

Supplementary Material

Brain Tissue-Derived Extracellular Vesicles in Alzheimer's Disease Display Altered Key Protein Levels Including Cell Type-Specific Markers

Supplementary Table 1. The human cortex tissues used in the study

Sample ID	Group	APOE genotype	Age	Sex (M ^a /F ^b)	RACE (W ^c /B ^d)	Braak	CERAD	PMI ^e (h)	Clinical pathological information	Cause of death	Sample inclusion (Y ^f /N ^g)	
											1st separation	2nd separation
AD1	AD	E2/3	68	M	W	6	C	4	AD	/	Y	Y
AD2	AD	E2/3	63	F	W	6	C	11	AD	AD	Y	Y
AD3	AD	E2/3	65	M	W	6	C	11	AD, Seizure disorder	AD	Y	Y
AD4	AD	E2/3	86	M	W	5	C	5	AD, hypercholesterolemia, hypothyroidism, urinary incontinence, peripheral vascular disease	AD	Y	Y
AD5	AD	E2/3	68	M	W	6	C	4	AD	/	Y	N
AD6	AD	E3/3	87	M	W	6	C	7	AD, congestive heart failure, bladder cancer	/	Y	Y
AD7	AD	E3/3	103	F	W	6	B	14	AD	/	Y	Y
AD8	AD	E3/3	88	F	W	6	C	8	AD, coronary artery disease, hypertension, hyperlipidemia	/	Y	Y
AD9	AD	E3/3	62	M	W	6	C	18	AD, hypertension, hyperlipidemia, myocardial infarction, cerebral amyloid angiopathy	/	Y	Y
AD10	AD	E3/3	85	F	W	6	C	17	AD, hypertension, congestive heart failure, coronary artery disease, peripheral neuropathy, scoliosis	Sepsis, cardiopulmonary arrest	Y	Y
AD11	AD	E3/4	56	F	W	6	C	19	AD	/	Y	N
AD12	AD	E3/4	90	F	W	6	C	21	AD, atherosclerosis in basilar artery	/	Y	Y
AD13	AD	E3/4	88	F	W	6	C	5	AD, atherosclerosis, old cystic infarct of the left occipital and parietal	/	Y	Y

									(posterior cerebral artery distribution), meningioma				
AD14	AD	E3/4	89	M	W	6	C	9.5	AD, hypertension, coronary artery disease, atrial fibrillation, prostate cancer	/	Y	Y	
AD15	AD	E3/4	68	F	W	6	C	5.5	AD, hypertension	AD	Y	Y	
AD16	AD	E3/4	85	M	W	6	C	18	AD, post lung pneumonectomy	AD	Y	Y	
AD17	AD	E4/4	61	M	W	4	C	20	AD, metastatic small cell carcinoma	Metastatic small cell carcinoma	Y	Y	
AD18	AD	E4/4	91	F	W	4	C	6	AD	Acute cardiac ischemic event	Y	Y	
AD19	AD	E4/4	79	F	W	4	C	6	AD	/	Y	Y	
AD20	AD	E4/4	90	F	W	6	C	6	AD, myocardial infarct, congestive heart failure, depression	/	Y	Y	
AD21	AD	E4/4	62	F	W	4	C	12	AD, seizure disorder, dementia	AD	Y	Y	
AD22	AD	E4/4	72	F	W	6	C	13	AD	Sepsis induced cardiopulmonary arrest	Y	Y	
AD23	AD	E3/4	74	M	W	5	C	9	AD, coronary artery disease, myocardial infarction, hyperlipidemia, hypertension, Type 2 diabetes mellitus, obstructive sleep apnea, atrial fibrillation, carotid artery stenosis	/	Y	N	
AD24	AD	E3/4	72	F	W	6	C	8	AD, hypertension, chronic obstructive pulmonary disease, depression, amyloid angiopathy	/	N	Y	
CTRL1	Control	E3/3	88	M	W	/	/	10	/	/	Y	Y	
CTRL2	Control	E3/3	85	M	B	/	/	6	Chronic kidney disease, congestive heart failure, diabetes mellitus, lung small cell carcinoma, right occipital infarct	Acute cardiac event	Y	Y	
CTRL3	Control	E3/3	58	F	W	/	/	6	Coronary artery disease, myocardial infarction, congestive heart failure	Acute cardiac event	Y	Y	

CTRL4	Control	E3/3	74	M	W	2	/	4	Coronary artery disease	Rupture of the right ventricular outflow	Y	Y
CTRL5	Control	E3/3	91	F	W	1		8	/	/	Y	Y
CTRL6	Control	E4/4	87	F	W	2	0	7	Osteoporosis, temporal lobe epilepsy, ophthalmic migraines	/	Y	Y
CTRL7	Control	E2/2	94	M	W	2	0	15	Subclinical Lewy body disease	Pancreatic cancer	Y	Y
CTRL8	Control	E3/3	76	M	W	/	/	3	Myocardial infarction, recurrent ventricular tachycardia, coronary artery disease, congestive Heart Failure	/	N	Y
CTRL9	Control	E3/3	68	M	W	/	/	14	Lung squamous and small cell carcinomas, Bronchopneumonia, acute bronchitis, pleural effusion, moderate emphysema	/	N	Y
CTRL10	Control	E3/3	62	M	W	/	/	19	Hypertension, aortic stenosis with valve replacement, bacterial endocarditis with subsequent aortic dissection, hemothorax, lung collapse	/	N	Y

^a M, male; ^b F, female; ^c W, White; ^d B, Black or African American; ^e postmortem interval; ^f Y, included; ^g N, not included; ^h1st batch of brain tissue used for proteomics study; ⁱ2nd batch of brain tissue used for proteomics data verification and EV surface marker profile

Supplementary Table 2. Protein number identified in samples

Sample ID	Group	BH	10K	EVs	ID	Group	BH	10K	EVs
CTRL1	Control	115	410	397	AD1	AD	1026	291	388
CTRL2	Control	1199	268	443	AD2	AD	621	372	476
CTRL3	Control	505	333	408	AD3	AD	976	311	353
CTRL4	Control	576	125	326	AD4	AD	504	388	342
CTRL5	Control	482	231	405	AD5	AD	459	230	399
CTRL6	Control	1239	296	730	AD6	AD	1121	420	426
CTRL7	Control	406	160	396	AD7	AD	1055	208	492
					AD8	AD	490	439	408
					AD9	AD	712	193	261
					AD10	AD	432	307	635
					AD11	AD	922	140	209
					AD12	AD	466	339	229
					AD13	AD	423	312	470
					AD14	AD	657	498	609
					AD15	AD	972	379	418
					AD16	AD	583	291	588
					AD17	AD	703	391	377
					AD18	AD	874	204	176
					AD19	AD	521	355	789
					AD20	AD	484	390	382
					AD21	AD	454	139	354
					AD22	AD	511	230	607
					AD23	AD	939	154	571

Supplementary Table 3. The up- and downregulated proteins in AD compared with control in 10K and bdEVs

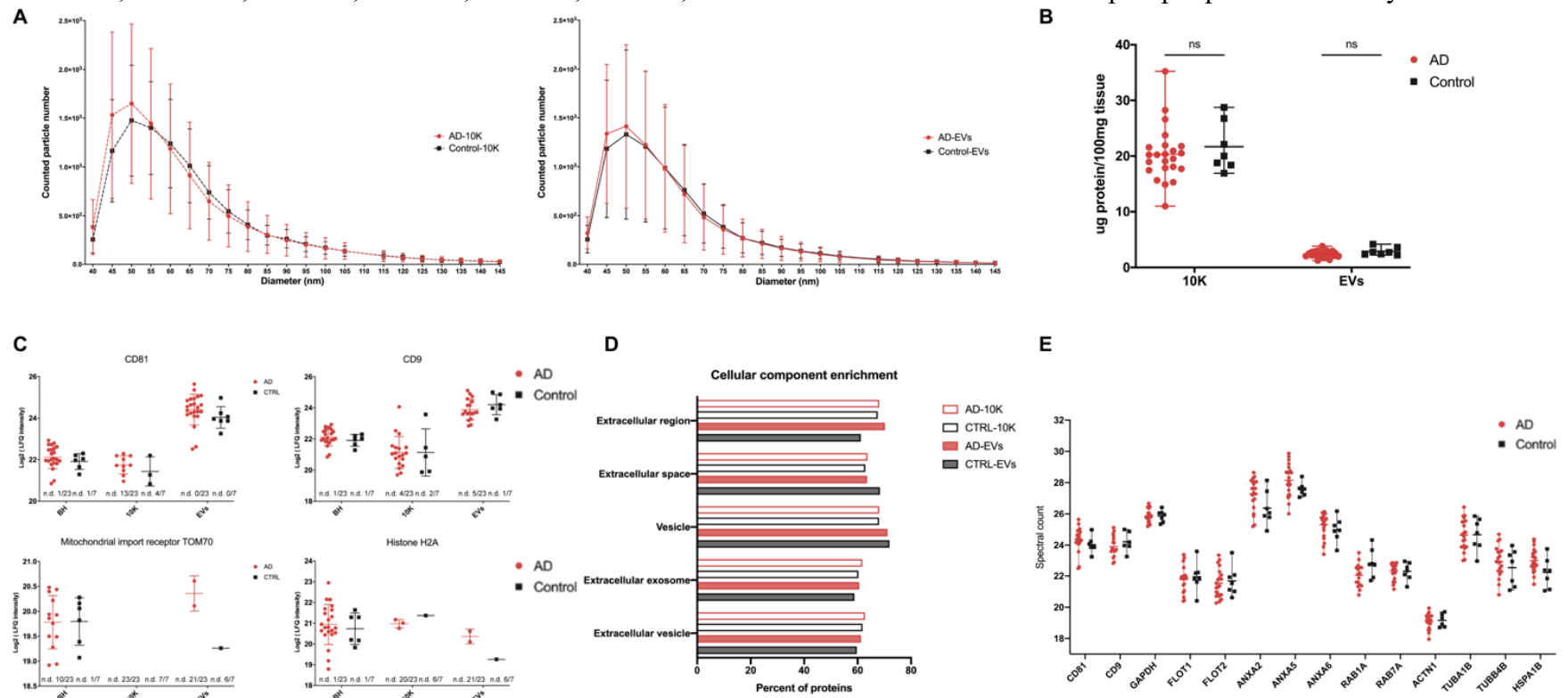
Fractions	Protein ID	Gene name	Protein name	log2Fold change	Average log2 (LFQ intensity)	p	Adjust p
	P00738	HP	Haptoglobin	2.47	22.39	0.00	0.10
	P29401	TKT	Transketolase	0.89	23.43	0.00	0.06
	P11142	HSPA8	Heat shock cognate 71 kDa protein	-0.30	25.15	0.04	0.61
	Q16555	DPYSL2	Dihydropyrimidinase-related protein 2	-0.34	26.98	0.05	0.61
10K	P23528	CFL1	Cofilin-1	-0.43	24.84	0.01	0.33
	P62873	GNB1	Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-1	-0.71	25.68	0.03	0.61
	P63261	ACTG1	Actin	-0.87	27.77	0.00	0.08
	P63104	YWHAZ	14-3-3 protein zeta/delta	-1.04	24.44	0.00	0.16
	P61981	YWHAG	14-3-3 protein gamma	-1.28	24.06	0.01	0.33
	P01857	IGHG1	Ig gamma-1 chain C region	2.06	24.45	0.01	0.08
	P01834	IGKC	Ig kappa chain C region	2.05	23.64	0.01	0.09
	P10636	MAPT	Microtubule-associated protein tau	1.52	23.19	0.00	0.05
	P30041	PRDX6	Peroxiredoxin-6	1.37	21.97	0.00	0.05
	P10768	ESD	S-formylglutathione hydrolase	1.16	22.41	0.00	0.05
	P62258	YWHAE	14-3-3 protein epsilon	1.09	23.16	0.00	0.04
	P07108	DBI	Acyl-CoA-binding protein	1.03	24.73	0.03	0.18
	P09211	GSTP1	Glutathione S-transferase P	1.00	23.10	0.01	0.09
bdEVs	P06454	PTMA	Prothymosin alpha; Prothymosin alpha	0.99	22.08	0.04	0.19
	Q13228	SELENBP1	Selenium-binding protein 1	0.97	21.98	0.00	0.05
	P55072	VCP	Transitional endoplasmic reticulum ATPase	0.95	23.56	0.00	0.04
	O14745	SLC9A3R1	Na(+)/H(+) exchange regulatory cofactor NHE-RF1	0.91	22.30	0.02	0.15
	Q06830	PRDX1	Peroxiredoxin-1	0.89	24.12	0.00	0.06
	P49189	ALDH9A1	4-trimethylaminobutyraldehyde dehydrogenase	0.80	21.97	0.04	0.19
	P0DMV9	HSPA1B; HSPA1A	Heat shock 70 kDa protein 1B	0.78	23.29	0.01	0.12
	P29401	TKT	Transketolase	0.74	23.43	0.00	0.05
	P50395	GDI2	Rab GDP dissociation inhibitor beta	0.72	21.68	0.02	0.16
	P32119	PRDX2	Peroxiredoxin-2	0.71	23.32	0.03	0.17

P52565	ARHGDI1	Rho GDP-dissociation inhibitor 1	0.69	23.13	0.04	0.19
O94760	DDAH1	N(G),N(G)-dimethylarginine dimethylaminohydrolase 1	0.47	24.92	0.02	0.15
P06733	ENO1	Alpha-enolase	0.35	26.65	0.03	0.18
P04075	ALDOA	Fructose-bisphosphate aldolase A	-0.33	25.75	0.04	0.21
P62873	GNB1	Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-1	-0.81	25.68	0.02	0.13
O94856	NFASC	Neurofascin	-0.90	21.77	0.04	0.19
O14594	NCAN	Neurocan core protein	-0.98	22.40	0.02	0.14
P63000	RAC1	Ras-related C3 botulinum toxin substrate 1	-1.03	22.54	0.00	0.04
P09471	GNAO1	Guanine nucleotide-binding protein G(o) subunit alpha	-1.27	25.66	0.00	0.05
Q12860	CNTN1	Contactin-1	-1.55	22.36	0.00	0.04

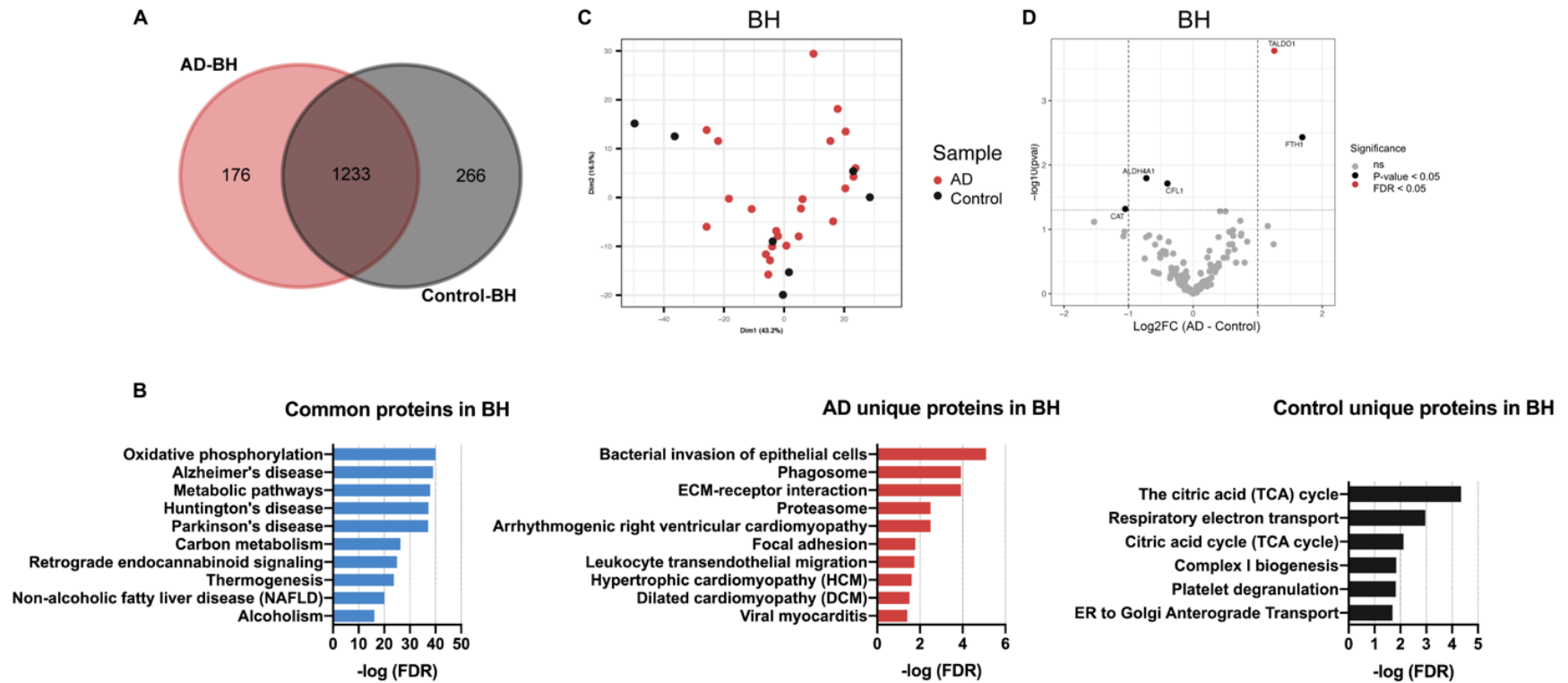
Supplementary Table 4. Cellular origin markers detected on bdEV

Spots	Panel 1	Protein name	Cell source	Panel 2	Protein name	Cell source	Panel 3	Protein name	Cell source	Panel 4	Protein name	Cell source
Spot 1	CD9	Cluster of Differentiation 9	EV markers	TSPO	Translocator protein	Astrocyte/ Microglia	TMEM119	Transmembrane Protein 119	Microglia	CD33 (Siglec-3)	Sialic acid binding Ig-like lectin 3	Microglia
Spot 2	CD81	Cluster of Differentiation 81	EV markers	CD36	Cluster of Differentiation 36 (platelet glycoprotein 4)	Microglia	GD1a	Ganglioside G1a	Neuron	CD18 (ITGB2)	Integrin beta chain-2	Microglia
Spot 3	CD63	Cluster of Differentiation 63	EV markers	CD38 (ADPRC1)	Cyclic ADP ribose hydrolase	Astrocyte/ Oligodendrocyte	CD31 (PECAM)	Platelet endothelial cell adhesion molecule-1	Endothelia	CD45 (PTPRC)	Protein tyrosine phosphatase receptor type C	Microglia
Spot 6	HLA-DR/DP/DQ	Human leukocyte antigens-DR/DP/DQ	Microglia/ Astrocyte	CD90 (Thy1)	THYmocyte differentiation antigen 1	Neuron	CD271 (LNGFR)	Low-affinity nerve growth factor receptor	Neuron	L1CAM	L1 cell adhesion molecule	Neuron
Spot 4	CD44 (HCAM)	Homing cell adhesion molecule	Astrocyte/ other glia	CD146 (MCAM)	Melanoma cell adhesion molecule	Endothelia	CD24	Cluster of Differentiation 24	Neuron	CD68	Cluster of Differentiation 68	Microglia
Spot 5	CD15 (sLeX)	Cluster of Differentiation 15/ Sialyl LewisX	Microglia	CD29 (ITGB1)	Integrin beta-1	Endothelia	CD40	Cluster of Differentiation 40	Endothelia	CD14	Cluster of Differentiation 14	Microglia
Spot 9	CD36	Cluster of Differentiation 36 (platelet glycoprotein 4)	Microglia	CD166 (hALCAM)	Activated leukocyte cell adhesion molecule	Neuron	CD163	Cluster of Differentiation 163	Microglia	CD11b (ITGAM)	Integrin alpha M	Microglia
Spot 7	GD2	Ganglioside G2	Neuron	CD64 (FcγRI)	Fc-gamma receptor 1	Microglia	GJA1	Gap junction alpha-1 protein	Astrocyte	IBA1	Ionized calcium-binding adapter molecule 1	Microglia
Spot 8	NCAM (CD56)	Neural cell adhesion molecule	Neuron	CD307d (FcRL4)	Fc-gamma receptor 4	Memory B cells	NRCAM	Neuronal Cell Adhesion Molecule	Neuron	GPNMB	Transmembrane glycoprotein NMB	Microglia
Spot 10	IgG1	/	Isotype control	IgG1	/	Isotype control	IgG1	/	Isotype control	IgG1	/	Isotype control

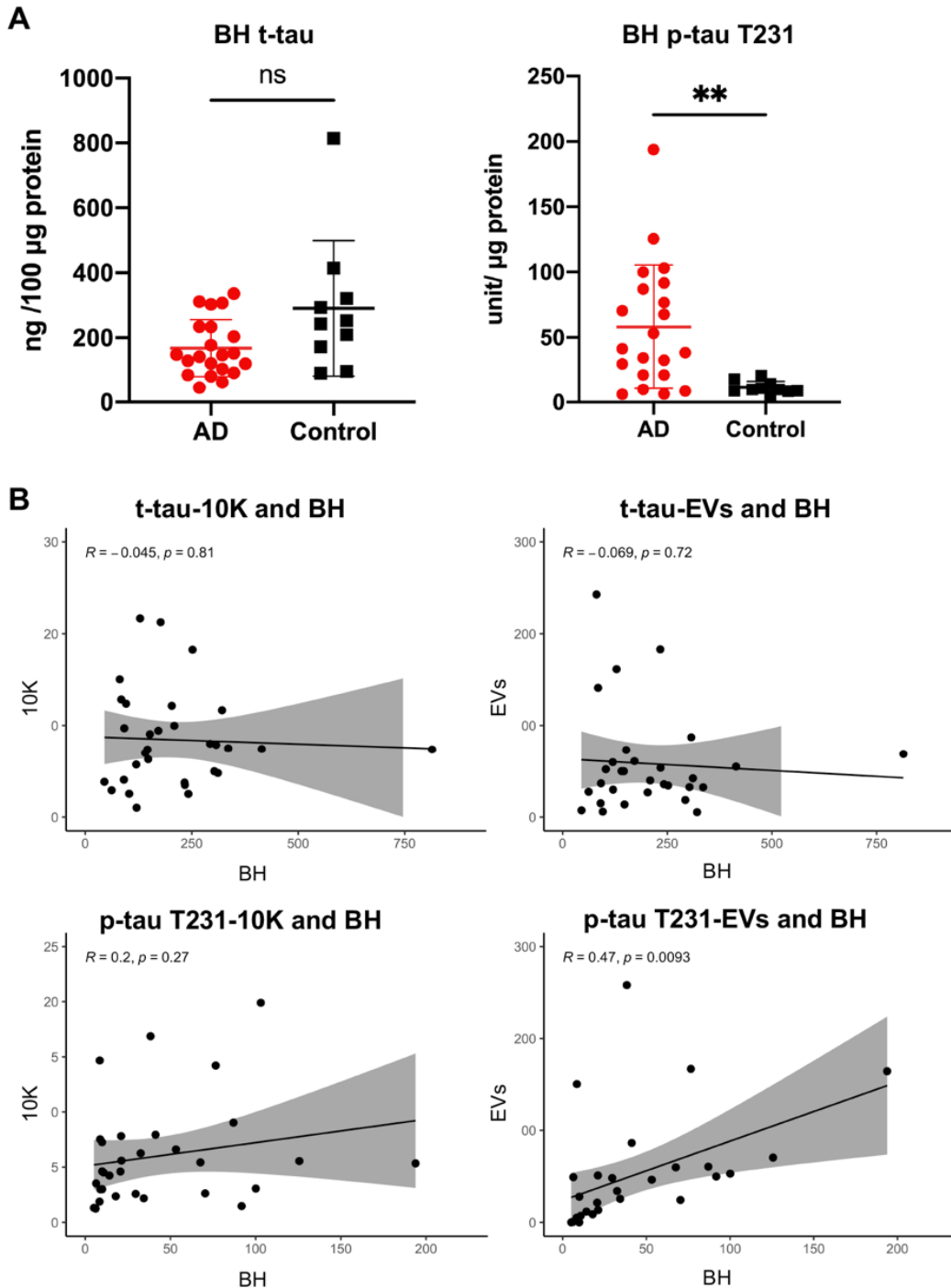
Supplementary Figure 1. Brain-derived 10K and EV characterization from AD and control samples. A) Size distributions of 10K and EV fractions (AD and control) were measured by NFCM and shown as particle number counted in a specific size bin. B) Protein concentration of 10K and EV fractions of AD and control samples were measured by BCA protein assay (normalized by per 100 mg tissue mass). C) Expression level of CD81, CD9, mitochondrial import receptor TOM70, and histone H2A per proteomics analysis in BH, 10K, and EVs from AD (n=23) and control (n=7) patients. Data are presented as mean log₂ (LFQ intensity) +/- SD. The number of patients without proteins detected were labelled in the figures as non-detected (n.d.). D) Cellular compartments of 10K and EV proteins identified in AD and control patients (enriched by STRING; the top 5 GO terms are ranked by FDR-corrected p-value and plotted as detected protein percent in 10K and EVs). E) The level of EV markers CD81, CD9, FLOT1, FLOT2, RAB1A, RAB7A, TUBA1B, TUBB4B, ANXA2, ANXA5, ANXA6, ACTN1, and GAPDH in AD and control samples per proteomics analysis.



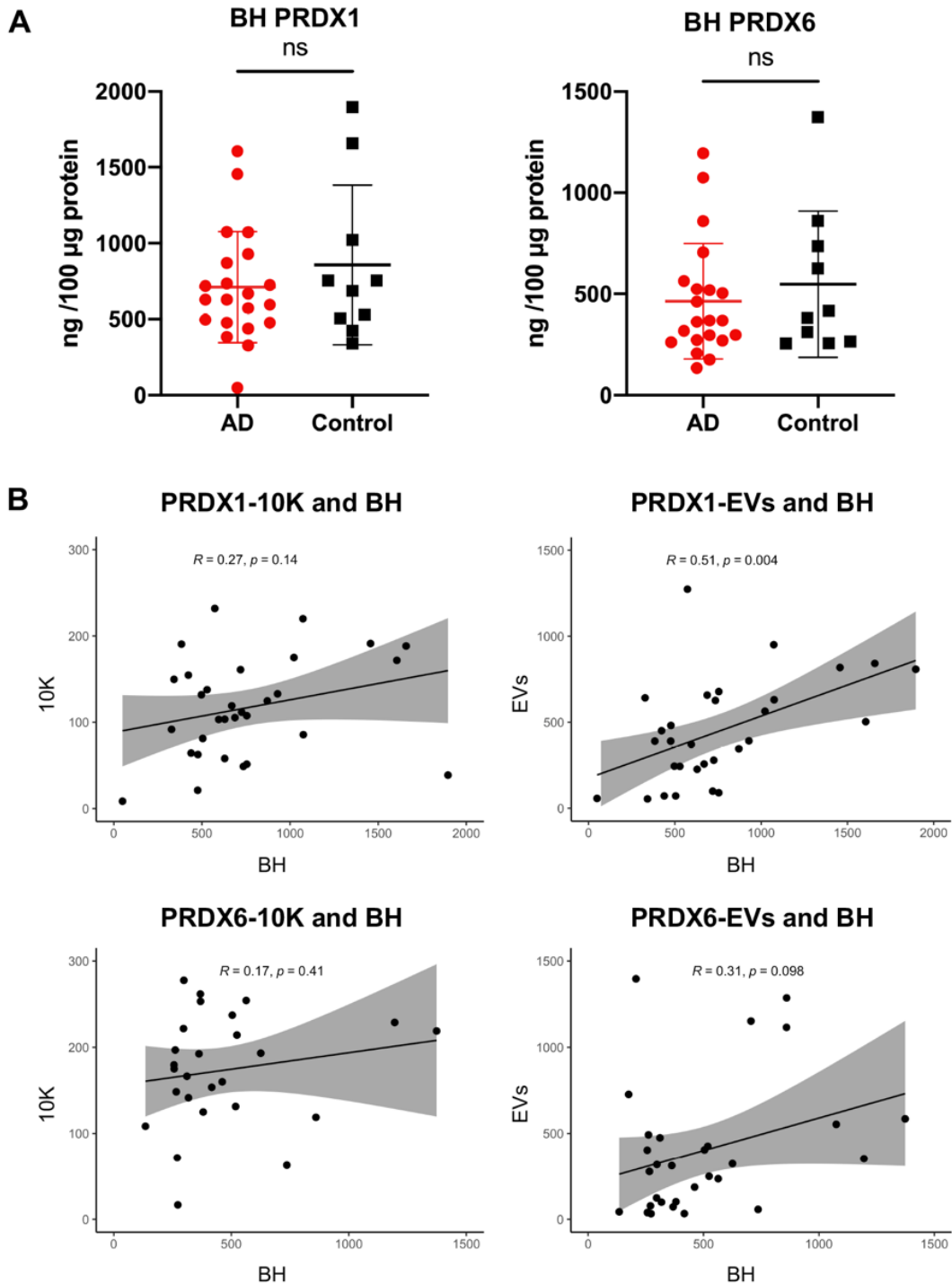
Supplementary Figure 2. Proteins related to AD pathology in brain tissues (BH). A) Venn diagram of proteins identified in BH of AD and control. B) The top 10 pathways ranked by FDR-corrected p value of BH proteins according to the Kyoto Encyclopedia of Genes and Genomes (KEGG). C) Principal component analysis (PCA) based on proteome content of BH. D) Volcano plots showing BH protein log₂ fold changes (Log₂FC) and p values (pval) for AD versus control. Thresholds for two-fold change and p value < 0.05 are indicated by dashed lines. Significant changes are indicated with different colors. Grey: non-significant (ns), black: p-value < 0.05, and red: FDR < 0.05.



Supplementary Figure 3. Total tau and phosphorylated tau at threonine 231 levels in brain tissues (BH) from AD patients and controls. A) The levels of total tau (t-tau) (left) and phosphorylated tau at threonine 231 (p-tau T231) (right) protein (per 100 μ g protein amount input) in BH as measured by ECL immunoassay. Data are presented as mean \pm SD. Ns, no significant difference ($p > 0.05$), $**p \leq 0.01$ by two-tailed Welch's t-test. B) Correlations of t-tau and p-tau T231 protein levels between BH with 10K (left), and BH with EVs (right) in all patients. Linear regression lines are shown in black. The grey area depicts 95% confidence intervals. Pearson correlation coefficient (R) and significance (p) are shown based on AD (n=21) and control (n=10) samples.



Supplementary Figure 4. Peroxiredoxin (PRDX) 1 and 6 levels in brain tissues (BH) from AD patients and controls. A) The levels of PRDX1 and PRDX6 (per 100 μg protein amount input) in BH as measured by ECL immunoassay. Data are presented as mean \pm SD. ns: no significant difference ($p > 0.05$) by two-tailed Welch's t-test. B) Correlations PRDX1 and PRDX6 protein levels between BH with 10K (left), and BH with EVs (right) in all patients. Linear regression lines are shown in black. The grey area depicts 95% confidence intervals. Pearson correlation coefficient (R) and significance (p) are shown based on AD ($n=21$) and control ($n=10$) samples.



Supplementary Figure 5. EV and cellular origin markers on the bdEV surface. A) The relative levels of EV marker proteins CD63, CD81, and CD9 after normalizing to the average of CD63, CD81, and CD9 signals. Data are presented as mean +/- SD. Ns, no significant difference ($p > 0.05$) by two-tailed Welch's t-test. B) The heatmap of top ten abundant markers among tested proteins. The average ECL signal intensities of correspondent markers were indicated by the colored bars in AD (n=21) and controls (n=10).

