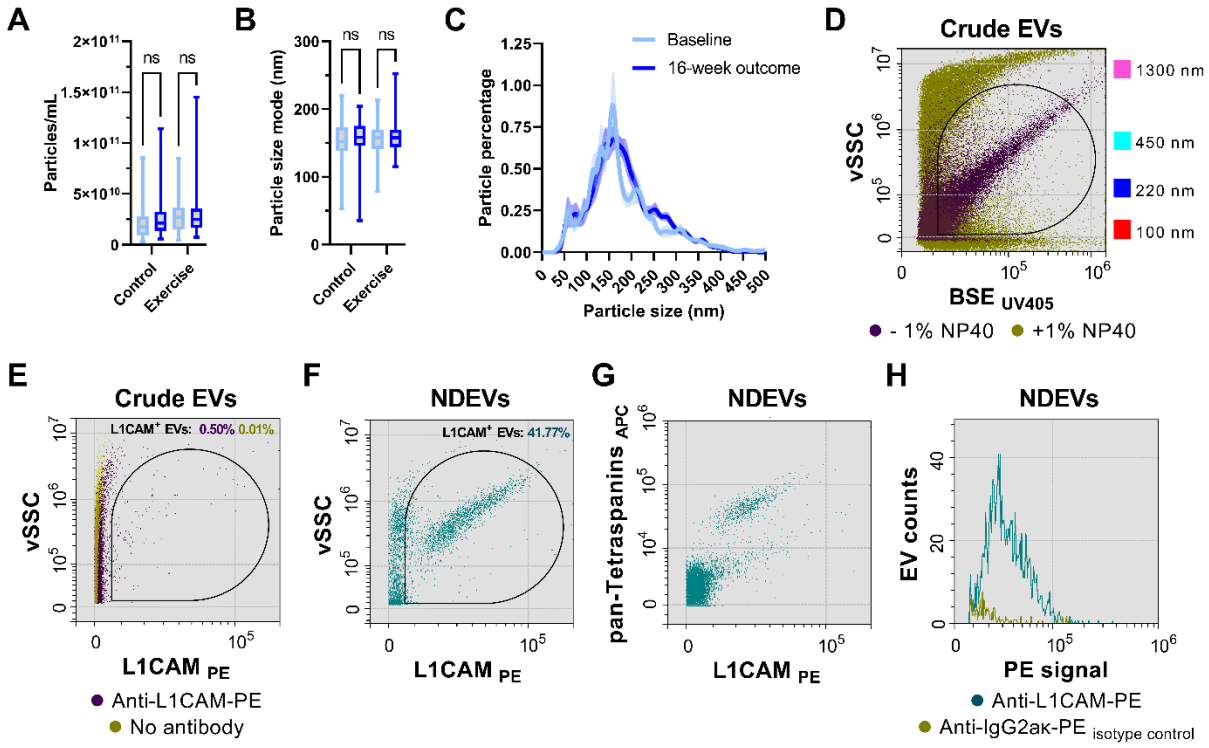
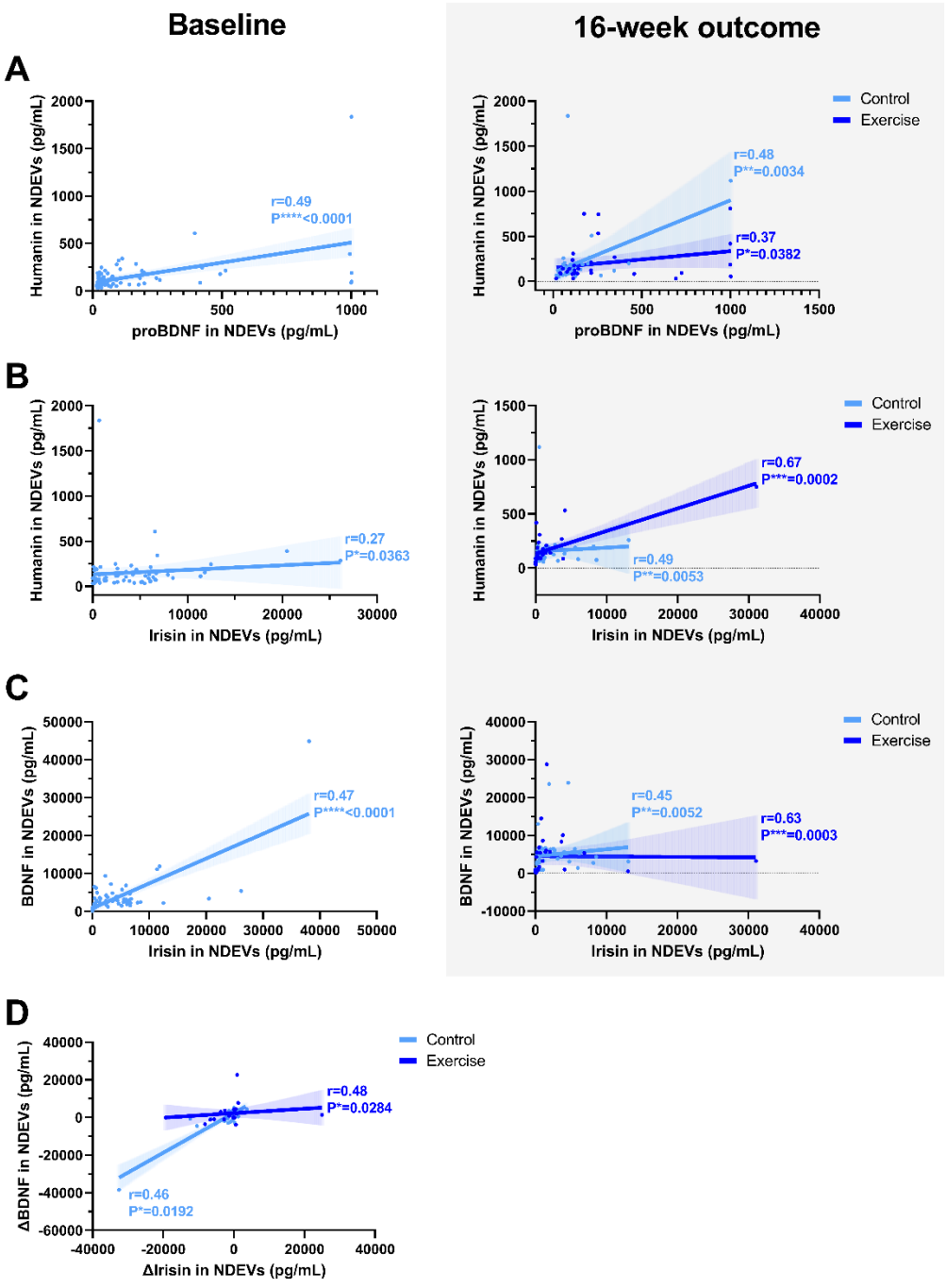
Supplementary Figures and Tables: *Delgado-Peraza F, Nogueras-Ortiz C, Simonsen AH, Knight DD, Yao PJ, Goetzl EJ, Jensen CS, Høgh P, Gottrup H, Vestergaard K, Hasselbalch SG, Kapogiannis D. Neuron-derived extracellular vesicles in blood reveal effects of exercise in Alzheimer's disease. Alzheimers Res Ther. 2023 Sep 20;15(1):156. doi: 10.1186/s13195-023-01303-9. PMID: 37730689; PMCID: PMC10510190.*



Supplementary Figure 1. Characterization of NDEVs. A and B) Box plots show the concentration (A) in particles/mL and size mode (B) in nanometers (nm) of NDEVs from the plasma of subjects in the control and exercise groups at baseline (light blue) and at the 16-week outcome (dark blue) determined using nanoparticle tracking analysis (NTA). Control group: baseline, N=37; 16-week outcome, N=37. Exercise group: baseline, N=42; 16-week outcome, N=34. Statistical analyses: mixed-effects linear model with Fisher's LSD test. **C)** Line graphs of the particle percentage in function of particle size (nm) determined using NTA show representative size profiles of NDEVs from subjects in the exercise group at baseline (light blue; N=10) and at the 16-week outcome (dark blue; N=10) visits. **D to H)** High-sensitivity nanoscale multiplex flow cytometry analysis (FCA) of pooled crude plasma EVs from multiple subjects sedimented using ExoQuick® before and after immunoprecipitation of NDEVs. Dot plots show the violet size scatter (vSSC) in function of the fluorescent signal of samples co-labeled with the fluorescent EV marker blue succinimidyl ester (BSE) (violet events in **D**) and PE-tagged anti-

L1CAM antibody (**E** and **F**). In **D**, a gate designated based on the background signal of negative controls encloses events positive for BSE in crude EVs, with similar results obtained for NDEVs (data not shown). The vSSC vs. BSE signal sensitivity to treatment with NP40 detergent (yellow events in **D**) confirms the membranous composition of detected events. A color-coded size range based on the vSSC of FITC-tagged beads is included on the right for the size comparison of events. Plots **E** and **F** compare the percentage of L1CAM-positive events in crude EVs and NDEVs, respectively, determined by the abundance of BSE-gated events within L1CAM-PE gates established based on the background signal of a negative control not incubated with anti-L1CAM antibody (yellow events in **E**). In **G**, a dot plot shows BSE-gated events of NDEVs co-stained with antibodies against L1CAM (PE-tagged, x-axis) and pan-Tetraspanins CD9, CD63 and CD81 (APC-tagged, y-axis). A histogram in **H** shows the abundance of BSE-gated PE-positive events in NDEVs labelled with PE-tagged anti-L1CAM antibody (turquoise) or its isotype control (yellow). The frequency of events in analyzed samples did not result in coinciding events as confirmed by swarming experiments (data not shown).



Supplementary Figure 2. NDEV protein level correlations are maintained after exercise. A to C) Scatter plots show the association between NDEV levels of selected protein pairs with significant correlations both at baseline (left) and at the 16-week outcome (right): (**A**) Humanin in function of proBDNF; (**B**) Humanin in function of Irisin; (**C**) BDNF in function of Irisin. In **D**, a scatter plot shows the relationship between BDNF and Irisin protein level changes from baseline

to the 16-week outcome for the control (light blue) and exercise groups (dark blue). Plots show the ‘r’ and P values of nonparametric Spearman correlations.

Supplementary table 1. NDEV protein levels in the exercise group by APOE4 status

Analyte	*APOE4 status*	*Visit*	*Number of samples*	*Mean*[†] *(pg/mL)*	*95% Confidence Interval*	
					Upper Bound	*Lower Bound*
proBDNF	Non-carriers	Baseline	14	131.93	280.95	-17.09
		16-week outcome	14	185.23	293.48	76.98
	Carriers	Baseline	29	165.89	268.11	63.67
		16-week outcome	23	355.45	516.04	194.86
BDNF	Non-carriers	Baseline	15	3018.18	4036.75	1999.60
		16-week outcome	14	5265.43	9245.15	1285.71
	Carriers	Baseline	28	2842.15	3751.02	1933.27
		16-week outcome	22	4645.19	7603.65	1686.74
Humanin	Non-carriers	Baseline	14	126.98	177.52	76.43
		16-week outcome	11	115.00	159.55	70.45
	Carriers	Baseline	29	120.77	162.54	79.01
		16-week outcome	20	254.25	374.44	134.07

[†] Estimated marginal means adjusted by age, sex, EV concentration and APOE status

Supplementary table 2. Protein levels of putative exerkines in NDEV lysates

Analyte	*Neuronal RNA expression (TPM)*[†]	*Randomization*	*Visit*	*Number of samples*	*Mean*[††] *(pg/mL or **ng/mL**)*	*95% Confidence Interval*		*Randomization*Visit Fixed Effects Test*			
						Lower Bound	*Upper Bound*	*Numerator df*	*Denominator df*	*F*	*Significance*
Fractalkine	24.3 ± 3.8	Control	Baseline	35	127.6	81.98	173.23	1	63.9	0.655	0.42
			16-week outcome	40	87.55	43.69	131.41				
		Exercise	Baseline	42	65.07	21.81	108.32				
			16-week outcome	33	50.75	3.38	98.12				
Fatty acid binding protein 3 (FABP3)	15.4 ± 0.8	Control	Baseline	33	400.88	218.47	583.3	1	77.95	0.281	0.6
			16-week outcome	41	362.3	194.55	530.04				
		Exercise	Baseline	40	340.74	177.04	504.44				
			16-week outcome	34	397.93	214.95	580.9				
FNDC5/Irisin	13.1 ± 2.8	Control	Baseline	29	4831.18	2644.17	7018.17	1	61.8	0.294	0.59
			16-week outcome	37	2373.75	745.57	4601.92				
		Exercise	Baseline	35	4279.87	2294.31	6265.43				
			16-week outcome	30	3100.52	901.01	5300.03				
Follistatin-like 1 protein (FSTL-1)	12.9 ± 3.3	Control	Baseline	32	202763.42	96341.15	309185.68	1	40.26	1.08	0.31
			16-week outcome	36	133786.86	30145.4	237428.32				
		Exercise	Baseline	33	110765.42	2565.36	218965.48				
			16-week outcome	32	102640.18	-7811.25	213091.62				
Osteonectin	3.6 ± 0.7	Control	Baseline	37	**8.79**	-49.87	67.44	1	94.58	1.57	0.21
			16-week outcome	39	**4.82**	-54.5	64.14				
		Exercise	Baseline	43	**-2.28**	-57.75	53.2				
			16-week outcome	34	**67.83**	4.69	130.97				
Osteocrin	3.5 ± 2.5	Control	Baseline	27	232.01	139.18	324.84	1	33.41	0.11	0.74
			16-week outcome	34	168.44	81.31	255.57				
		Exercise	Baseline	28	112.4	23.14	201.66				
			16-week outcome	30	64.79	-25.73	155.32				
Interleukin-15	3.4 ± 1.4	Control	Baseline	37	1.93	0.03	3.84	1	68.61	0.1	0.76
			16-week outcome	43	1.26	-0.59	3.1				
		Exercise	Baseline	44	1.6	-0.16	3.36				
			16-week outcome	36	0.31	-1.68	2.29				
Myostatin	1.3 ± 0.1	Control	Baseline	37	939.52	184.36	1694.67	1	94.78	1.27	0.26
			16-week outcome	42	111.91	-621.56	845.38				

		Exercise	Baseline	43	-36.37	-736.42	663.69				
			16-week outcome	36	-25.38	-813.27	762.52				
Apelin	0.3 ± 0.2	Control	Baseline	31	7463.84	1505.38	13422.3	1	36.99	0.05	0.83
			16-week outcome	38	4865.52	-650.66	10381.69				
		Exercise	Baseline	35	5766.9	132.88	11400.91				
			16-week outcome	30	2183.91	-4001.79	8369.6				
Oncostatin M	0.1 ± 0.0	Control	Baseline	32	44.22	13.29	75.14	1	89.53	0.29	0.59
			16-week outcome	39	15.97	-12.35	44.28				
		Exercise	Baseline	37	22.07	-6.79	50.93				
			16-week outcome	32	9.55	-22.17	41.27				
Erythropoeitin	0.1 ± 0.0	Control	Baseline	22	862.12	482.82	1241.42	1	43.39	0.21	0.65
			16-week outcome	28	639.96	282.48	997.43				
		Exercise	Baseline	23	881.76	493.26	1270.27				
			16-week outcome	16	826.57	374.42	1278.72				

† Nucleotide transcripts per million (TPM) data extracted from The Human Protein Atlas

†† Estimated marginal means adjusted by age, sex, EV concentration and APOE status

Supplementary table 3. Correlations between analyte levels in NDEVs at baseline

	Analyte											
	proBDNF				*BDNF*				*Humanin*			
Analyte	*Spearman r*	*95% C.I.†*	*P value*	*Sample size*	*Spearman r*	*95% C.I.†*	*P value*	*Sample size*	*Spearman r*	*95% C.I.†*	*P value*	*Sample size*
BDNF	0.17	-0.07 to 0.38	0.14876	78	-	-	-	-	-	-	-	-
Humanin	0.49	0.29 to 0.64	**0.00001**	77	0.17	-0.07 to 0.39	0.14665	77	-	-	-	-
Irisin	0.16	-0.11 to 0.40	0.22948	61	0.47	0.26 to 0.66	**0.00009**	63	0.27	0.02 to 0.50	**0.03628**	61

† Confidence interval

- redundant

Correlations with P values in bold are statistically significant

Supplementary table 4. Correlations between analyte levels in NDEVs at 16-week outcome

Control group

	Analyte											
	proBDNF				*BDNF*				*Humanin*			
Analyte	*Spearman r*	*95% C.I.†*	*P value*	*Sample size*	*Spearman r*	*95% C.I.†*	*P value*	*Sample size*	*Spearman r*	*95% C.I.†*	*P value*	*Sample size*
BDNF	0.21	-0.10 to 0.49	0.16701	43	-	-	-	-	-	-	-	-
Humanin	0.48	0.16 to 0.70	**0.00336**	36	0.29	-0.06 to 0.57	0.08379	36	-	-	-	-
Irisin	0.21	-0.13 to 0.51	0.21048	37	0.45	0.14 to 0.68	**0.00518**	37	0.49	0.15 to 0.72	**0.00533**	31

Exercise group

	Analyte											
	proBDNF				*BDNF*				*Humanin*			
Analyte	*Spearman r*	*95% C.I.†*	*P value*	*Sample size*	*Spearman r*	*95% C.I.†*	*P value*	*Sample size*	*Spearman r*	*95% C.I.†*	*P value*	*Sample size*
BDNF	-0.11	-0.43 to 0.24	0.53556	36	-	-	-	-	-	-	-	-
Humanin	0.37	0.01 to 0.65	**0.03823**	31	0.32	-0.06 to 0.61	0.08368	31	-	-	-	-
Irisin	0.00	-0.38 to 0.38	0.98580	29	0.63	0.33 to 0.81	**0.00025**	29	0.67	0.38 to 0.85	**0.00017**	26

† Confidence interval

- redundant

Correlations with P values in bold are statistically significant

Supplementary table 5. Correlations between analyte level changes in NDEVs from baseline to 16-week outcome

Control group

	Analyte											
	proBDNF				*BDNF*				*Humanin*			
Analyte	*Spearman r*	*95% C.I.†*	*P value*	*Sample size*	*Spearman r*	*95% C.I.†*	*P value*	*Sample size*	*Spearman r*	*95% C.I.†*	*P value*	*Sample size*
BDNF	0.35	-0.01 to 0.63	**0.04750**	32	-	-	-	-	-	-	-	-
Humanin	-0.01	-0.41 to 0.39	0.94582	26	0.31	-0.10 to 0.63	0.11886	26	-	-	-	-
Irisin	0.10	-0.34 to 0.49	0.65661	24	0.46	0.07 to 0.73	**0.01929**	25	0.25	-0.22 to 0.62	0.27312	21

Exercise group

	Analyte											
	proBDNF				*BDNF*				*Humanin*			
Analyte	*Spearman r*	*95% C.I.†*	*P value*	*Sample size*	*Spearman r*	*95% C.I.†*	*P value*	*Sample size*	*Spearman r*	*95% C.I.†*	*P value*	*Sample size*
BDNF	0.06	-0.32 to 0.42	0.75509	30	-	-	-	-	-	-	-	-
Humanin	0.30	-0.09 to 0.61	0.11723	28	0.03	-0.37 to 0.41	0.88702	27	-	-	-	-
Irisin	0.01	-0.44 to 0.47	0.95232	20	0.48	0.04 to 0.76	**0.02843**	21	0.26	-0.27 to 0.67	0.31270	17

† Confidence interval

- redundant

Correlations with P values in bold are statistically significant