UC San Diego

Evaluation of Blood-Based, Exosome Cargo as Biomarkers for AD and Other Neurodegenerative Diseases

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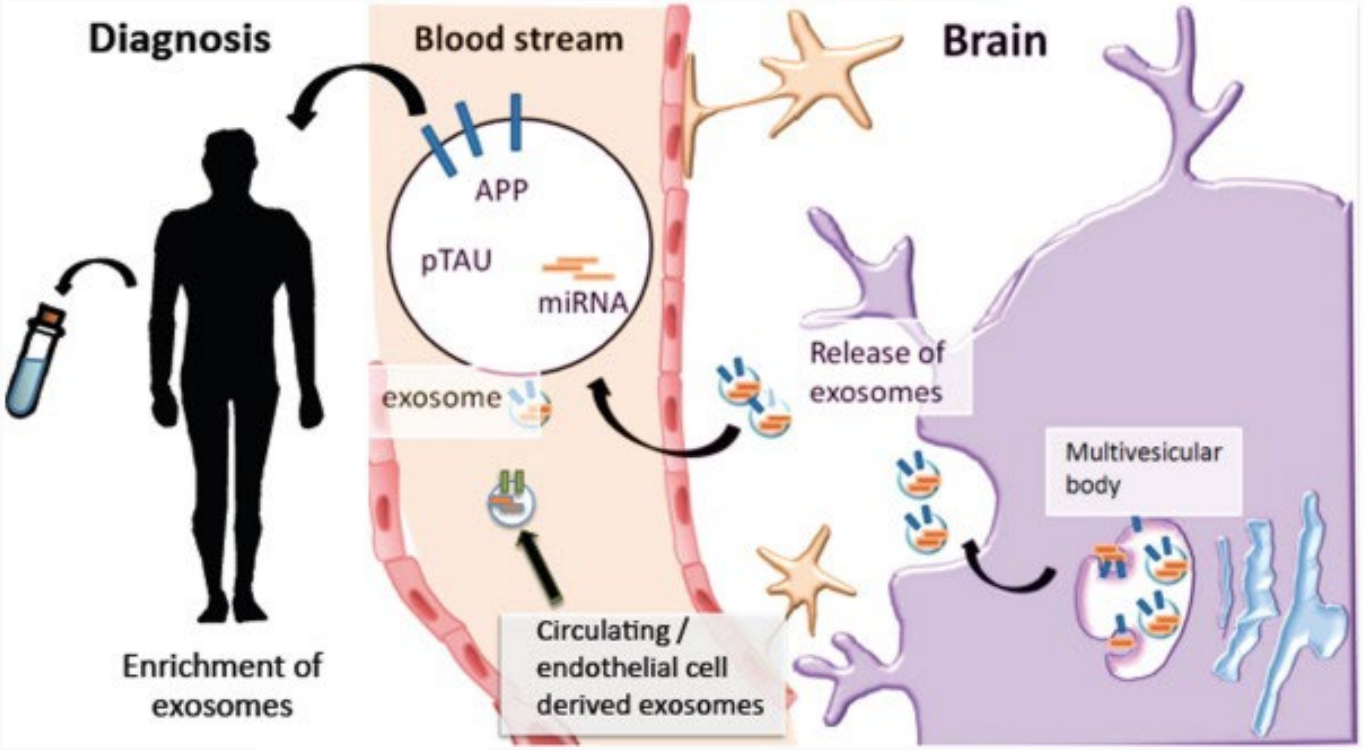
NEURONAL & GLIAL DERIVED EXOSOMES IN BLOOD



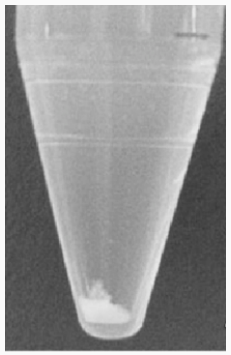
- Exosomes are nano-sized microvesicles of endosomal origin.
- Secreted from a variety of cell types including neurons.
  - Function not well understood
    - Intracellular communication, waste removal
- Exosomes contain range of cargo including proteins, lipids, miRNA and offer a snapshot of the cellular health

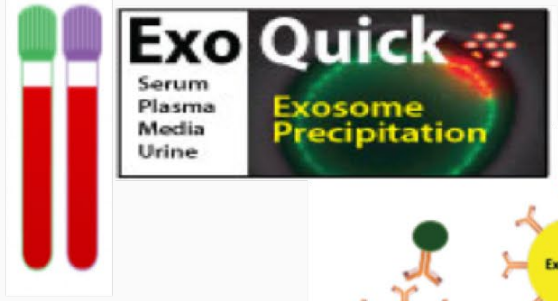
Protein cargo extracted from neuronal and astrocyte-derived exosomes offers predictive ability to distinguish stages of AD and other diseases

EXOSOME ISOLATION & ENRICHMENT

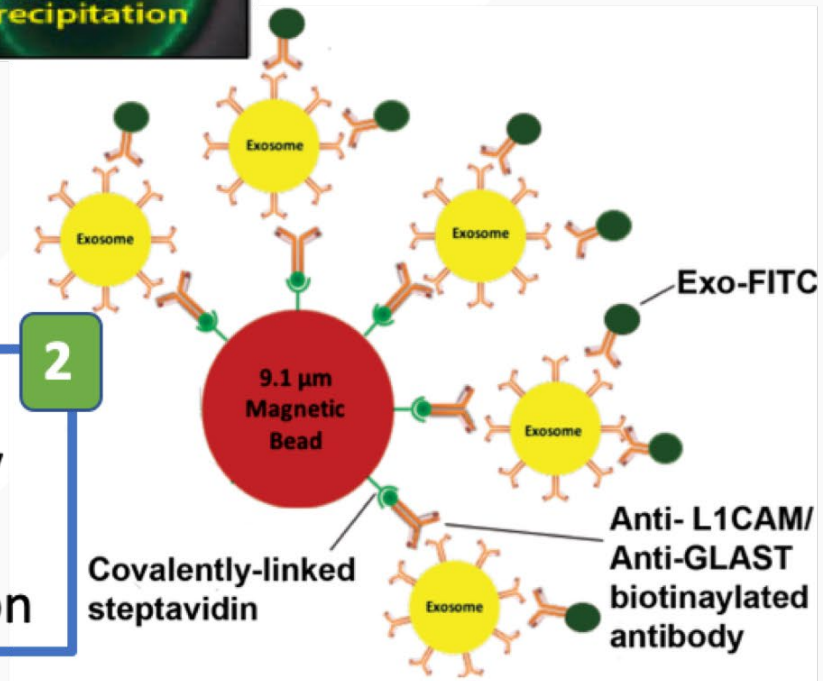
1 Exosome Isolation

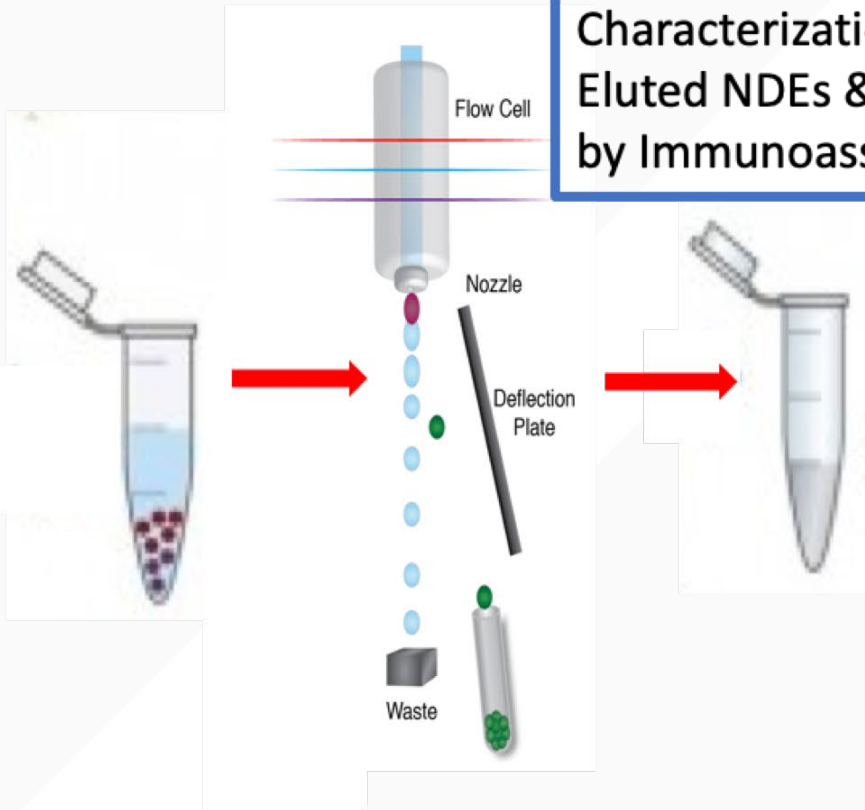
Precipitation





2 Bead Antibody Exosome Formation



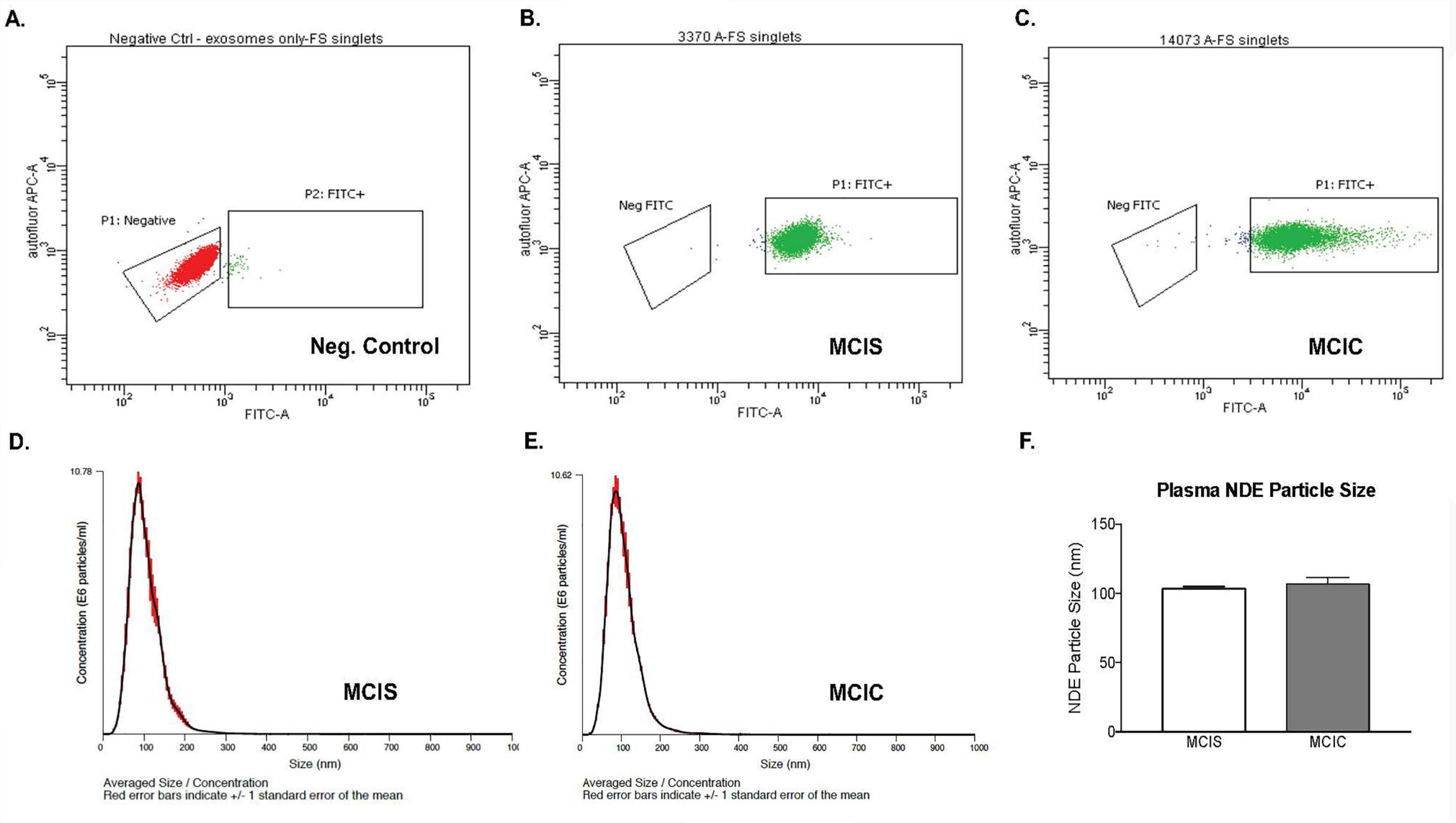


4 Characterization of Eluted NDEs & ADEs by Immunoassay

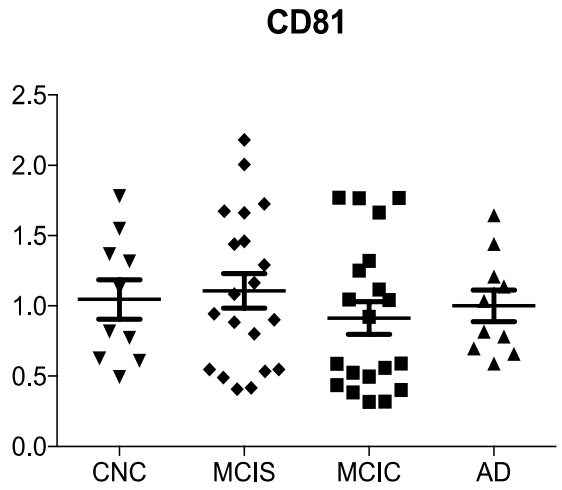
3 Fluorescence-activated cell sorting (FACS) & Neural (NDE) & Astrocyte (ADE) collection

EXOSOME CHARACTERIZATION VIA FACS & NANOSIGHT

Exosome confirmation by size (100 nm)



PLASMA NDE CARGO DIFFERENTIATE MCI AND AD FROM CONTROLS



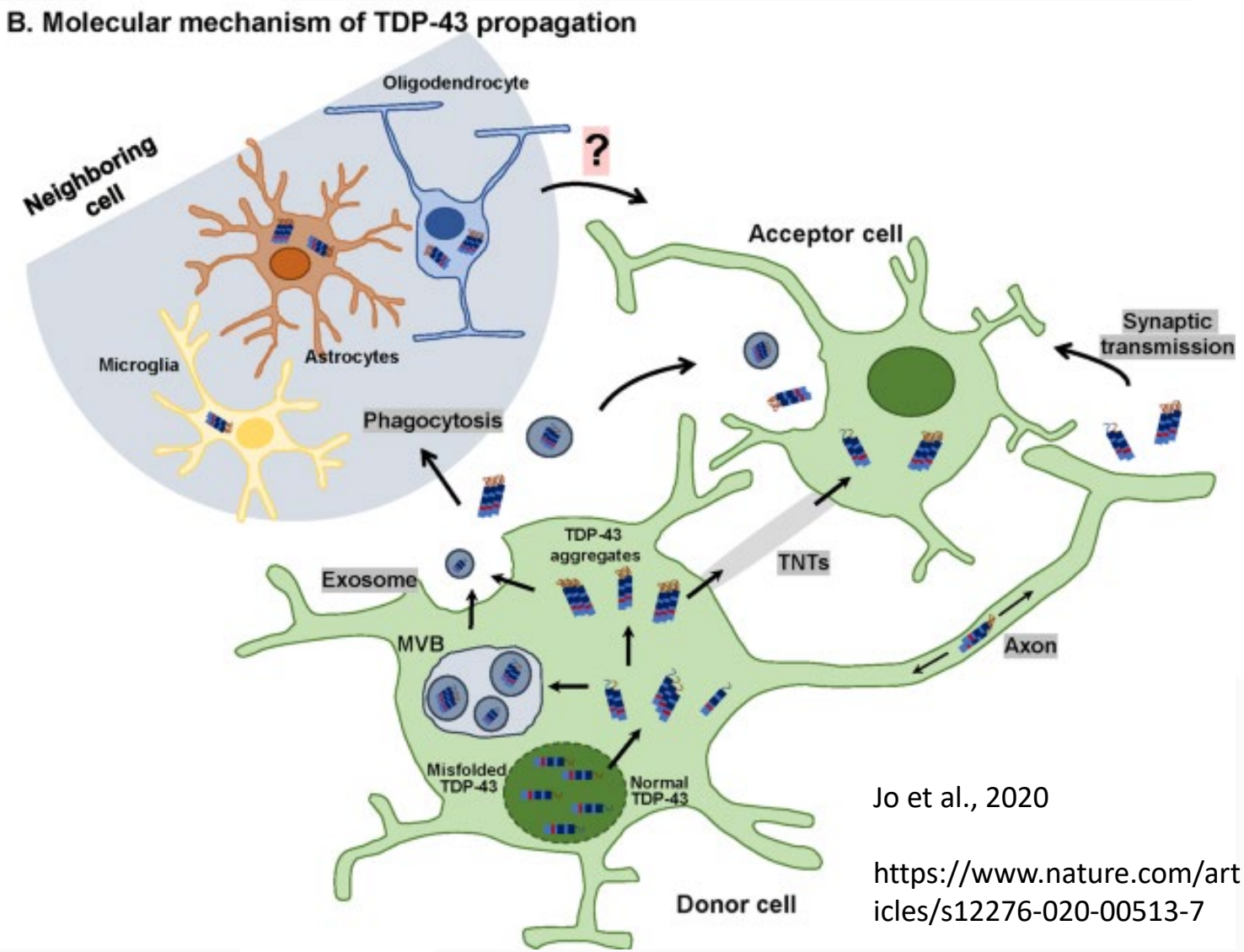
Winston et al., 2016

BIOMARKER POTENTIAL OF EXOSOME CARGO

- Plasma NDE protein profiles can accurately differentiate AD from control patients and accurately predict conversion of MCI to AD (Winston et al., 2016).
- Synaptic proteins contained in plasma NDEs were significantly decreased in patients with MCI, independent of GHRH treatment (Winston et al., 2018).
- Plasma NDEs and ADEs (Aβ & NRGN) can distinguish mTBI from controls. (Winston et al., 2019)

TDP-43 ACCUMULATION IN EXOSOMES

- Propagation of TDP-43 protein occurs in several neurodegenerative diseases including AD, FTD, and ALS
- Neuron-to-glia or glia-to-neuron transfer of TDP-43 has been observed to occur via phagocytosis, exosomes, tunneling nanotubes or synaptic transmission
- We developed a plasma bioassay to detect TDP43 in exosomes



Jo et al., 2020

https://www.nature.com/articles/s12276-020-00513-7

DONOR SAMPLES: DEMOGRAPHIC DATA

Patient Characteristic		LATE – NC (-) (N = 42)	LATE – NC (+) (N = 22)	Combined (N = 64)
Age of Death, mean (SD)		80 (9.6)	84 (9.7)	81 (9.8)
MMSE, mean (SD)		22 (8.0)	17 (11)	21 (9.2)
Years of Education, mean (SD)		16 (3.6)	18 (2.9)	17 (3.4)
Last Clinical Index	Control	13 (31%)	3 (13.6%)	16 (25%)
	MCI or Impaired	9 (21.4%)	3 (13.6%)	12 (18.75%)
	Demented	20 (47.6%)	16 (72.7%)	36 (56.25%)
Sex	Female	21 (50.0%)	9 (40.9%)	30 (46.9%)
	Male	21 (50.0%)	13 (59.1%)	34 (53.1%)
APOEε4	Negative	24 (57.1%)	10 (45.5%)	35 (54.7%)
	Positive	17 (40.5%)	11 (50%)	28 (43.8%)
	(Missing)	1 (2.4%)	1 (4.5%)	2 (1.5%)
Race	White	40 (95.2%)	21 (95.5%)	61 (95.3%)
	Black or African American	2 (4.8%)	1 (4.5%)	3 (4.7%)
	Hispanics	0 (0.0%)	0 (0.0%)	0 (0.0%)
	Asian	0 (0.0%)	0 (0.0%)	0 (0.0%)
	American Indian or Alaska Native	0 (0.0%)	0 (0.0%)	0 (0.0%)

Plasma samples from Univ of KY ADRC/Pete Nelson.

*NC – Neuropathological Changes

TDP43 CAPTURE AND DETECTION ANTIBODIES

labnr.	Materials	isotype	putative epitope	Species recognized
1	mAb 205.25.26 (3/16/09)	IgG2a	aa 261-393	Human preferable
2	mAb 5060.47.61(7-12-16)	IgG2a	aa 183-203	Human/mouse
3	mAb 5044.48.57.52.55 (7-12-16)	IgG2a	aa287-322	Human/mouse
4	mAb 5044.48.42(7-12-16)	IgG1	aa 261-393	Human preferable
5	mAb 5195.180.14(3-22-18)	IgG2b	aa394-414	Human/mouse

Gifts from Drs. J Trojanowski and V. Lee, UPenn

Detection Antibody: Recombinant Anti-TDP43 antibody (ab255922) +
Biotinylation Kit / Biotin Conjugation Kit (Type A) (ab201795)

EXOSOME ENRICHMENT & ELISA PLATE PROTOCOLS

Exosome Isolation & Enrichment

PEG-Based Exosome Isolation

↓

Magnetic Bead Immunocapture

↓

FACS Sort and Bead Collection

↓

Exosome Elution and Collection

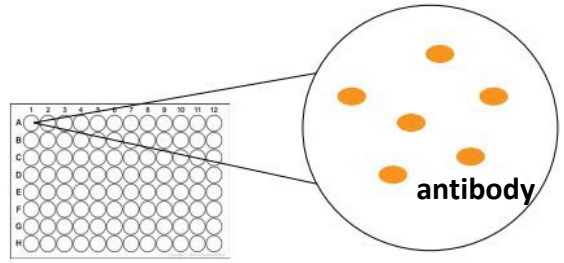
Eluted NDEs, ADEs, MDEs

Sandwich ELISA Plate Protocol

DuoSet Ancillary Reagent Kit 2 (Bio-Techne)

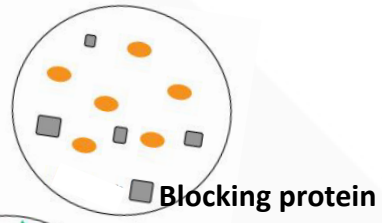
Coating

- Overnight coating with Capture Ab: Hu Recombinant TDP-43 (UPenn)



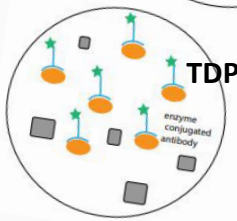
Blocking

- 1% BSA in PBS for 1hr at RT



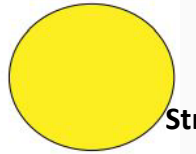
Detection

- Hu TDP-43-biotin (Abcam), 2hr at RT

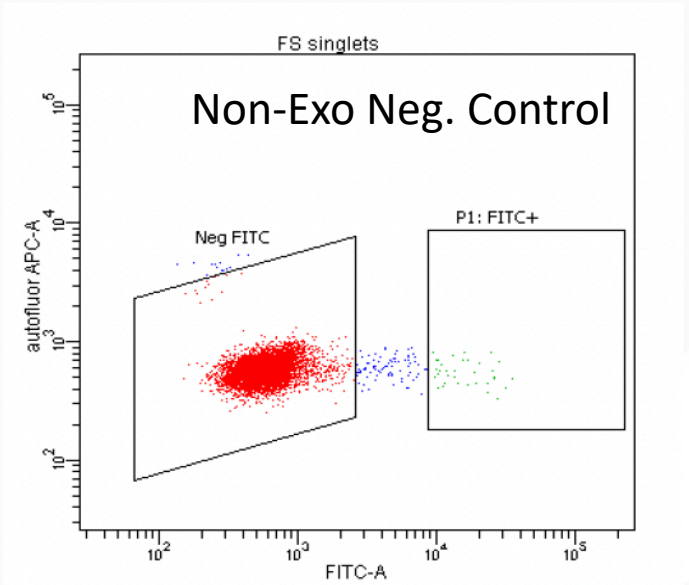


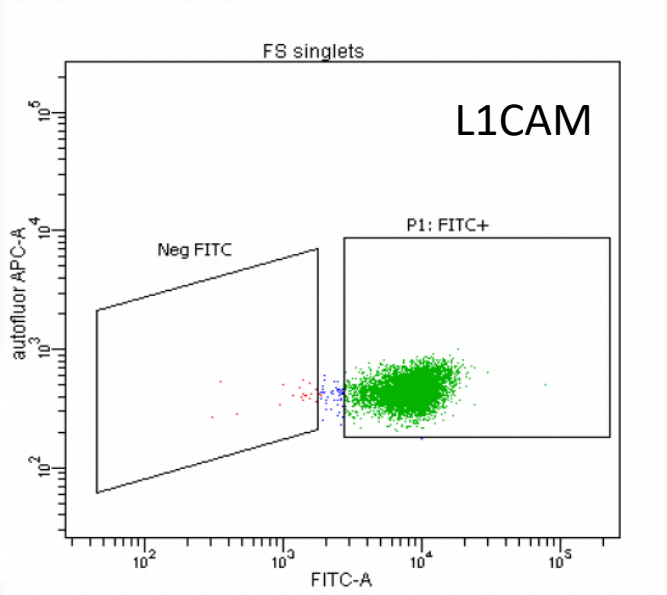
Signal Quantification

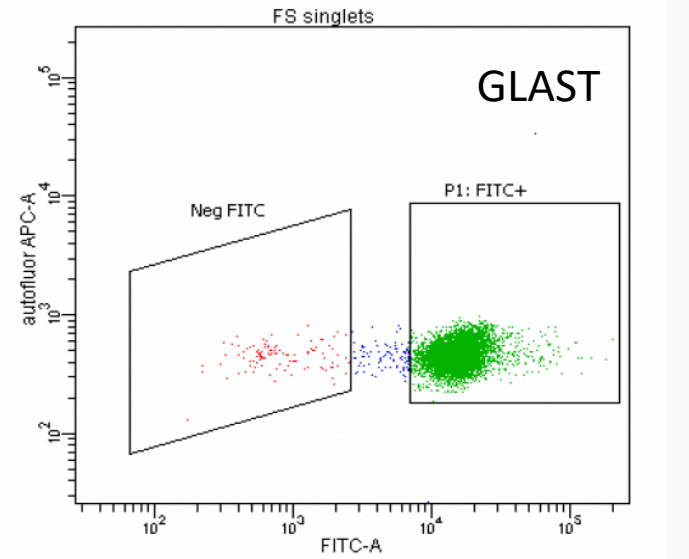
- Streptavidin-HRP at 37 °
- Read at 450 nm

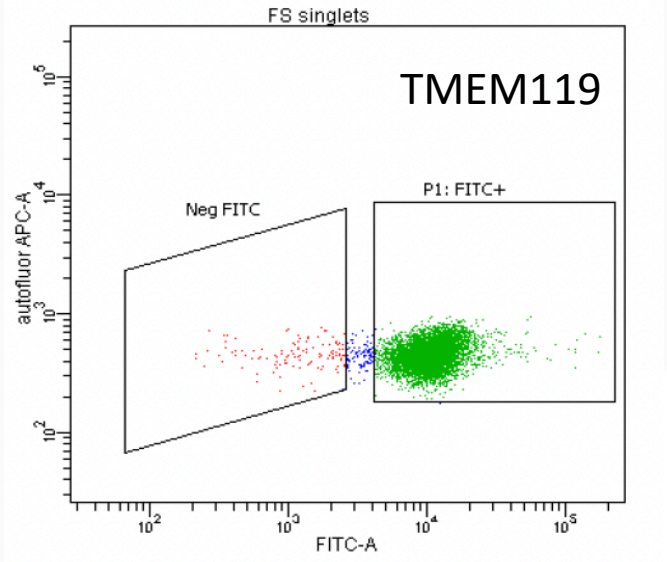


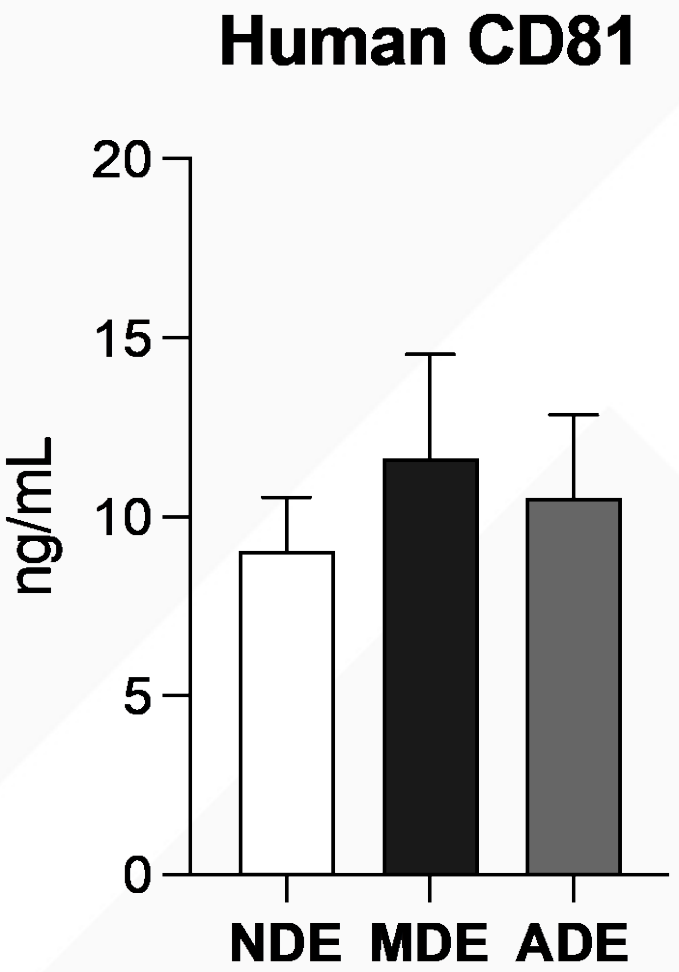
CHARACTERIZATION OF BLOOD BASED EXOSOMES VIA FACS & ELISA





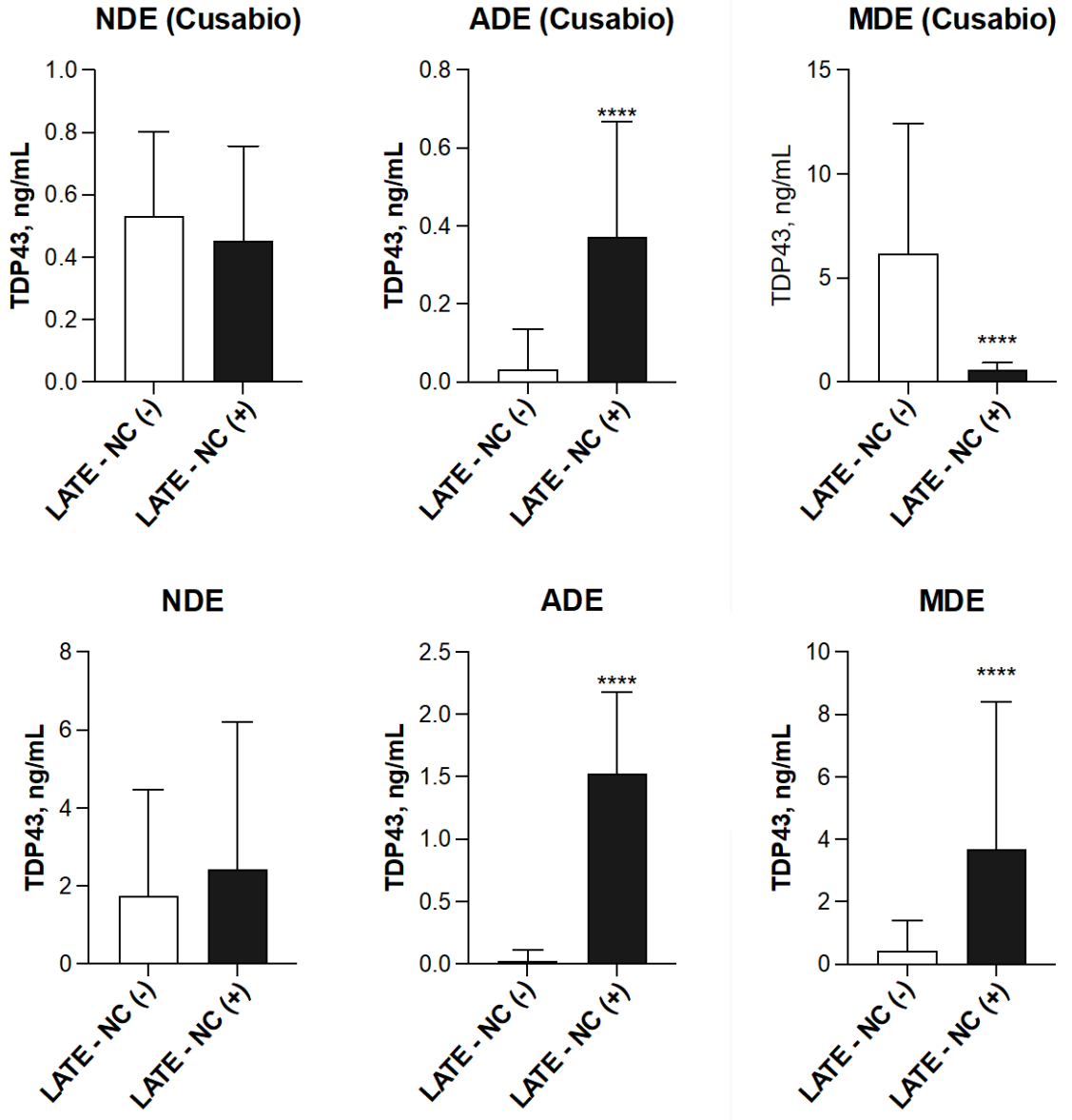




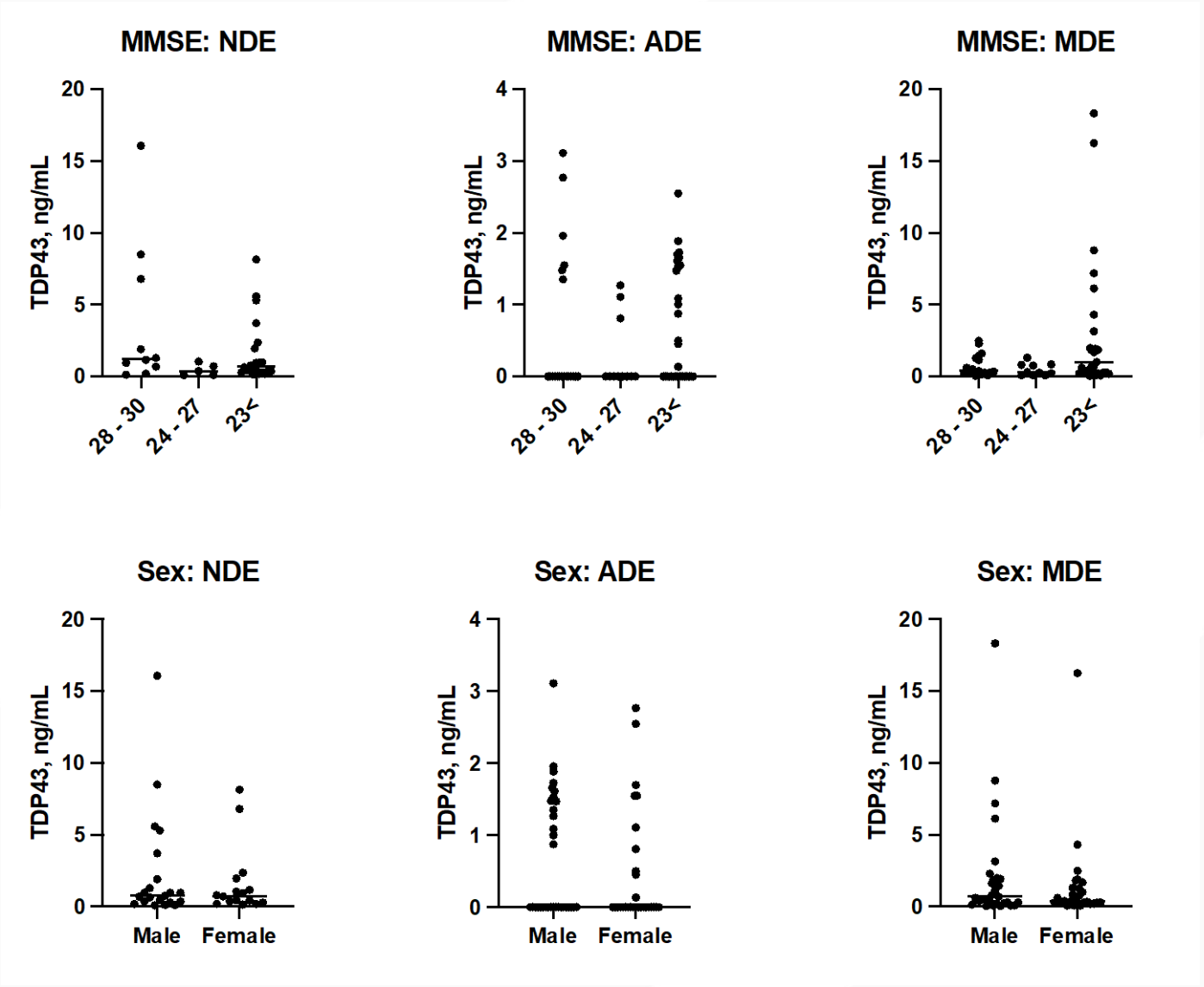


TDP-43 IS SIGNIFICANTLY INCREASED IN ASTROCYTE EXOSOMES DERIVED FROM CONFIRMED LATE-NC DONORS

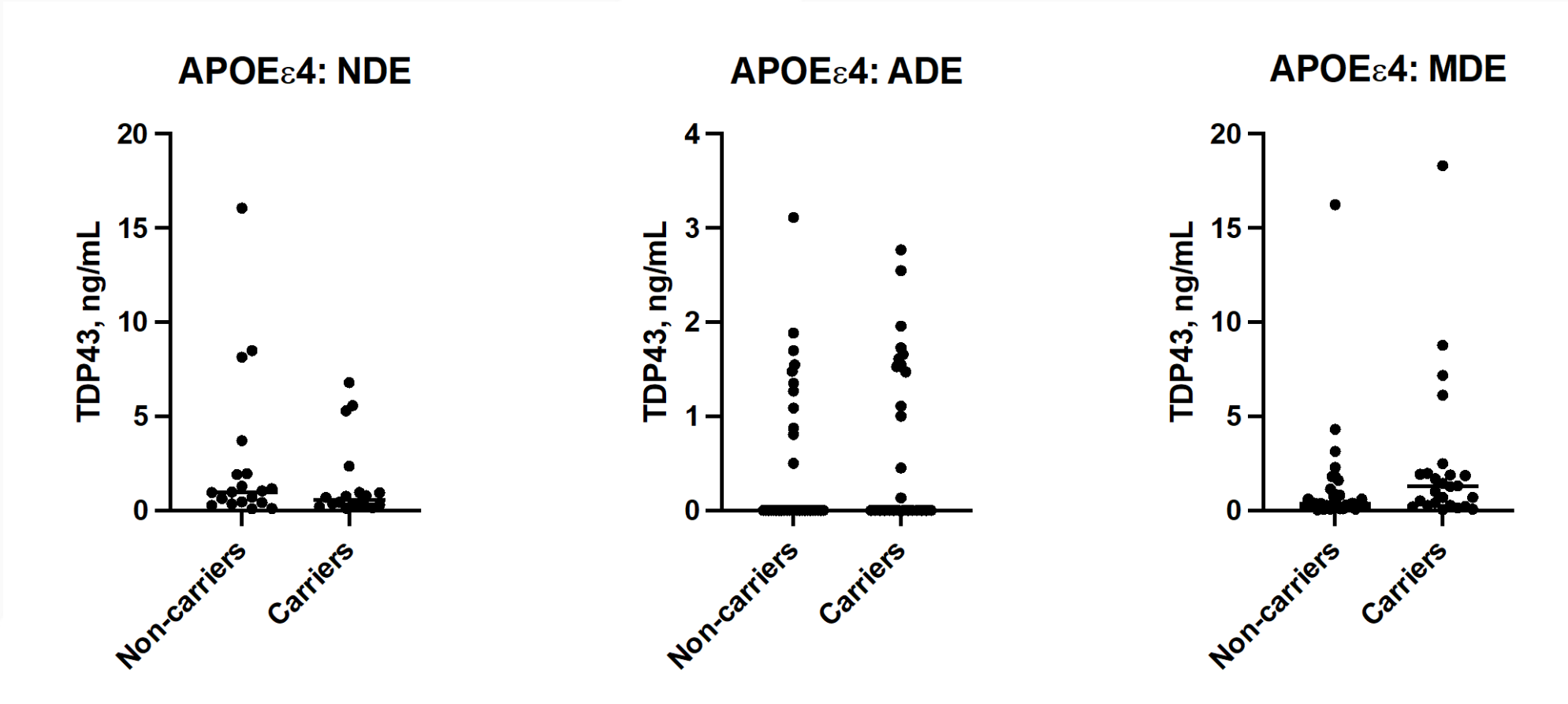
- Direct comparison between our in-house ELISA and the ELISA kit from Cusabio-American Research Products, Inc.
- Both are sandwich ELISAs, in which detected TDP-43 in the exosome preparations or standards are sandwiched between pre-coated TDP43 antibody (UPenn) and Biotin-conjugated TDP-43 antibody (Abcam).



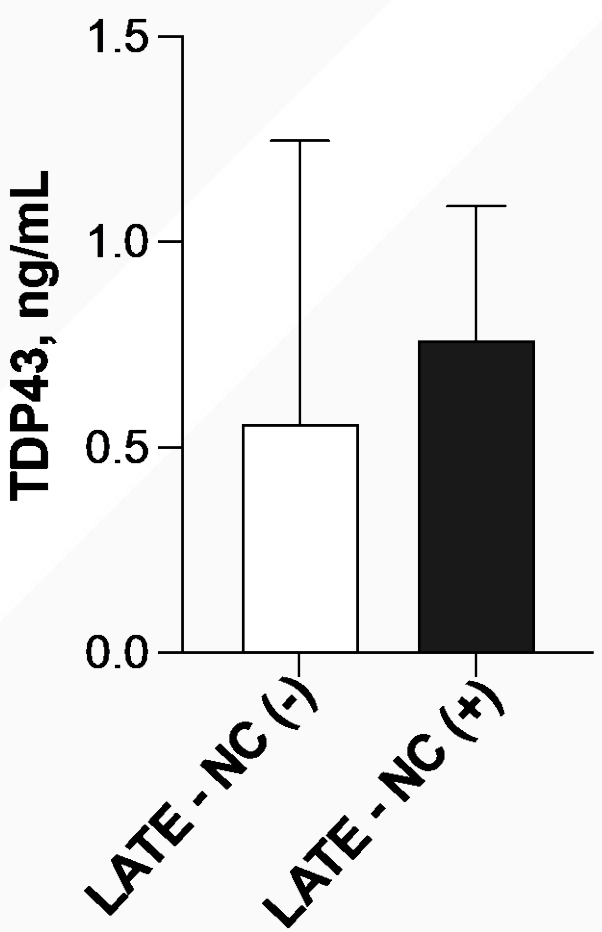
EXOSOMAL TDP-43 LEVELS ARE NOT INFLUENCED BY COGNITION-BASED VARIABLES & SEX

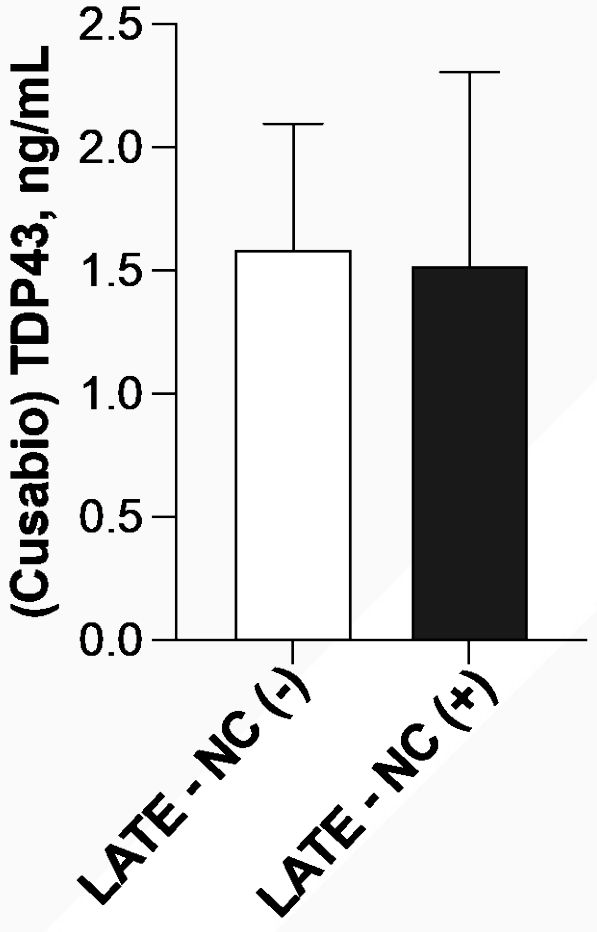


EXOSOMAL TDP-43 LEVELS ARE NOT INFLUENCE BY APOE GENOTYPE



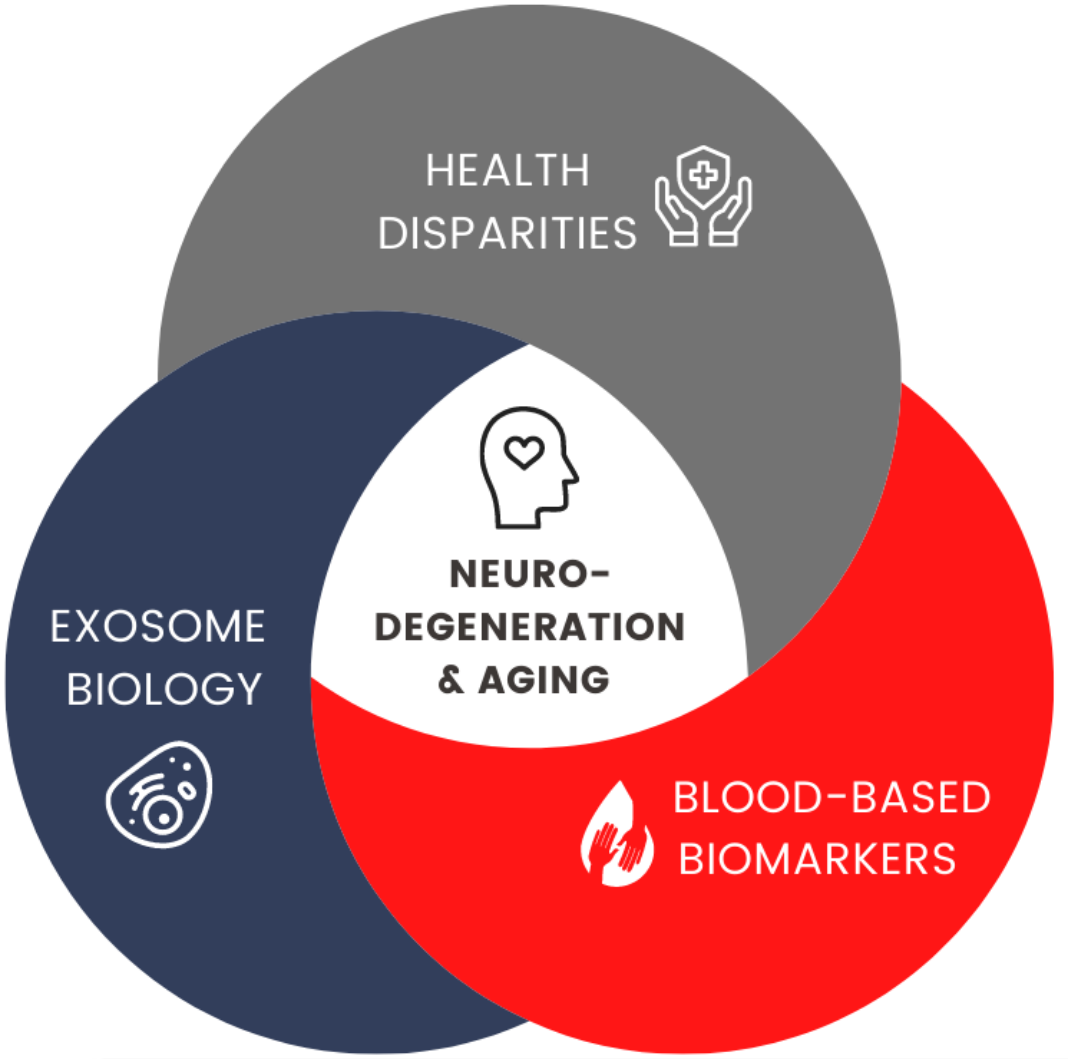
TDP-43 DETECTED IN EXOSOME DEPLETED PLASMA





SUMMARY AND FUTURE DIRECTIONS

- TDP-43 can be detected in NDEs, ADEs, and MDEs and in exosome depleted plasma of from LATE-NC (-) and (+) patients
- ADE levels of TDP-43 are significantly elevated in patients with LATE
- Exosomal levels of TDP-43 do not correlate with cognition-based variables, sex and APOE genotype
- *Future studies*: Assess the ethnoracial impact on exosomes as biomarkers for AD and other neurodegenerative diseases



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