

Supplementary Material

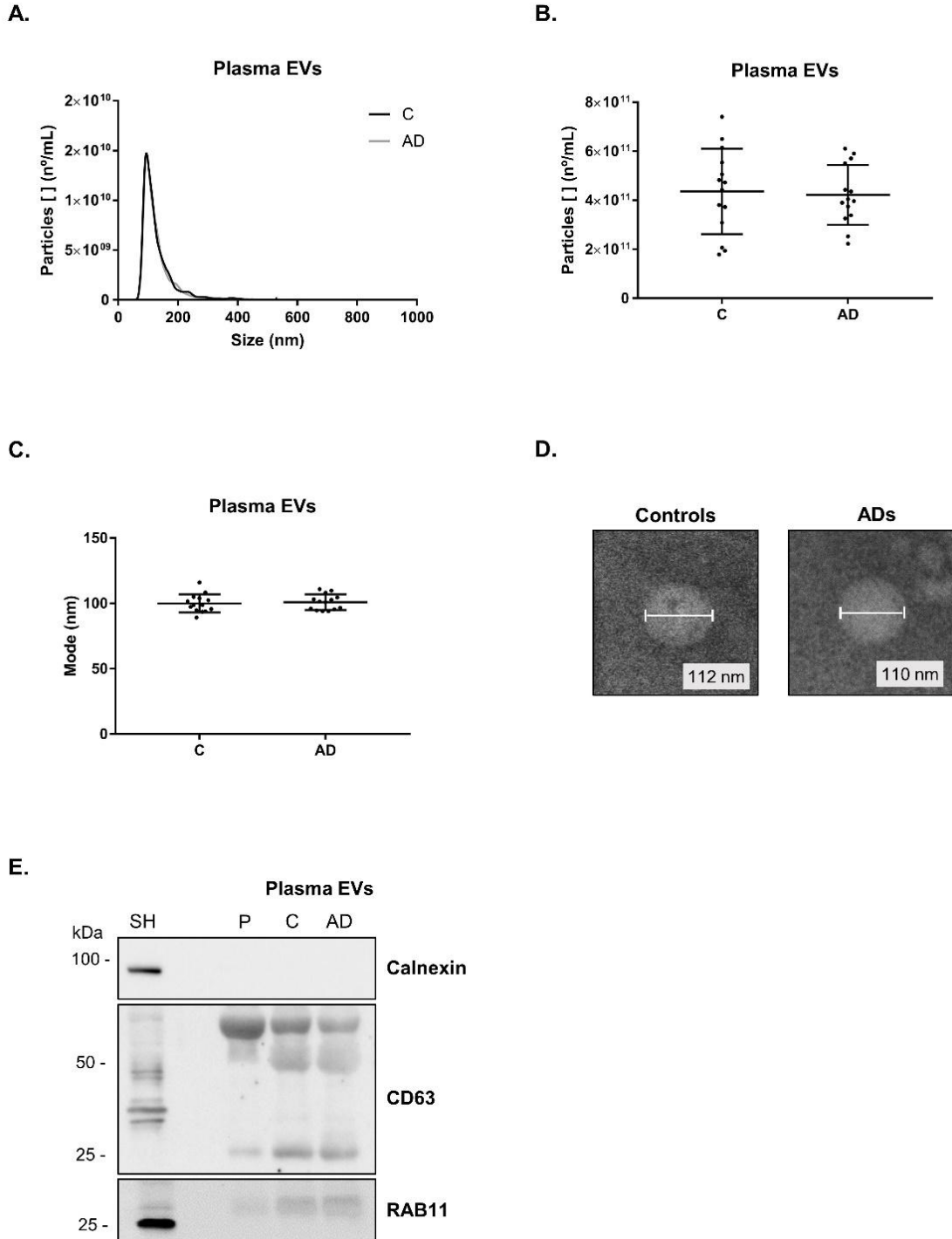
FTIR Spectroscopy and Blood-Derived Extracellular Vesicles Duo in Alzheimer's Disease

Supplementary Table 1. Demographics and clinical data of serum- and plasma-derived EVs participants.

	Serum group			Plasma group		
	C (N=12)	AD (N=12)	p	C (N=14)	AD (N=14)	p
Age (mean±SD)	67.58±7.74	73.17±10.66	0.12	66.64±8.83	66.79±10.15	0.97
MMSE scores (mean±SD)	26.42±2.84	24.83±3.24	0.18	27.56±3.21	22.86±4.04	<0.01*
CDT (points) (mean±SD)	2.58±1.08	3.08±1.38	0.26	1.67±0.87	3.00±1.16	<0.01**
CSF Aβ₁₋₄₂ (ng/mL) (mean±SD)	1029.0±461.7	592.0±204.1	<0.01	1327.0±506.6	618.4±114.6	<0.001
CSF Aβ₁₋₄₀ (ng/mL) (mean±SD)	12670±6362	17274±5542	0.08	10096±2713	13313±4415	0.03
CSF Aβ_{1-42/1-40} (mean±SD)	0.07±0.007	0.03±0.006	<0.001	1.34±0.39	0.50±0.20	<0.001
CSF P-tau 181 (pg/mL) (mean±SD)	277.70±129.10	695.0±336.90	<0.001	46.14±13.77	95.36±32.25	<0.001
CSF T-tau (pg/mL) (mean±SD)	43.33±15.65	86.98±31.79	<0.001	216.40±79.65	788.90±451.10	<0.001

AD, Alzheimer's disease cases; C, Controls; CDT, Clock-Drawing Test; CSF, Cerebrospinal fluid; MMSE, Mini-Mental State Examination; SD, Standard deviation. * Data available only for n=9 Controls and n=14 ADs. ** Data available only for n=9 Controls and n=13 ADs.

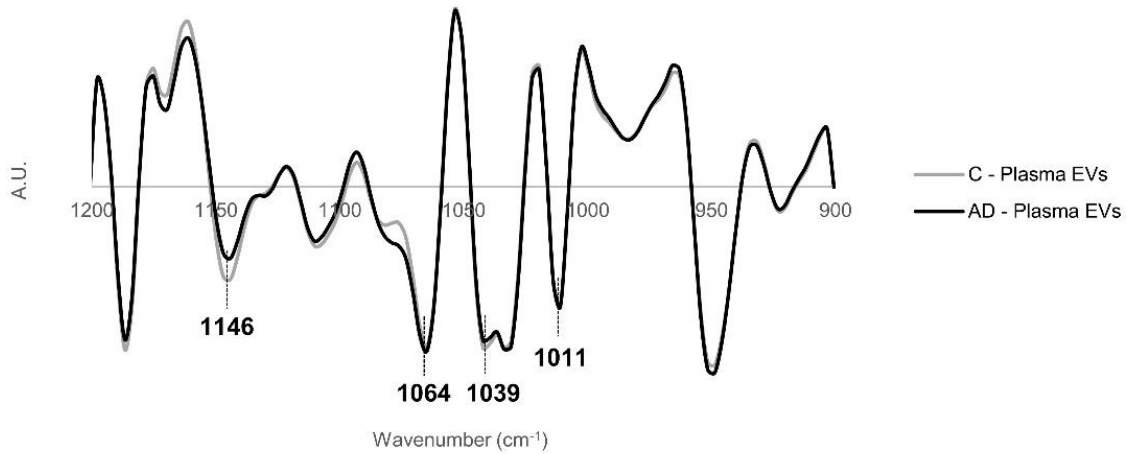
Supplementary Figure 1. Characterization of plasma-derived EVs.



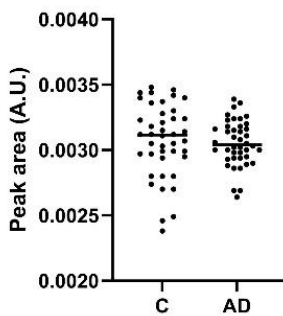
EVs size distribution (A), particle concentration (B) and mode size (C) determined by Nanoparticle Tracking Analysis (NTA). Transmission electron microscopy (TEM) of isolated nanovesicles (D) from Controls or AD cases. Western blot (WB) analysis (E) of EV markers CD63, RAB11 and negative marker Calnexin. Membrane includes a pool of SH-SY5Y cell lysates, a pool of total plasma and pools of plasma-derived EVs isolated from Controls or AD cases. AD, Alzheimer's disease; C, Controls; EVs, Extracellular vesicles; P, Plasma; SH, SH-SY5Y cell lysates.

Supplementary Figure 2. FTIR spectra of plasma-derived EVs in the 1200-900 cm^{-1} region.

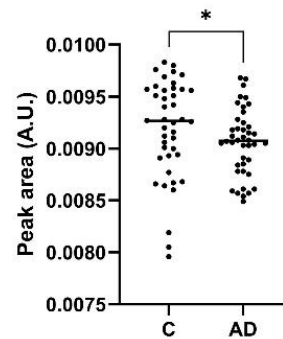
A. 2nd derivatives of plasma-derived EVs spectra



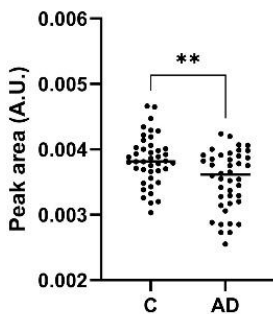
B. 1011 cm^{-1} peak



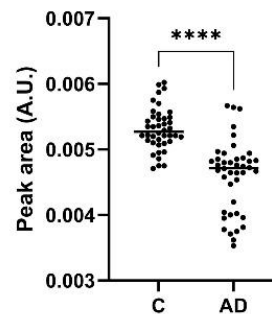
C. 1039 cm^{-1} peak



D. 1064 cm^{-1} peak

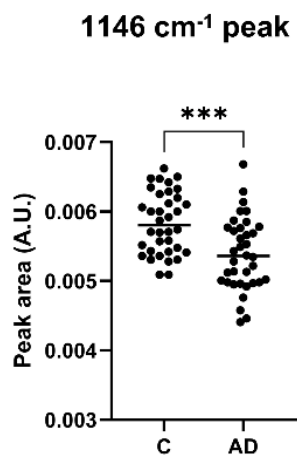


E. 1146 cm^{-1} peak



Mean second derivative of plasma-derived EVs spectra of Controls and AD cases (A) in the 1200-900 cm^{-1} region, mainly assigned to the carbohydrates and nucleic acids content, and peak area analysis (B-F) in plasma-derived EVs spectra. Previous data by the group support a decrease in the 1011 cm^{-1} , 1030 cm^{-1} , and 1064 cm^{-1} peaks in AD cases, for serum-derived EVs spectra. Three replicates were analyzed for each sample. * $p \leq 0.05$, ** $p \leq 0.01$; **** $p \leq 0.0001$ AD, Alzheimer's disease; A.U., Arbitrary units; C, Controls; EVs, Extracellular vesicles.

Supplementary Figure 3. Peak area analysis of serum-derived EVs spectra at 1146 cm⁻¹.



Three replicates were analyzed for each sample. *** $p \leq 0.001$
AD, Alzheimer's disease; A.U., Arbitrary units; C, Controls.