Supplementary Material

Extracellular Vesicle-Enriched miRNA-Biomarkers Show Improved Utility for Detecting Alzheimer's Disease Dementia and Medial Temporal Atrophy

Supplementary Table 1. Summary of Neuropsychological Battery and Component Tests

Cognitive Domain	Component Test(s)
i. Executive Function	Frontal Assessment Battery [1]
ii. Attention	Digit Span, Visual Memory Span [2], and Auditory Detection [3]
iii. Language	Modified Boston Naming Test [4] and Verbal Fluency [5]
iv. Visuomotor Speed	Symbol Digit Modality Test [6], Maze Task [7], and Digit Cancellation [8]
v. Visuoconstruction	Weschler Memory Scale – Revised (WMS-R) Visual Reproduction Copy task [2], Clock Drawing [9] and Weschler Adult Intelligence Scale – Revised (WAIS-R) subtest of Block Design [10]
vi. Verbal Memory	Word List Recall & Recognition Tasks [11], and Story Recall Task
vii. Visual Memory	Picture Recall & Recognition Tasks, and WMS-R Visual Reproduction Recall & Recognition Task [2]

REFERENCES

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[4] Mack WJ, Freed DM, Williams BW, Henderson VW (1992) Boston Naming Test: shortened versions for use in Alzheimer's disease. *J Gerontol* **47**, P154-158.

[5] Isaacs B, Kennie AT (1973) The Set test as an aid to the detection of dementia in old people. *Br J Psychiatry* **123**, 467-470.

[6] Smith A (1973) *Symbol Digit Modalities Test*, Los Angeles, CA.

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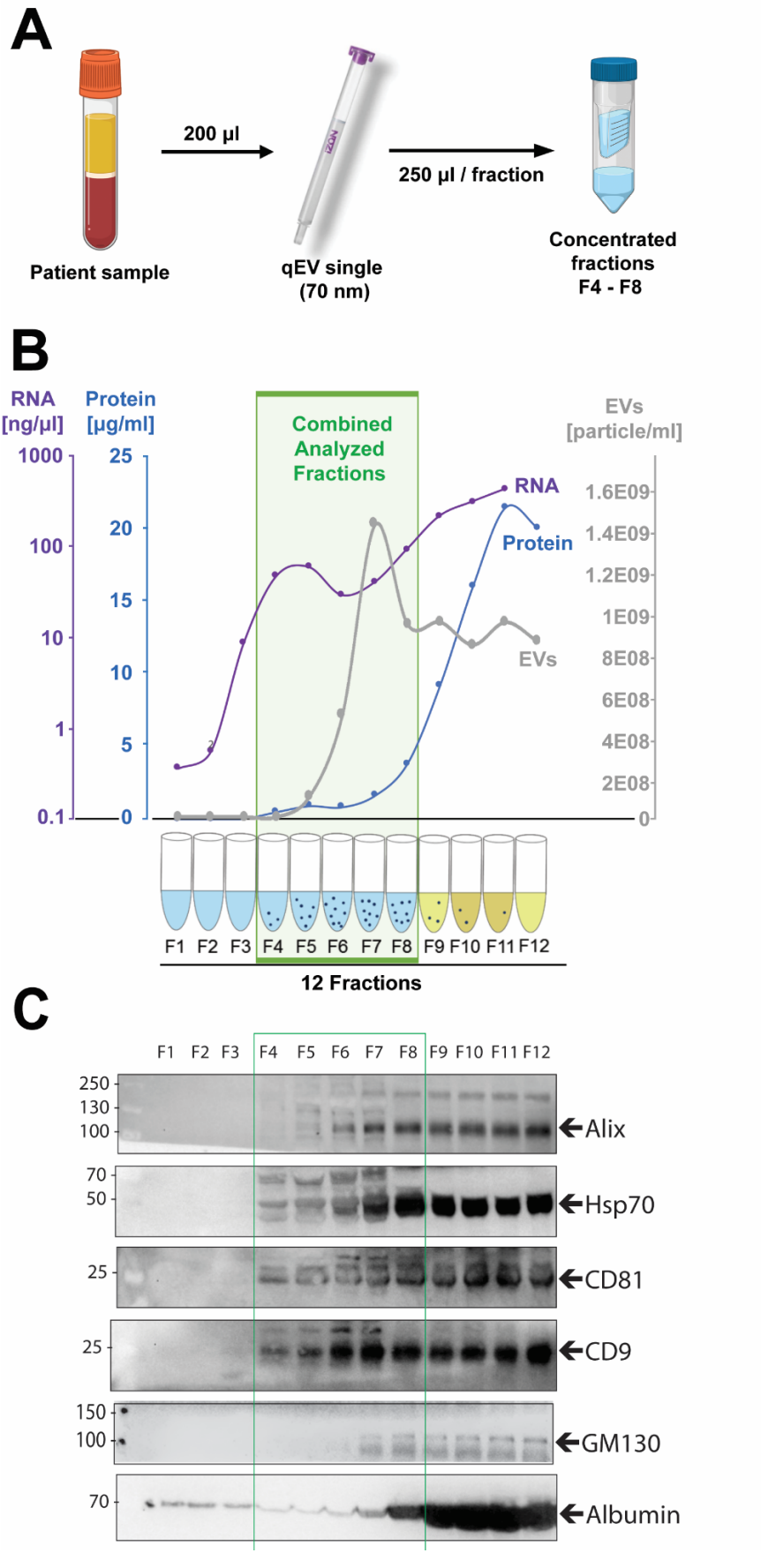
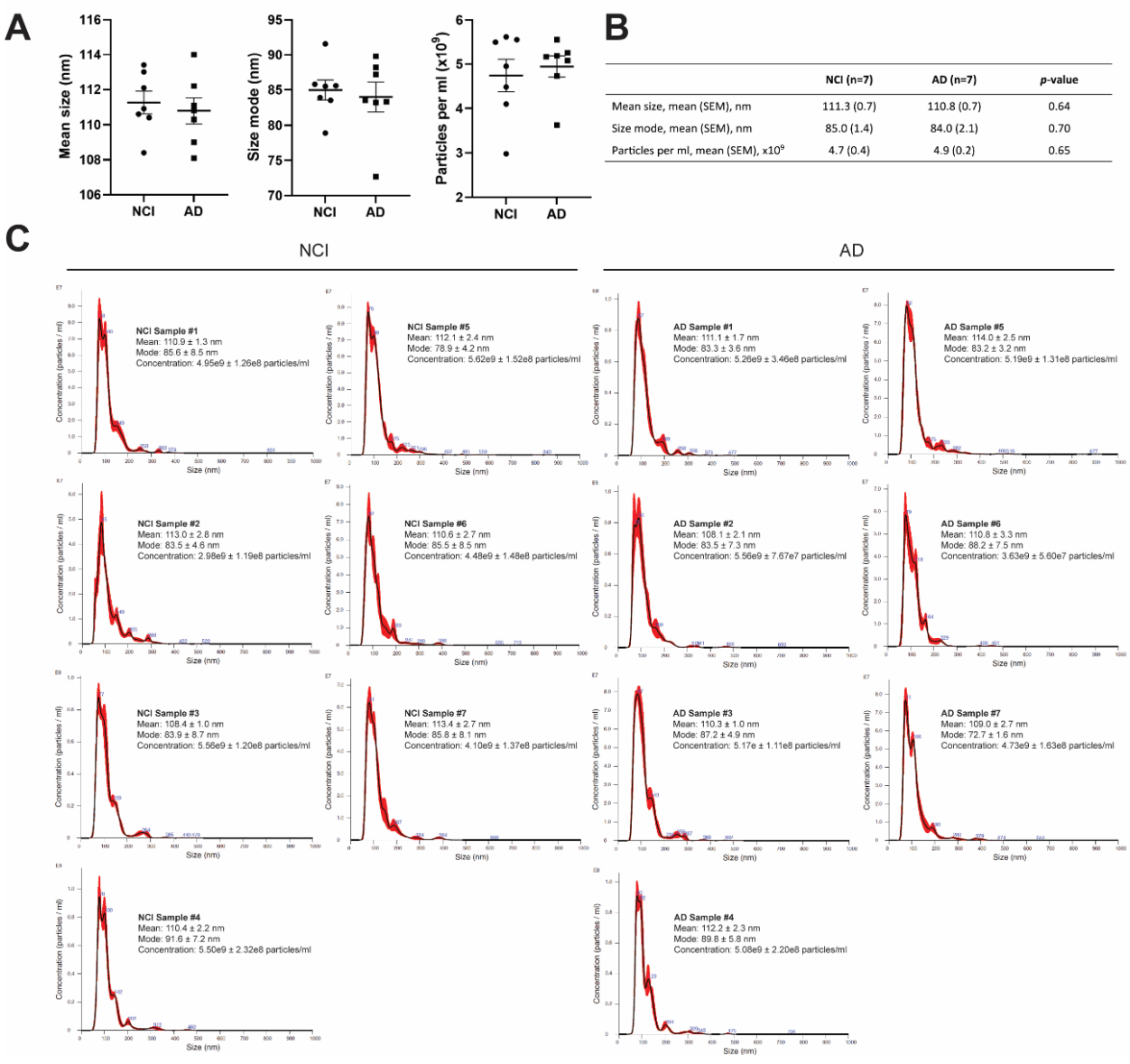
[10] Wechsler D (1981) *Wechsler Adult Intelligence Scale-Revised*, Harcourt Brace Jovanovich, San Antonio, TX.

[11] Sahadevan S, Tan NJ, Tan T, Tan S (1997) Cognitive testing of elderly Chinese people in Singapore: influence of education and age on normative scores. *Age Ageing* **26**, 481-486.

Supplementary Figure 1. Isolation of patient sample-derived EVs. (A) Schematic overview of EV isolation via size exclusion columns (qEV single, 70 nm, IZON) and centrifugation concentrators (>100 kDa). (B) Measurement of protein, RNA, and EV particle concertation in individual fractions. Fractions F4–F8 were combined for miRNA analysis containing most EVs, the bulk of the RNA molecules, and little protein contamination. Albumin protein contamination is visible as yellow-colored fractions, as indicated by yellow shading. (C) Western blots showing classical EV markers (Alix, Hsp70, CD81, CD9) as well as non-EV markers (albumin and GM130) in individual fractions. Molecular weights (in kDa) are indicated on the right of each blot (all unedited blots can be found in Supplementary Figure 4). We detected classical EV markers in all fractions analysed with little albumin contamination, indicating successful EV separation.

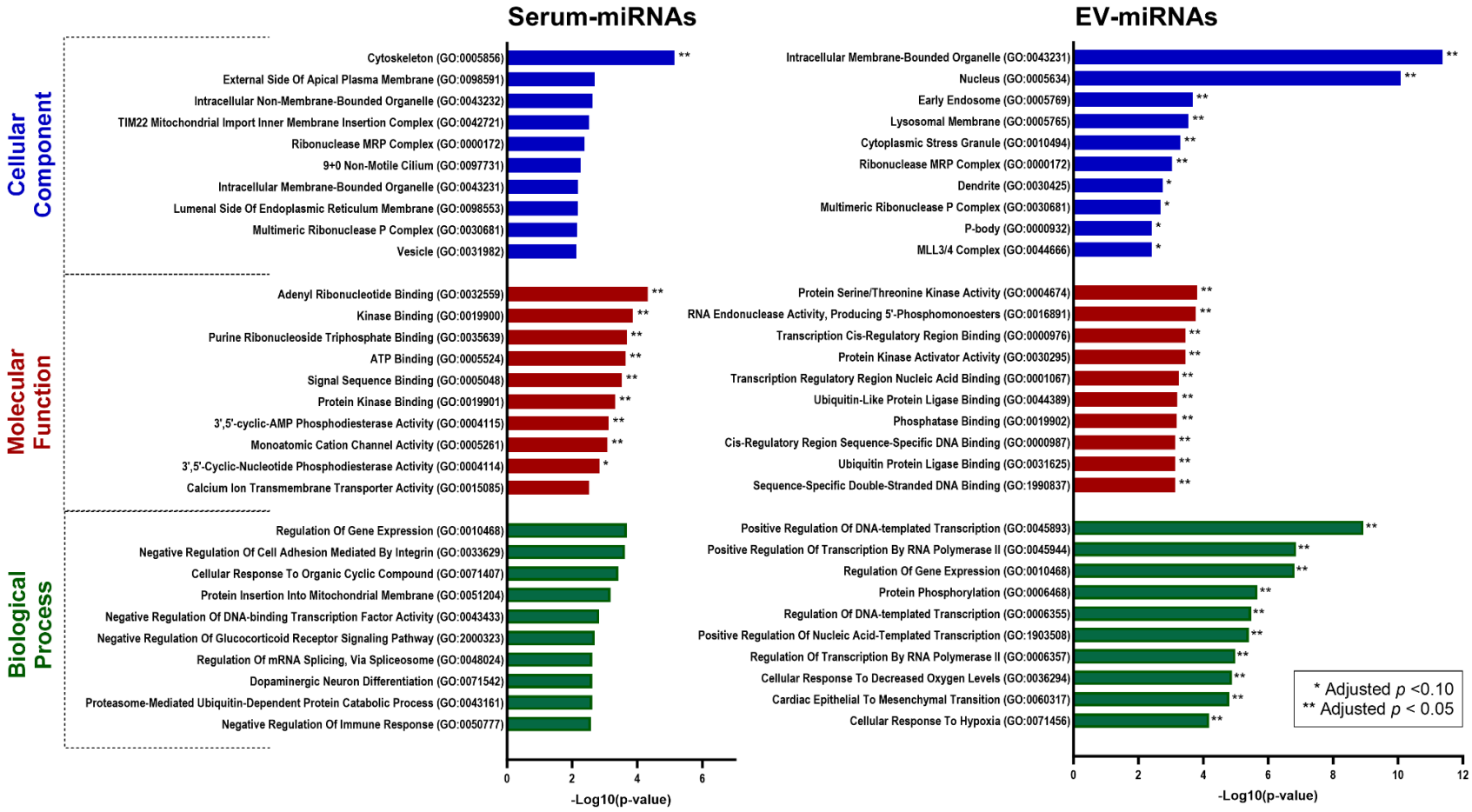

A
200 µl
250 µl / fraction
Patient sample
qEV single
(70 nm)
Concentrated
fractions
F4 - F8
B
RNA
[ng/µl]
Protein
[µg/ml]
EVs
[particle/ml]
Combined
Analyzed
Fractions
RNA
Protein
EVs
1000
100
10
1
0.1
25
20
15
10
5
0
1.6E09
1.4E09
1.2E09
1E09
8E08
6E08
4E08
2E08
0
F1 F2 F3 F4 F5 F6 F7 F8 F9 F10 F11 F12
12 Fractions
C
F1 F2 F3 F4 F5 F6 F7 F8 F9 F10 F11 F12
250
130
100
Alix
70
50
Hsp70
25
CD81
25
CD9
150
100
GM130
70
Albumin

Supplementary Figure 2. Nanoparticle tracking analysis of patient sample-derived EVs. Measurement of isolated EVs via nanoparticle tracing analysis (NTA, NanoSight), indicating the presence of exosomes and micro-vesicles. (A) Dot plots and (B) tabulated analyses based on the individual NTA (C, n=7 each) show that no significant differences were observed between the NCI and AD groups in terms of particle mean size (nm), size mode (nm), and particle per ml (10^9).



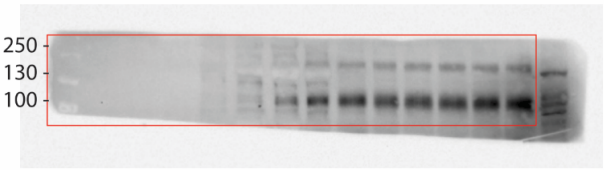
	NCI (n=7)	AD (n=7)	*p*-value
Mean size, mean (SEM), nm	111.3 (0.7)	110.8 (0.7)	0.64
Size mode, mean (SEM), nm	85.0 (1.4)	84.0 (2.1)	0.70
Particles per ml, mean (SEM), x10^9	4.7 (0.4)	4.9 (0.2)	0.65

Supplementary Figure 3. Gene Ontology (GO) enrichment analysis of serum- and EV-miRNAs. GO enrichment analyses were performed on the validated targets of serum- (hsa-miR-431-3p, hsa-miR-1290, and hsa-miR-181a-2-3p) and EV-miRNAs (hsa-miR-1290 and hsa-miR-128-3p) in the combination panels separately. The top ten GO terms with $p < 0.05$ in each aspect, namely "cellular component", "molecular function", and "biological process", were displayed with their significance expressed in -Log10(p-value). p-value adjustment was further performed, as expressed in False Discovery Rate (FDR) at * 10% and ** 5%.

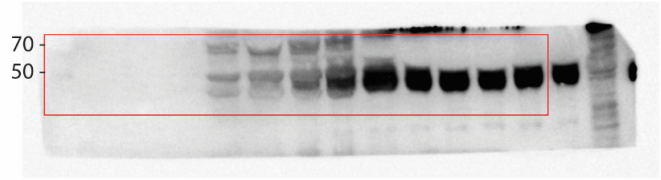


Supplementary Figure 4. Full unedited blots for Supplementary Figure 1. Representative blots on the relevant lanes were cropped (boxed in red) and presented in Supplementary Figure 1. Molecular weights (in kDa) are indicated on the right of each blot.

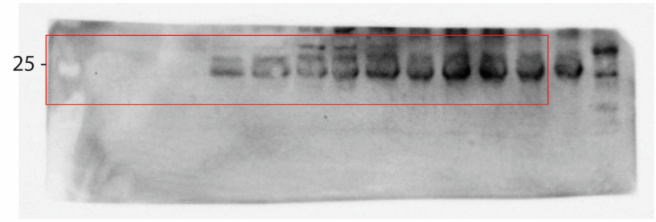
Full unedited blot for Alix



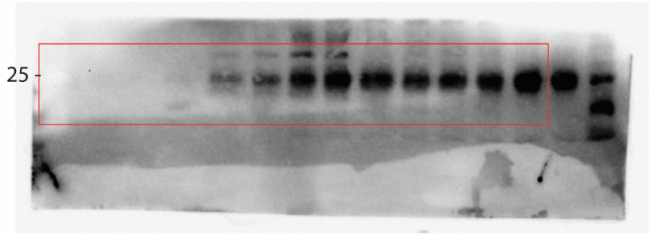
Full unedited blot for Hsp70



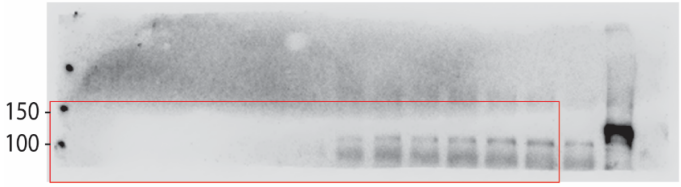
Full unedited blot for CD81



Full unedited blot for CD9



Full unedited blot for GM130



Full unedited blot for Albumin