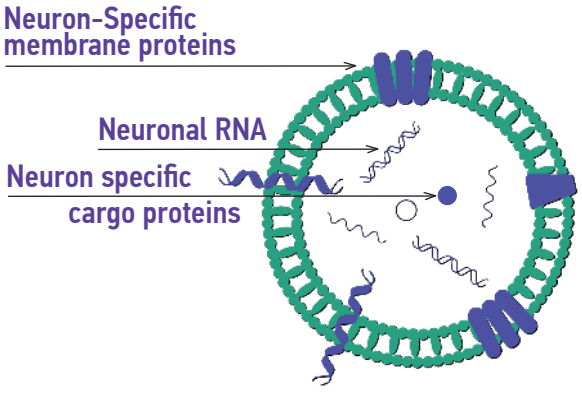
Affinity-based isolation and characterization of extracellular vesicles from cell culture and plasma

O Volpert[1], K Elgart[1], E Tordoff[1], S Rau[1], A Elsherbini[1], E Eren[2], D Kapogiannis[2], E Eitan[1].

[1] - NeuroDex Inc., Natick, MA; [2] - National Institute of Aging, Baltimore, MD.

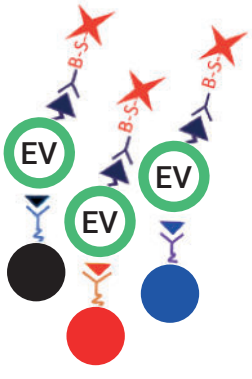
Neuronal extracellular vesicles (NDE's)

- Nano-sized membrane vesicles shed by neurons into blood
- Stable through 3+ freeze-thaw cycles)
- Cargo comprised of RNA, proteins, and lipids



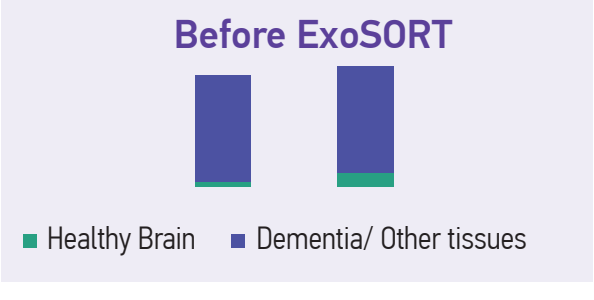
LuminEV

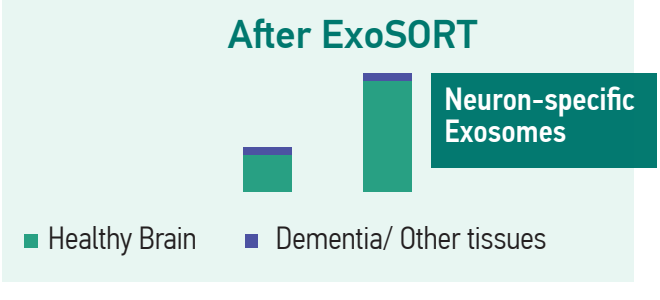
- A proprietary NeuroDex technology for intact EV analysis
- Detects EVs in plasma and culture media; does not require EV enrichment/purification
- Multiplexing ability: sensitive simultaneous detection of multiple EV surface markers, co-localization analysis
- Built-in negative control
- Medium-throughput format (96-well plates)



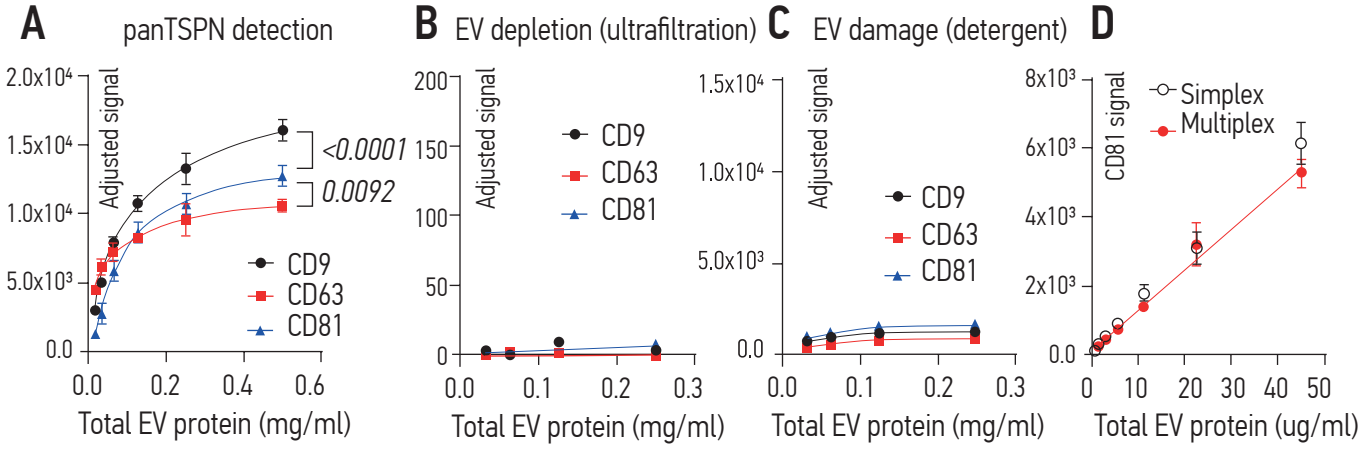
ExoSORT™

- A proprietary NeuroDex technology for enriching neuronal Evs from 300+ uL of plasma.
- Immunoaffinity capture (surface GAP43 and NLGN3 antigens).
- Proprietary recombinant antibodies produced in-house.
- Up to 100-fold increase in signal-to-noise ratio
- Low to medium throughput (50-90 samples per day).

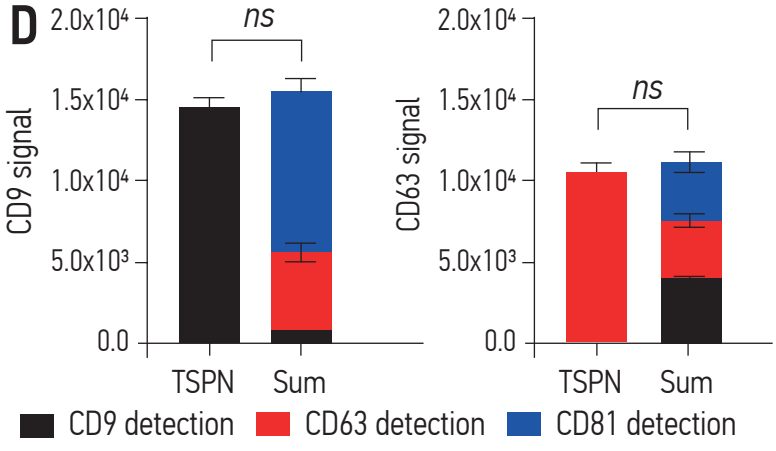




LuminEV assay qualification using purified EVs



LuminEV assay qualification using purified EVs



All of the experiments were performed with similar results using U87 and HEK293 EVs. A clear demonstration of isolated EV detection.

A. Linearity, and saturation with increased EV loading (total protein).

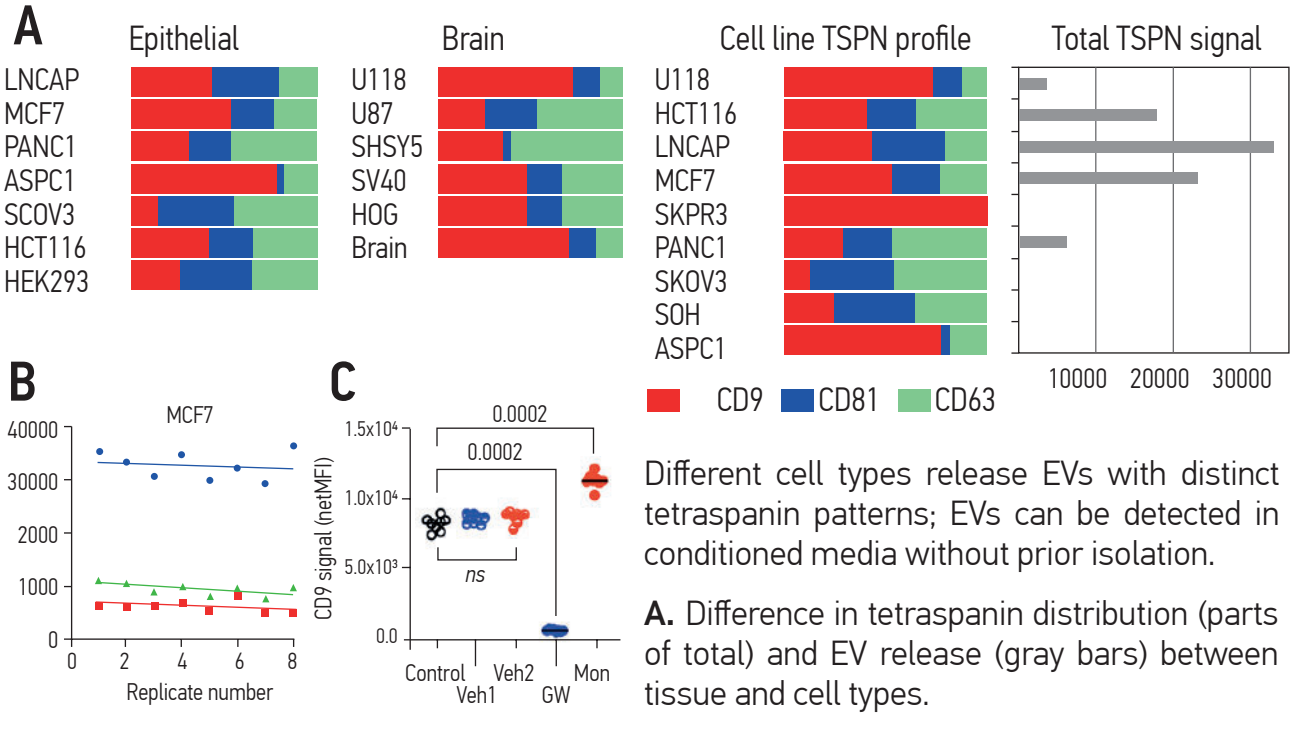
B. EV specificity corroborated by signal reduction upon EV depletion/ lysis.

C. Multiplex (CD9, CD81, and CD63) and simplex assays demonstrate linearity and similar signal intensity for individual analytes.

D. The combined signal intensity generated using CD9, CD63, or CD81 as detection antibodies is equivalent to the signal elicited with a combination of all three antibodies.

Similar data generated using CD9, CD63 and CD81 capture antibodies.

LuminEV detects differences in tetraspanin profiles between distinct cell types/treatments



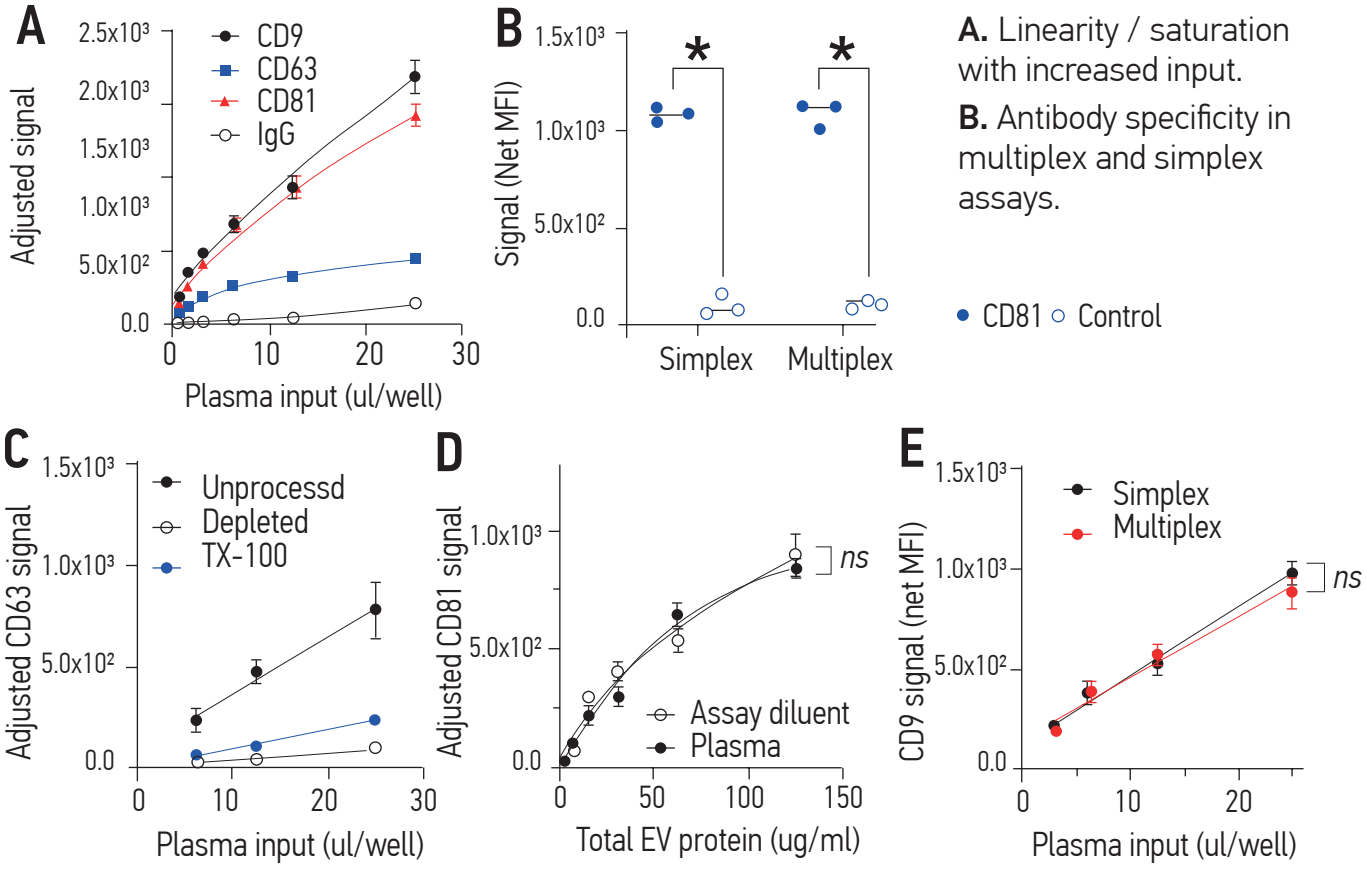
Different cell types release EVs with distinct tetraspanin patterns; EVs can be detected in conditioned media without prior isolation.

A. Difference in tetraspanin distribution (parts of total) and EV release (gray bars) between tissue and cell types.

B. Compatibility with analysis of EV release in 96-well plate format: was demonstrated for MCF7, HEK293, U87 cell lines, and IPS-derived neurons. Variability between replicates under 10%.

C. Detection of treatment effects in 96-well format. EV release (tetraspanin signal) was reduced by over 90% by GW4869 and increased by Monensin. Similar results were shown for CD63 and CD81 with multiple EV release modulators.

LuminEV is applicable for Plasma EV analysis



A. Linearity / saturation with increased input.

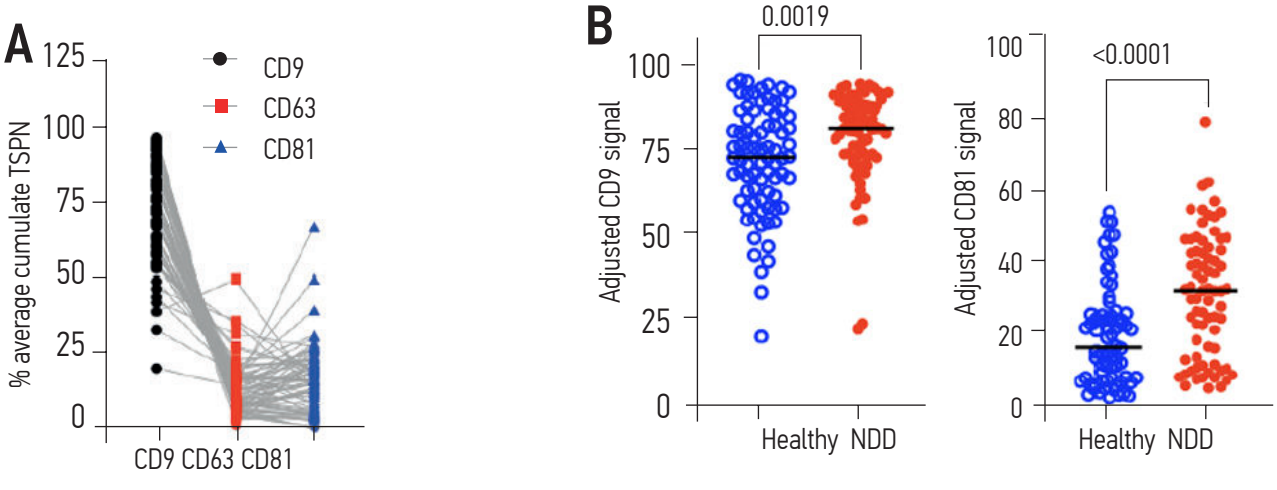
B. Antibody specificity in multiplex and simplex assays.

C. Specificity for EV specificity confirmed by signal reduction by EV depletion/lysis.

D. Lack of matrix effect, shown using U87 EVs spiking in plasma.

E. Multiplex (CD9, CD81, CD63) and simplex assays maintain linearity, equivalent signals (shown for CD81 and CD63).

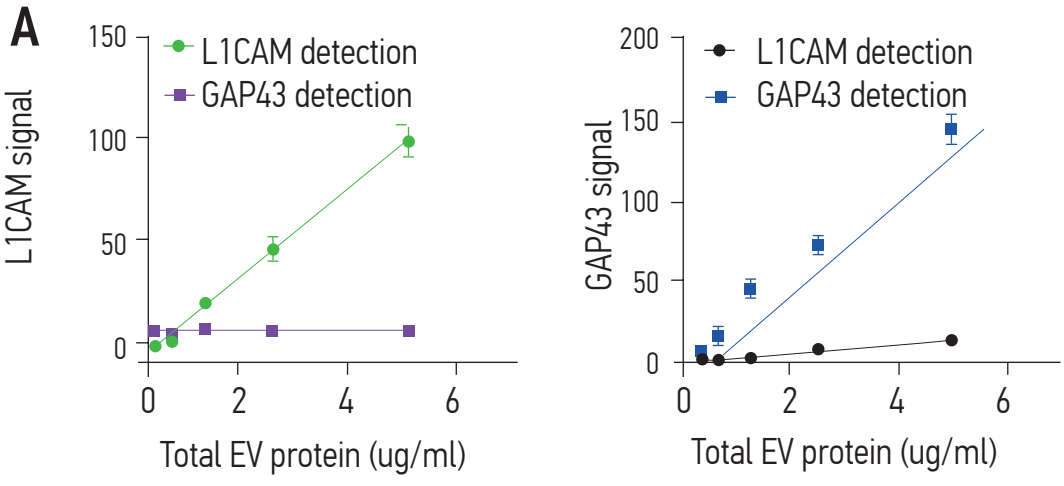
LuminEV for EV analysis in unprocessed plasma

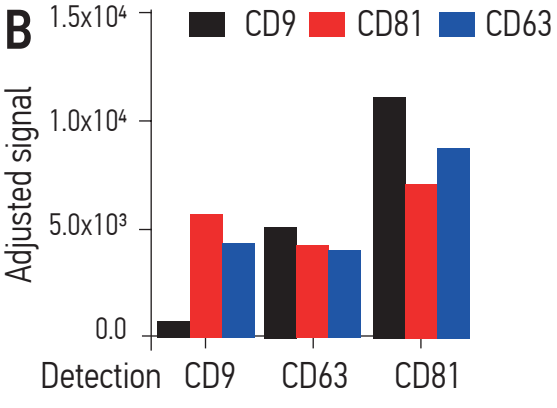


A. Plasma from heathy donors (N=80) shows CD9 as a dominant EV marker followed by CD63 and CD81. Biological variability 22-82%.

B. Higher content of CD9+, CD81+ EVs in neurological disease (NDD, N=80). Although significant, the differences do not allow clear separation. Not suitable as biomarker, but may be improved by normalization

LuminEV detects EV surface protein co-localization

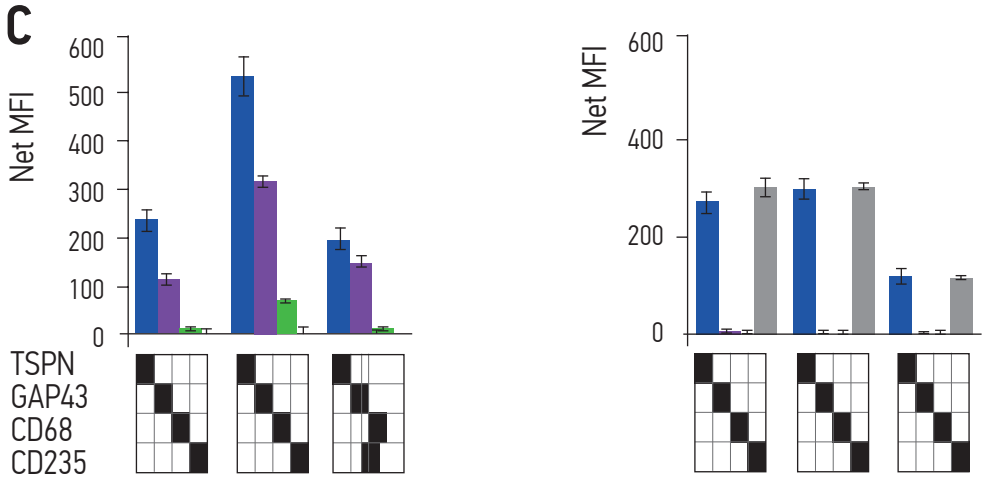




A. HEK293 cells overexpressing L1CAM (top), or GAP43 (bottom) detectable only with appropriate antibody w/o cross-reactivity.

B. EV from HEK293 cells captured with CD9, CD63 or CD81 (color-coded) and detected with the same antibodies (shown on X axis).

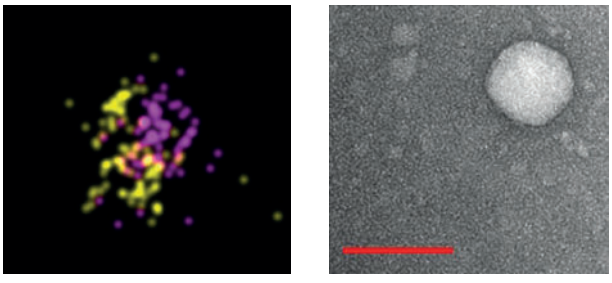
Co-localization analysis in cell culture, demonstration of specificity.



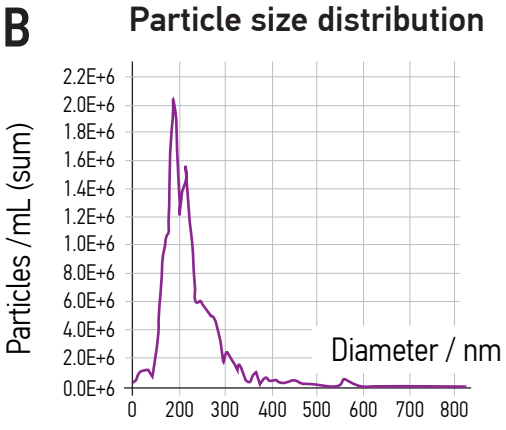
C. Plasma EVs captured with GAP43 (left) or CD235a antibodies (right) and detected with dell-distinct cell-specific or tetraspanin antibodies (X-axis). Only the combination of antibodies against co-localized markers generates a detectable signal.

ExoSORT™

A



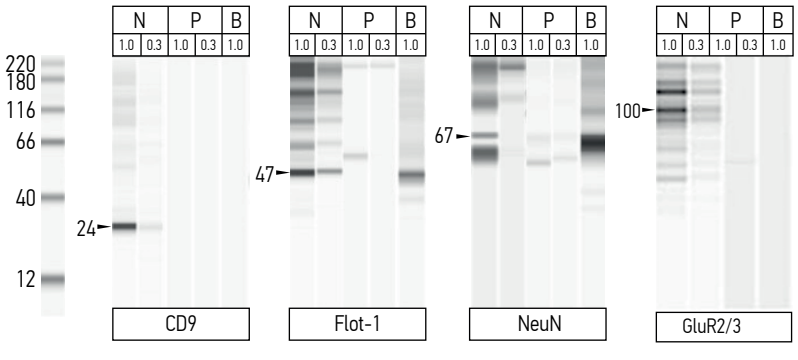
B



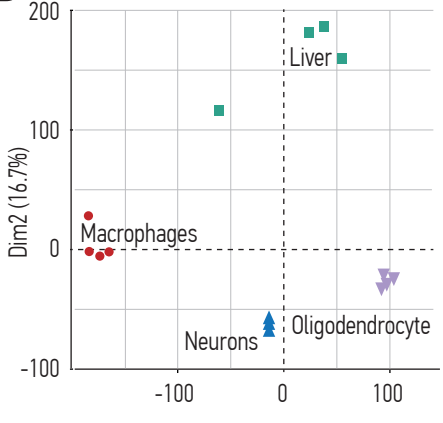
A. ONI and TEM of plasma NDEs.

B. Size distribution (Zeta-view).

C



D



C. Western blot analysis (JESS) of EV and neuronal markers.

D. mRNAseq analysis of NDEs and other plasma EV subtypes. Extended analysis ongoing (>1000 samples).

E

Tissue	Total EVs	NDEs
Erythrocyte	4E-35	
Liver	6.5E-27	
T-cell	1E-21	
Brain		2.3E-08
Fetal brain	1	5E-06
		1.5E-3

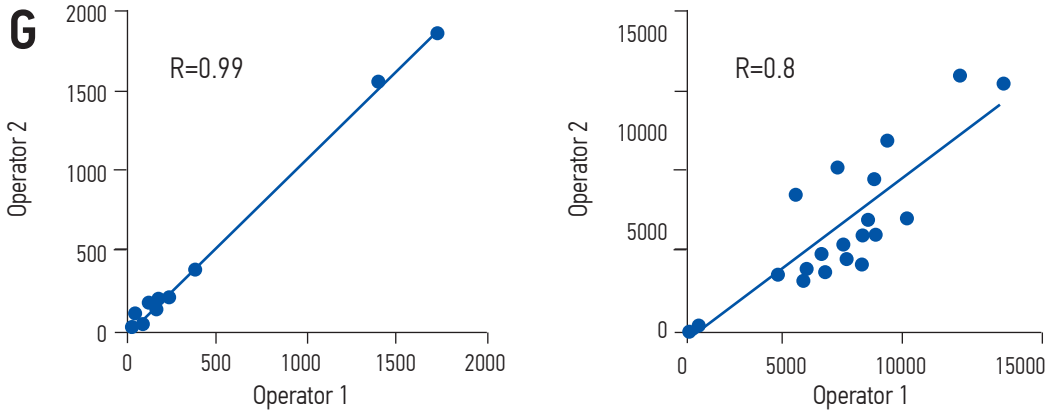
F

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.67	0.14	0.51	0.16	0.55	0.16	0.53	0.17	0.45	0.15	0.45	0.15
B	0.15	0.53	0.15	0.61	0.16	0.45	0.15	0.48	0.15	0.60	0.15	0.53
C	0.72	0.16	0.55	0.17	0.48	0.16	0.53	0.16	0.57	0.17	0.42	0.15
D	0.17	0.55	0.15	0.52	0.15	0.47	0.15	0.53	0.18	0.43	0.16	0.43
E	0.66	0.17	0.63	0.15	0.58	0.16	0.64	0.16	0.55	0.17	0.60	0.15
F	0.16	0.61	0.16	0.55	0.16	0.51	0.17	0.51	0.18	0.48	0.18	0.45
G	0.74	0.18	0.69	0.17	0.53	0.17	0.58	0.17	0.52	0.17	0.30	0.15
H	0.17	0.67	0.17	0.51	0.17	0.56	0.19	0.51	0.19	0.59	0.20	0.39

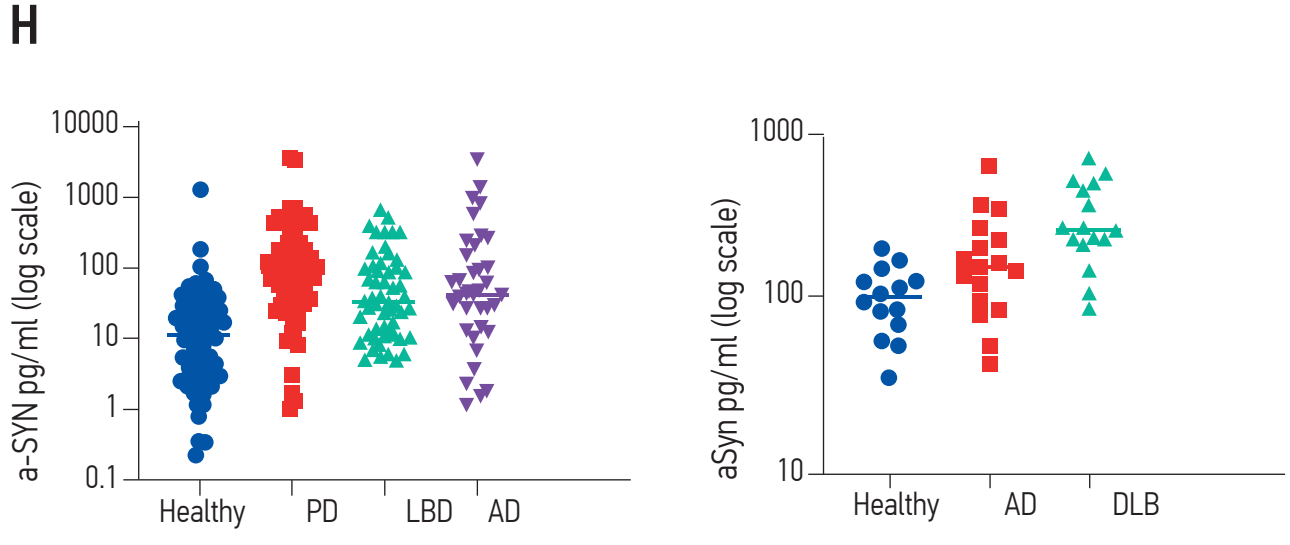
CV<12%
Fold from control >4

E. Gene ontology analysis, P value of tissue enrichment.

F. High intra-assay reproducibility (CV<15%).



G. High reproducibiliyu between experiments/operators: Alpha-synuclein and LC3 measurements.



H. Biomarker potential of ExoSORT platform: NDE alpha-synuclein measurement (clinically and PM diagnosed disease) > 900 sample analysis in progress.