Supplementary material

Profiling inflammatory extracellular vesicles in plasma and cerebrospinal fluid: an optimized diagnostic model for Parkinson's disease

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Summary:

Table S1. Characteristics of patients in plasma discovery cohort

Variable	HC [n=36]	PD [n=29]	AP		Overall *P*-value	*Pairwise Comparisons*					
			MSA [n=9]	AP-Tau [n=10]		HC vs. PD	HC vs. MSA	HC vs. AP-Tau	PD vs. MSA	PD vs. AP-Tau	MSA vs. AP-Tau
Age (years)	62 ± 8.6	67 ± 11.6	66 ± 8.7	72 ± 8.6	**0.025**	0.341	1.000	**0.027**	1.000	0.774	1.000
Sex (ref. male)	18 (50.0)	18 (62.1)	3 (33.3)	5 (50.0)	0.474	-	-	-	-	-	-
Disease duration (years)	-	4.0 [2.5-8.5]	5.0 [1.0-7.5]	3.5 [2.8-5.3]	0.682	-	-	-	-	-	-
H&Y	-	2.0 [1.0-3.0]	4.5 [3.3-5.0]	4.0 [3.0-5.0]	**<0.001**	-	-	-	**0.001**	**0.001**	1.000
MDS-UPDRS	-	24.0 [13.5-37.3]	42.0 [26.5-42.0]	47.0 [30.5-99.0]	**0.029**	-	-	-	0.568	**0.041**	1.000
Beck Depression	-	5.0 [3.0-8.0]	6.0 [3.0-14.0]	15.0 [12.5-18.0]	**0.003**	-	-	-	1.000	**0.002**	0.151
MMSE	-	30.0 [29.0-30.0]	27.5 [24.3-29.8]	27.0 [22.5-28.0]	**<0.001**	-	-	-	0.061	**0.001**	0.699
MOCA	-	27.0 [23.3-29.0]	23.0 [18.0-26.0]	19.5 [14.5-22.5]	**0.006**	-	-	-	0.292	**0.008**	0.726
Olfactory test	-	7.0 [4.0-8.6]	9.0 [6.0-10.0]	7.0 [4.5-8.5]	0.305	-	-	-	-	-	-
RBD	-	3.0 [2.0-5.0]	4.0 [1.0-5.5]	2.5 [0.8-4.3]	0.678	-	-	-	-	-	-
LEDD	-	563 [180-750]	-	-	-	-	-	-	-	-	-

Clinical characteristics of patients from the plasma discovery cohort (n=84): healthy controls (HC) are compared to patients with PD (Parkinson's disease), MSA (multisystem atrophy), or AP-Tau (atypical parkinsonism with tauopathies). Variables are reported as mean ± SD, median [interquartile range], absolute number (percentage), as appropriated. A $P<0.05$ was considered significant and shown in bold.

Abbreviations: H&Y (Hoehn and Yahr scale), MDS-UPDRS (Movement Disorder Society-Unified Parkinson's Disease Rating Scale), BDI-II (Beck Depression Inventory II), MMSE (Mini-Mental State Examination), MoCA (Montreal Cognitive Assessment), RBD (Rem Behavior Disorder scale), LEDD (LevoDopa equivalent Dose).

Table S2. NTA and MACSPlex evaluation of CSF-derived EVs

Variable	HC [n=4]	PD [n=4]	AP		Overall *P*-value	*Pairwise Comparisons*					
			MSA [n=4]	AP-Tau [n=4]		HC vs. PD	HC vs. MSA	HC vs. AP-Tau	PD vs. MSA	PD vs. AP-Tau	MSA vs. AP-Tau
N° of EVs/mL*E9 (all vesicles)	0.9 [0.7-1.6]	7.4 [5.8-10.2]	9.4 [3.1-17.3]	6.2 [4.7-15.9]	**0.036**	**0.048**	0.155	0.105	1.000	1.000	1.000
N° of EVs/mL*E9 (30-150nm)	0.5 [0.4-0.6]	2.2 [2.0-3.2]	2.8 [0.6-11.2]	2.4 [1.2-4.8]	0.065	-	-	-	-	-	-
N° of EVs/mL*E9 (151-500nm)	0.4 [0.3-1.1]	5.4 [3.5-7.0]	3.6 [2.3-9.2]	3.6 [2.6-12.4]	**0.033**	**0.045**	0.188	0.128	1.000	1.000	1.000
Diameter (nm)	155 [135-188]	211 [194-228]	228 [160-262]	221 [147-242]	0.203	-	-	-	-	-	-
CD9 (MFI; FC)	4.8 [3.5-7.1]	18.1 [9.5-39.2]	24.0 [10.7-39.3]	16.2 [8.6-37.5]	0.080	-	-	-	-	-	-
CD63 (MFI; FC)	3.3 [2.6-5.9]	9.6 [6.7-13.7]	3.6 [2.2-9.6]	4.7 [1.4-7.2]	0.142	-	-	-	-	-	-
CD81 (MFI; FC)	14.9 [12.6-19.9]	22.2 [9.4-28.4]	16.8 [9.1-26.2]	15.6 [4.8-36.0]	0.936	-	-	-	-	-	-
Mean MFI CD9, CD63, CD81 (FC)	7.7 [6.3-10.9]	15.7 [14.5-18.7]	17.8 [7.9-21.5]	16.3 [5.7-22.1]	0.281	-	-	-	-	-	-
CD3 (nMFI; %)	0.0 [0.0-0.0]	13.6 [3.8-41.2]	20.8 [15.2-24.7]	11.7 [0.2-58.2]	0.052	-	-	-	-	-	-
CD4 (nMFI; %)	2.7 [1.2-14.9]	7.4 [0.8-28.8]	11.0 [1.2-20.1]	1.7 [0.0-45.1]	0.909	-	-	-	-	-	-
CD19 (nMFI; %)	4.6 [1.0-9.5]	0.8 [0.0-6.0]	1.0 [0.0-12.3]	0.0 [0.0-15.7]	0.783	-	-	-	-	-	-
CD8 (nMFI; %)	0.0 [0.0-6.5]	13.5 [2.5-30.9]	8.2 [0.8-17.0]	2.1 [0.0-4.5]	0.162	-	-	-	-	-	-
HLA-II (nMFI; %)	3.4 [0.0-9.2]	4.2 [2.1-14.2]	3.5 [0.0-11.7]	9.6 [2.1-15.1]	0.667	-	-	-	-	-	-
CD56 (nMFI; %)	0.0 [0.0-0.0]	0.0 [0.0-178.0]	0.0 [0.0-0.0]	0.0 [0.0-95.2]	0.542	-	-	-	-	-	-
CD105 (nMFI; %)	4.1	306.4	297.9	19.6	0.228	-	-	-	-	-	-

	[0.6-6.2]	[44.7-771.2]	[52.6-1,330.6]	[1.1-188.8]								
CD2 (nMFI; %)	0.0 [0.0-0.0]	2.9 [0.1-22.7]	15.3 [4.1-30.6]	6.3 [0.9-18.1]	0.059	-	-	-	-	-	-
CD1c (nMFI; %)	0.0 [0.0-5.9]	11.4 [0.3-28.0]	20.3 [9.3-25.0]	9.9 [3.2-63.7]	0.165	-	-	-	-	-	-
CD25 (nMFI; %)	1.2 [0.0-5.7]	0.0 [0.0-3.9]	0.0 [0.0-7.9]	0.0 [0.0-12.8]	0.935	-	-	-	-	-	-
CD49e (nMFI; %)	0.0 [0.0-0.0]	0.0 [0.0-1.7]	1.2 [0.0-10.1]	0.0 [0.0-0.5]	0.366	-	-	-	-	-	-
ROR1 (nMFI; %)	4.7 [0.8-25.8]	2.1 [0.1-18.3]	10.6 [2.3-13.7]	1.1 [0.0-4.8]	0.614	-	-	-	-	-	-
CD209 (nMFI; %)	1.9 [0.0-27.9]	0.0 [0.0-4.2]	0.0 [0.0-10.7]	0.0 [0.0-16.9]	0.846	-	-	-	-	-	-
CD9 (nMFI; %)	61.3 [51.3-69.8]	109.5 [57.6-238.0]	135.4 [120.9-197.2]	178.5 [87.1-198.8]	0.147	-	-	-	-	-	-
SSEA-4 (nMFI; %)	4.5 [2.5-14.4]	4.1 [0.8-356.5]	155.5 [32.2-203.5]	25.9 [8.4-163.2]	0.162	-	-	-	-	-	-
HLA-I (nMFI; %)	0.0 [0.0-40.5]	22.9 [2.8-217.6]	11.3 [0.0-57.3]	13.8 [0.0-54.9]	0.702	-	-	-	-	-	-
CD63 (nMFI; %)	45.3 [39.7-53.5]	60.5 [45.8-73.3]	28.0 [19.8-48.1]	27.5 [16.7-45.2]	0.065	-	-	-	-	-	-
CD40 (nMFI; %)	0.0 [0.0-2.7]	0.0 [0.0-7.1]	0.0 [0.0-12.2]	0.0 [0.0-0.0]	0.756	-	-	-	-	-	-
CD62P (nMFI; %)	0.0 [0.0-0.0]	0.0 [0.0-0.0]	0.0 [0.0-7.2]	0.0 [0.0-0.0]	0.392	-	-	-	-	-	-
CD11c (nMFI; %)	3.1 [1.1-6.4]	0.0 [0.0-0.0]	0.0 [0.0-9.5]	0.0 [0.0-0.7]	0.149	-	-	-	-	-	-
CD81 (nMFI; %)	194.5 [180.9-203.7]	131.3 [60.6-183.4]	131.8 [81.0-137.9]	89.4 [82.7-174.1]	0.185	-	-	-	-	-	-
MCSP (nMFI; %)	0.0 [0.0-5.9]	0.0 [0.0-0.0]	5.1 [0.0-11.8]	0.0 [0.0-4.9]	0.366	-	-	-	-	-	-
CD146 (nMFI; %)	2.1 [0.0-4.5]	0.0 [0.0-5.8]	0.8 [0.0-12.0]	0.0 [0.0-7.6]	0.905	-	-	-	-	-	-
CD41b (nMFI; %)	0.0 [0.0-0.4]	4.2 [0.0-25.4]	2.1 [0.0-18.0]	2.8 [0.6-5.7]	0.516	-	-	-	-	-	-
CD42a (nMFI; %)	0.8	0.0	0.0	0.0	0.532	-	-	-	-	-	-

	[0.0-8.6]	[0.0-9.6]	[0.0-0.0]	[0.0-1.4]							
CD24 (nMFI; %)	9.9 [4.3-45.4]	10.0 [4.7-41.6]	11.9 [2.7-18.4]	7.3 [0.0-33.5]	0.902	-	-	-	-	-	-
CD86 (nMFI; %)	0.0 [0.0-2.9]	4.4 [0.0-47.6]	21.2 [18.3-41.4]	24.2 [3.3-85.4]	0.113	-	-	-	-	-	-
CD44 (nMFI; %)	0.6 [0.0-8.1]	9.4 [0.0-30.9]	16.4 [7.4-22.0]	2.9 [0.0-29.3]	0.410	-	-	-	-	-	-
CD326 (nMFI; %)	0.0 [0.0-7.1]	0.0 [0.0-840.7]	121.3 [27.3-468.5]	48.4 [0.2-135.5]	0.130	-	-	-	-	-	-
CD133/1 (nMFI; %)	2.4 [0.7-20.1]	8.1 [1.8-12.8]	8.5 [0.3-34.3]	10.4 [2.0-29.4]	0.962	-	-	-	-	-	-
CD29 (nMFI; %)	3.7 [0.0-7.4]	1.3 [0.0-5.2]	2.7 [0.0-16.0]	0.0 [0.0-1.5]	0.652	-	-	-	-	-	-
CD69 (nMFI; %)	0.0 [0.0-0.0]	0.0 [0.0-1.4]	0.0 [0.0-10.5]	0.0 [0.0-1.5]	0.756	-	-	-	-	-	-
CD142 (nMFI; %)	0.3 [0.0-10.4]	0.5 [0.0-17.4]	3.2 [0.0-15.5]	0.1 [0.0-7.5]	0.967	-	-	-	-	-	-
CD45 (nMFI; %)	0.0 [0.0-0.6]	4.6 [0.2-61.5]	22.5 [17.6-26.2]	14.5 [0.4-51.1]	0.104	-	-	-	-	-	-
CD31 (nMFI; %)	0.0 [0.0-8.7]	0.0 [0.0-5.0]	0.0 [0.0-9.5]	0.0 [0.0-0.4]	0.992	-	-	-	-	-	-
CD20 (nMFI; %)	1.3 [0.0-9.2]	10.4 [2.5-28.6]	17.1 [3.4-30.7]	8.1 [0.0-20.7]	0.555	-	-	-	-	-	-
CD14 (nMFI; %)	0.5 [0.0-4.8]	17.6 [3.8-111.2]	24.3 [5.4-41.0]	0.2 [0.0-47.3]	0.426	-	-	-	-	-	-

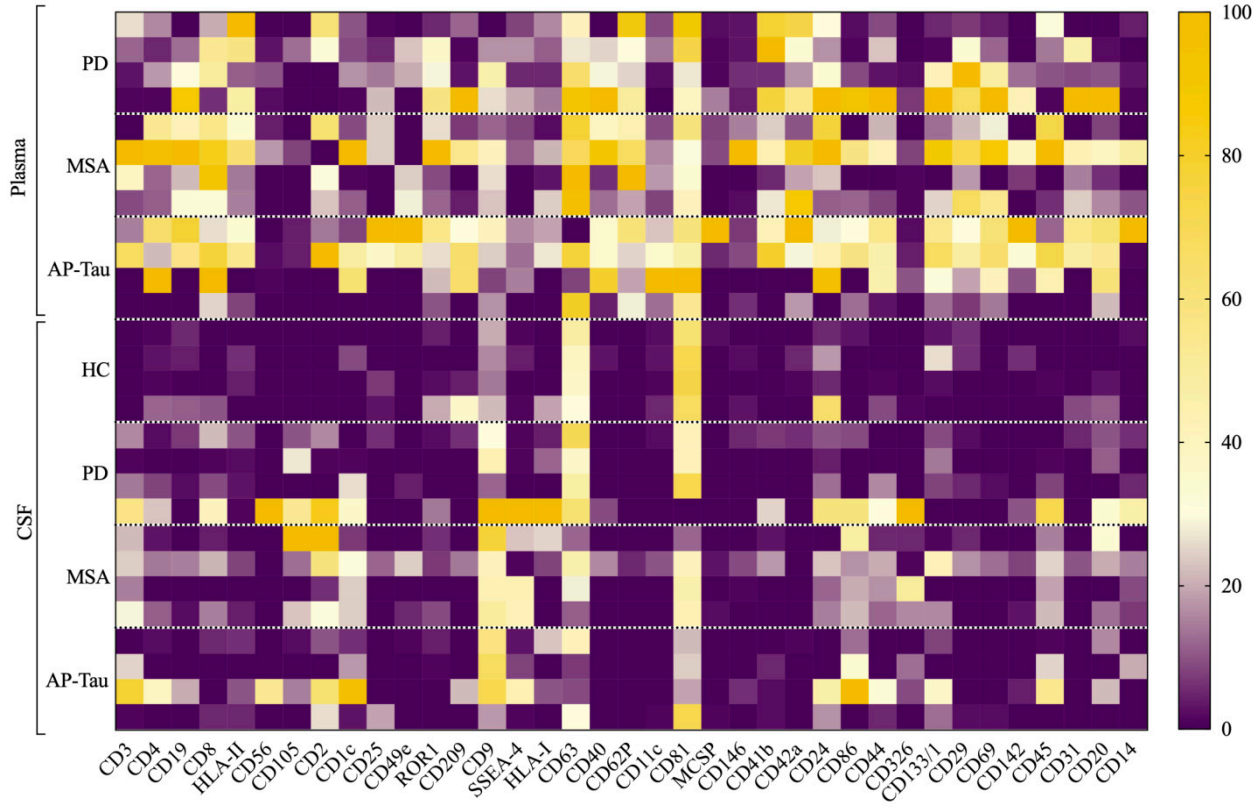
The table reports EV parameters derived by nanoparticle tracking analysis (NTA) and MACSPlex flow cytometry assay in cerebrospinal fluid (CSF) of healthy controls (HC) and patients with Parkinson's disease (PD), multisystem atrophy (MSA), or atypical parkinsonism with tauopathies (AP-Tau). We considered EV concentration and diameter at NTA, median fluorescence intensity (MFI) and mean MFI for CD9, CD63, and CD81 at flow cytometry, and normalized MFI (nMFI; %) for 37 EV surface antigens at flow cytometry. Data are expressed as median and interquartile range. A $P<0.05$ was considered significant and shown in bold.

Table S3. EV quantitative analysis in plasma and CSF

Variable	**Plasma** [n=12]	**CSF** [n=12]	***P*-value**
N° of EVs/mL (all vesicles*E11)	20.8 [12.0-23.3]	0.07 [0.05-0.14]	**<0.001**
N° of EVs/mL (30-150nm *E11)	9.3 [6.3-12.3]	0.02 [0.01-0.04]	**<0.001**
N° of EVs/mL (151-500nm *E11)	8.6 [5.9-11.8]	0.04 [0.03-0.07]	**<0.001**
Diameter (nm)	171 [153-177]	221 [194-234]	**0.005**
CD9 (MFI; FC)	28.8 [17.5-38.4]	19.1 [8.6-39.1]	0.374
CD63 (MFI; FC)	22.6 [18.4-58.0]	6.0 [3.2-10.4]	0.071
CD81 (MFI; FC)	58.0 [32.4-143.0]	19.7 [8.4-27.4]	**<0.001**
Mean MFI CD9, CD63, CD81 (MFI; FC)	43.0 [25.4-77.2]	16.0 [12.7-20.4]	**<0.001**

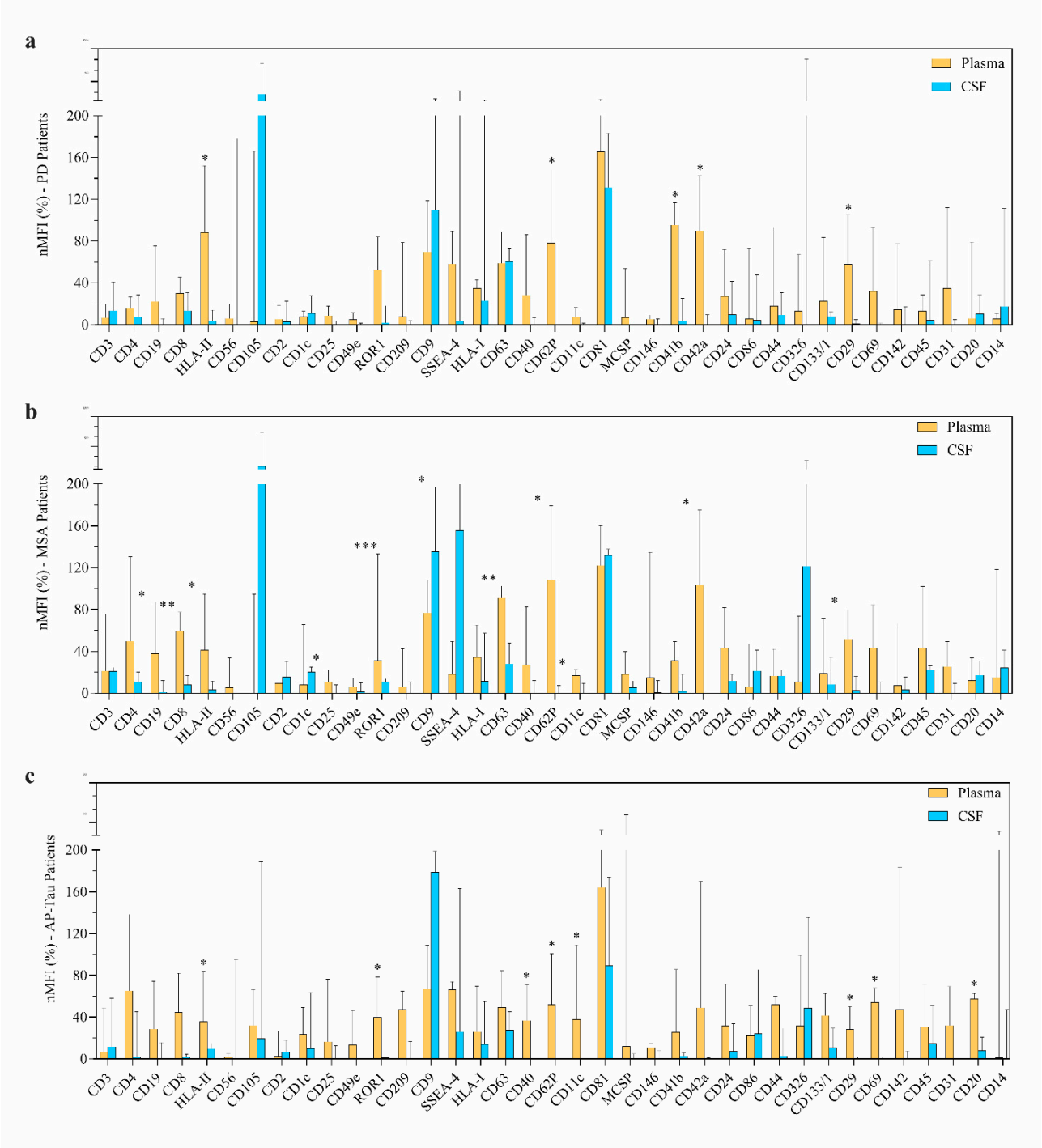
Extracellular vesicle (EV) concentration and diameter at nanoparticle tracking analysis (NTA), median fluorescence intensity (MFI) and mean MFI for CD9, CD63, and CD81 after flow cytometry are reported for plasma and cerebrospinal fluid (CSF). Data are expressed as median and interquartile range. $P<0.05$ was considered significant and shown in bold.

Figure S1. EV surface markers expression in plasma and CSF



Stratification of patients for diagnosis and expression of extracellular vesicle (EV) surface antigens in plasma and cerebrospinal fluid (CSF). The heat map shows normalized median fluorescence intensity (purple = low nMFI; yellow = high nMFI) for the 28 analyzed samples: 12 plasma samples from patients with PD (Parkinson's disease), MSA (multisystem atrophy), AP-Tau (atypical parkinsonism with tauopathies; n=4 per group) were compared to the respective CSF sample, plus 4 CSF samples from HC.

Figure S2. Evaluation of EV surface antigens in pathological groups



The expression of 37 extracellular vesicle (EV) surface antigens was assessed by flow cytometry and expressed as normalized median fluorescence intensity (nMFI; %); 12 plasma samples (yellow) from patients with PD (Parkinson's disease; **a**), MSA (multisystem atrophy; **b**) and AP-Tau (atypical parkinsonism with tauopathies; **c**; (n=4 per group) were compared to the respective cerebrospinal fluid (CSF; light blue) samples. Bars show median and inter-quartile range (**P*<0.05; ***P*<0.01; ***P*<0.001). Data and statistics are reported in Table S4.

Table S4. EV surface antigens in plasma and CSF in pathological groups

Variable	**PD** [n=4]			**MSA** [n=4]			**AP-Tau** [n=4]		
	CSF	**Plasma**	***P*-value**	**CSF**	**Plasma**	***P*-value**	**CSF**	**Plasma**	***P*-value**
N° of EVs/mL *E11 (all vesicles)	0.07 [0.06-0.10]	19.0 [16.1-20.9]	**0.020**	0.09 [0.03-0.17.]	22.6 [12.6-23.8]	**0.021**	0.06 [0.05-0.16]	17.1 [5.3-24.8]	**0.025**
N° of EVs/mL*E11 (30-150nm)	0.02 [0.02-0.03]	9.0 [7.7-11.7]	**0.027**	0.03 [0.01-0.11]	10.4 [6.8-12.2]	**0.029**	0.02 [0.01-0.05]	8.6 [2.1-16.0]	**0.031**
N° of EVs/mL*E11 (151-500nm)	0.05 [0.04-0.07]	8.6 [8.4-10.7]	**0.028**	0.04 [0.02-0.09]	11.8 [5.5-12.5]	**0.031**	0.04 [0.03-0.12	5.9 [3.2-11.4]	**0.029**
Diameter (nm)	211 [194-228]	175 [155-183]	**0.033**	228 [160-262]	161 [153-171]	0.343	221 [147-242]	173 [139-192]	0.343
CD9 (MFI; FC)	18.1 [9.5-39.2]	33.7 [22.3-122.7]	0.200	24.0 [10.7-39.3]	27.8 [17.0-59.2]	0.886	16.2 [8.6-37.5]	21.5 [15.8-35.0]	0.686
CD63 (MFI; FC)	9.6 [6.7-13.7]	41.8 [18.4-149.7]	**0.028**	3.6 [2.2-9.6]	32.5 [22.4-62.5]	**0.029**	4.7 [1.4-7.2]	15.5 [4.5-25.0]	0.200
CD81 (MFI; FC)	22.2 [9.4-28.4]	177.4 [48.7-300.4]	0.114	16.8 [9.1-26.2]	45.4 [26.0-122.5]	0.057	15.6 [4.8-36.0]	49.9 [41.7-121.0]	0.057
Mean MFI CD9, CD63, CD81 (FC)	15.7 [14.5-18.7]	96.7 [33.5-174.9]	**0.021**	17.8 [7.9-21.5]	39.8 [21.9-76.7]	0.061	16.3 [5.7-22.1]	32.0 [27.3-48.5]	**0.029**
CD3	13.6 [3.8-41.2]	6.8 [1.7-20.0]	0.426	20.8 [15.2-24.7]	21.1 [2.0-76.0]	0.555	11.7 [0.2-58.2]	6.7 [0.0-48.9]	0.686
CD4	7.4 [0.8-28.8]	15.7 [2.4-26.9]	0.801	11.0 [1.2-20.1]	49.7 [17.4-130.6]	0.129	1.7 [0.0-45.1]	65.3 [8.4-138.4]	0.343
CD19	0.8 [0.0-6.0]	22.3 [3.4-75.5]	0.166	1.0 [0.0-12.3]	37.9 [24.8-87.5]	**0.047**	0.0 [0.0-15.7]	28.7 [0.0-74.3]	0.486
CD8	13.5 [2.5-30.9]	30.2 [7.9-45.6]	0.367	8.2 [0.8-17.0]	59.4 [32.9-77.5]	**0.008**	2.1 [0.0-4.5]	44.7 [21.8-82.0]	**0.029**
HLA-II	4.2 [2.1-14.2]	88.2 [33.5-152.1]	**0.037**	3.5 [0.0-11.7]	41.3 [24.2-94.9]	**0.048**	9.6 [2.1-15.1]	35.8 [3.2-83.9]	0.343
CD56	0.0 [0.0-178.0]	6.0 [1.3-20.2]	0.432	0.0 [0.0-0.0]	5.2 [0.0-33.8]	0.234	0.0 [0.0-95.2]	1.8 [0.2-5.0]	0.486
CD105	306.4 [44.7-771.2]	3.0 [0.0-166.1]	0.162	297.9 [52.6-1,330.6]	0.0 [0.0-94.5]	0.205	19.6 [1.1-188.8]	31.9 [0.0-66.4]	1.000

CD2	2.9 [0.1-22.7]	5.5 [0.0-18.4]	0.946	15.3 [4.1-30.6]	9.3 [2.0-18.6]	0.444	6.3 [0.9-18.1]	2.3 [0.0-26.5]	1.000
CD1c	11.4 [0.3-28.0]	7.8 [2.8-13.0]	0.530	20.3 [9.3-25.0]	8.2 [2.8-65.4]	0.731	9.9 [3.2-63.7]	23.3 [1.6-49.1]	1.000
CD25	0.0 [0.0-3.9]	8.9 [2.1-18.0]	0.104	0.0 [0.0-7.9]	10.9 [0.0-22.0]	0.268	0.0 [0.0-12.8]	16.5 [0.0-76.4]	0.486
CD49e	0.0 [0.0-1.7]	5.2 [0.0-11.7]	0.177	1.2 [0.0-10.1]	6.4 [0.0-14.4]	0.555	0.0 [0.0-0.5]	13.1 [0.0-46.5]	0.486
ROR1	2.1 [0.1-18.3]	52.9 [17.4-84.2]	0.051	10.6 [2.3-13.7]	31.1 [15.6-133.2]	0.197	1.1 [0.0-4.8]	39.9 [21.0-78.5]	**0.029**
CD209	0.0 [0.0-4.2]	7.7 [1.8-78.7]	0.287	0.0 [0.0-10.7]	5.6 [1.0-42.4]	0.370	0.0 [0.0-16.9]	47.3 [7.6-64.8]	0.114
CD9	109.5 [57.6-238.0]	69.9 [25.1-118.7]	0.292	135.4 [120.9-197.2]	76.8 [52.6-108.2]	**0.034**	178.5 [87.1-198.8]	97.3 [40.1-129.1]	0.114
SSEA-4	4.1 [0.8-356.5]	58.2 [26.2-89.9]	0.619	155.5 [32.2-203.5]	18.2 [0.2-49.4]	0.069	25.9 [8.4-163.2]	66.0 [15.6-73.9]	0.886
HLA-I	22.9 [2.8-217.6]	34.8 [17.7-43.2]	0.487	11.3 [0.0-57.3]	34.3 [6.8-65.1]	0.603	13.8 [0.0-54.9]	25.8 [0.0-69.7]	0.868
CD63	60.5 [45.8-73.3]	58.8 [37.6-88.8]	0.907	28.0 [19.8-48.1]	91.0 [76.6-102.4]	**0.001**	27.5 [16.7-45.2]	49.1 [11.3-84.4]	0.886
CD40	0.0 [0.0-7.1]	28.5 [6.6-86.3]	0.143	0.0 [0.0-12.2]	26.9 [7.9-82.5]	0.146	0.0 [0.0-0.0]	36.9 [12.6-71.2]	**0.029**
CD62P	0.0 [0.0-0.0]	78.0 [51.9-148.2]	**0.012**	0.0 [0.0-7.2]	108.5 [49.8-179.2]	**0.017**	0.0 [0.0-0.0]	51.9 [39.9-100.8]	**0.031**
CD11c	0.0 [0.0-0.0]	7.4 [0.8-16.4]	0.091	0.0 [0.0-9.5]	16.8 [10.5-22.7]	**0.025**	0.0 [0.0-0.7]	37.5 [20.6-109.1]	**0.030**
CD81	131.3 [60.6-183.4]	165.5 [99.6-231.0]	0.410	131.8 [81.0-137.9]	121.9 [110.2-160.3]	0.555	89.4 [82.7-174.1]	164.0 [144.4-241.6]	0.200
MCSP	0.0 [0.0-0.0]	7.3 [5.2-54.0]	0.208	5.1 [0.0-11.8]	18.1 [0.0-39.9]	0.286	0.0 [0.0-4.9]	12.1 [0.6-356.4]	0.200
CD146	0.0 [0.0-5.8]	5.4 [4.5-9.5]	0.112	0.8 [0.0-12.0]	14.7 [0.8-134.8]	0.305	0.0 [0.0-7.6]	10.8 [2.6-15.0]	0.114
CD41b	4.2 [0.0-25.4]	95.4 [28.9-116.7]	**0.037**	2.1 [0.0-18.0]	31.0 [12.1-49.4]	0.076	2.8 [0.6-5.7]	25.6 [0.0-85.7]	0.886
CD42a	0.0	90.1	**0.014**	0.0	103.0	**0.045**	0.0	49.1	0.114

	[0.0-9.6]	[43.0-142.6]		[0.0-0.0]	[25.0-175.1]		[0.0-1.4]	[9.4-170.0]	
CD24	10.0 [4.7-41.6]	27.3 [17.6-72.0]	0.342	11.9 [2.7-18.4]	43.4 [12.6-81.9]	0.114	7.3 [0.0-33.5]	31.6 [6.2-71.6]	0.486
CD86	4.4 [0.0-47.6]	5.9 [0.9-73.6]	0.739	21.2 [18.3-41.4]	6.0 [0.0-47.0]	0.573	24.2 [3.3-85.4]	22.0 [3.3-51.1]	0.686
CD44	9.4 [0.0-30.9]	18.1 [4.7-92.7]	0.392	16.4 [7.4-22.0]	16.6 [2.3-42.2]	0.667	2.9 [0.0-29.3]	51.9 [15.4-60.2]	0.114
CD326	0.0 [0.0-840.7]	13.0 [2.1-67.2]	0.403	121.3 [27.3-468.5]	10.8 [1.9-73.7]	0.211	48.4 [0.2-135.5]	31.8 [8.2-99.4]	0.886
CD133/1	8.1 [1.8-12.8]	22.5 [1.5-83.7]	0.260	8.5 [0.3-34.3]	18.8 [3.1-71.5]	0.464	10.4 [2.0-29.4]	41.5 [16.9-62.8]	0.200
CD29	1.3 [0.0-5.2]	57.8 [15.5-105.3]	**0.049**	2.7 [0.0-16.0]	51.6 [21.6-80.1]	**0.038**	0.0 [0.0-1.5]	28.2 [11.1-50.1]	**0.029**
CD69	0.0 [0.0-1.4]	32.2 [6.5-92.9]	0.112	0.0 [0.0-10.5]	43.5 [7.5-84.3]	0.085	0.0 [0.0-1.5]	53.8 [22.1-68.0]	**0.026**
CD142	0.5 [0.0-17.4]	14.5 [0.0-77.3]	0.321	3.2 [0.0-15.5]	7.2 [0.0-66.6]	0.406	0.1 [0.0-7.5]	47.1 [5.7-183.6]	0.200
CD45	4.6 [0.2-61.5]	13.1 [3.8-28.9]	0.747	22.5 [17.6-26.2]	43.2 [1.8-102.1]	0.359	14.5 [0.4-51.1]	30.6 [3.4-71.7]	0.686
CD31	0.0 [0.0-5.0]	34.7 [2.9-112.0]	0.154	0.0 [0.0-9.5]	25.2 [4.8-49.2]	0.100	0.0 [0.0-0.4]	32.1 [0.0-69.4]	0.486
CD20	10.4 [2.5-28.6]	6.1 [0.5-79.1]	0.586	17.1 [3.4-30.7]	11.9 [6.8-33.7]	0.975	8.1 [0.0-20.7]	57.4 [30.4-62.9]	**0.029**
CD14	17.6 [3.8-111.2]	5.9 [0.5-11.1]	0.288	24.3 [5.4-41.0]	15.0 [0.0-118.3]	0.588	0.2 [0.0-47.3]	1.0 [0.0-231.6]	0.885

The table reports plasma and cerebrospinal fluid (CSF)-derived extracellular vesicle (EV) parameters derived by nanoparticle tracking analysis (NTA) and flow cytometry for patients with PD (Parkinson's disease), MSA (multisystem atrophy), or AP-Tau (atypical parkinsonism with tauopathies). We considered EV concentration and diameter at NTA, median fluorescence intensity (MFI) and mean MFI for CD9, CD63, and CD81 at flow cytometry, and normalized MFI (nMFI; %) for 37 EV surface antigens at flow cytometry. Data are expressed as median and interquartile range. A $P<0.05$ was considered significant and shown in bold.