

Supplementary Information

Proteomics profiling of neuron-derived small extracellular vesicles from human plasma: enabling single subject analysis

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Supplementary Figures

Figure S1. NES cells and neuronal progeny characterization. (A-C) NES cells are immunopositive for neural stem cell markers Nestin and Vimentin (VIM), and express transcription factors SOX1 and SOX2, typically found in neuronal progenitors. Phospho-vimentin (pVIM) labels cells in the M phase of the cell cycle. (D-F) Upon three months of differentiation, NES cell-derived neurons appear immunodecorated with pan-neuronal markers neurofilament (NF), MAP2, and TUBB3, mostly expressed in neurites. Neuronal somas are immunopositive for RBFOX3. Scale bar is 20 μm .

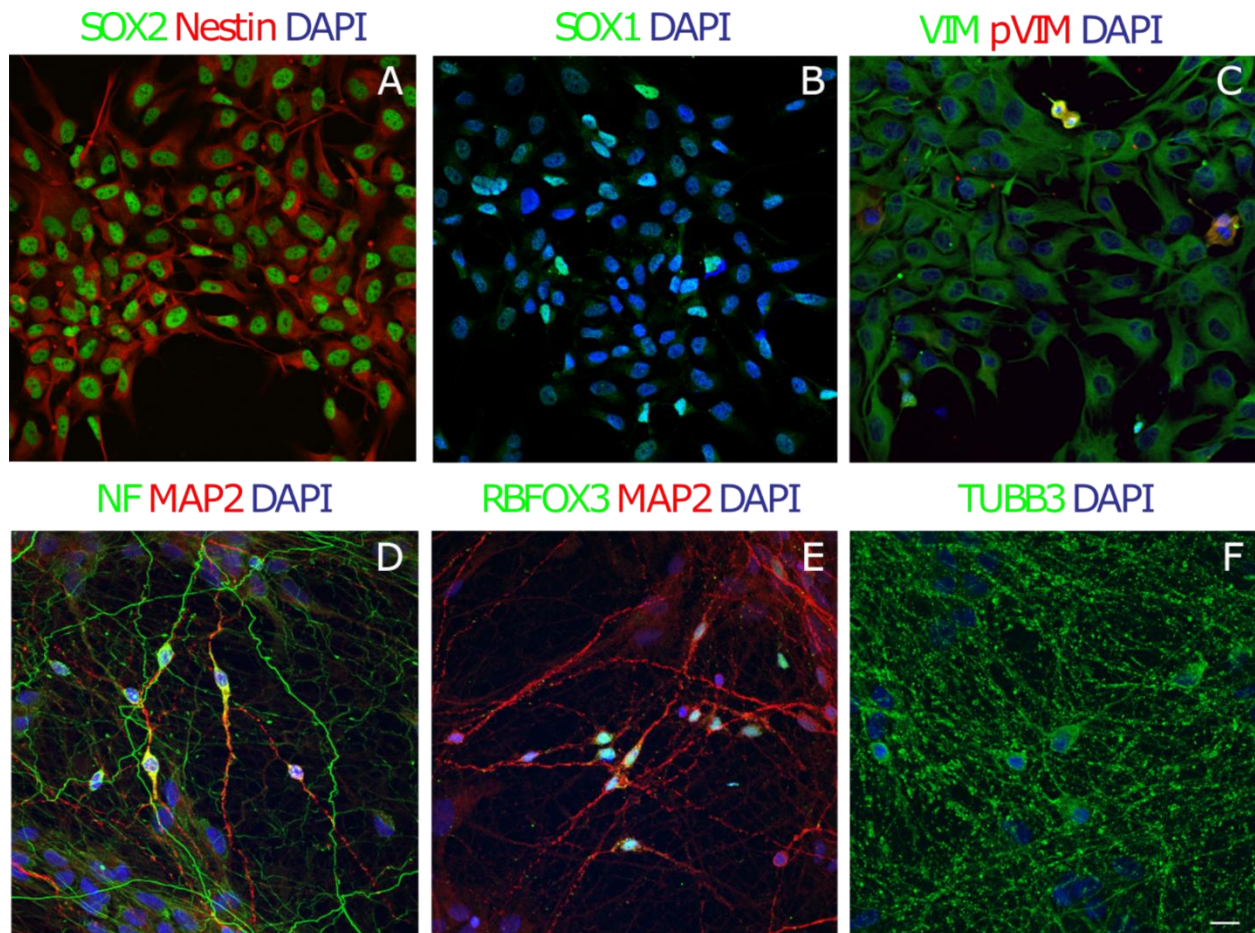


Figure S2. Human neurons' culture media SEC elution profile. The analysis was performed using a Superose 6 Increase 10/300 SEC column in-line with a Zetasizer Dynamic Light Scattering. The graph represents the vesicles diameter size (d.nm) in blue, the derived count rate in red and the signal detected at λ 220 nm in black. The light-orange box represents the selected fraction for NDsEV analysis (14-22 min).

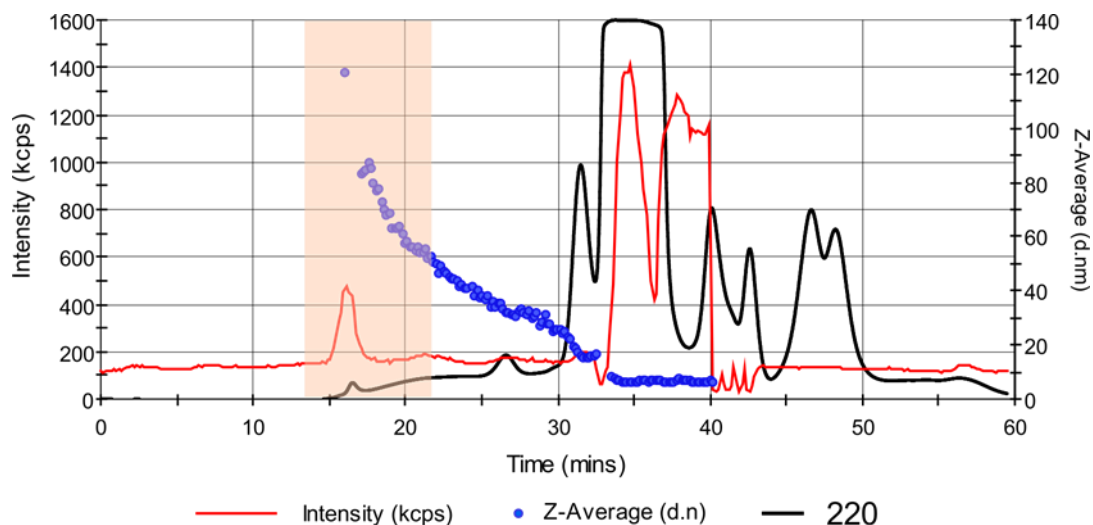
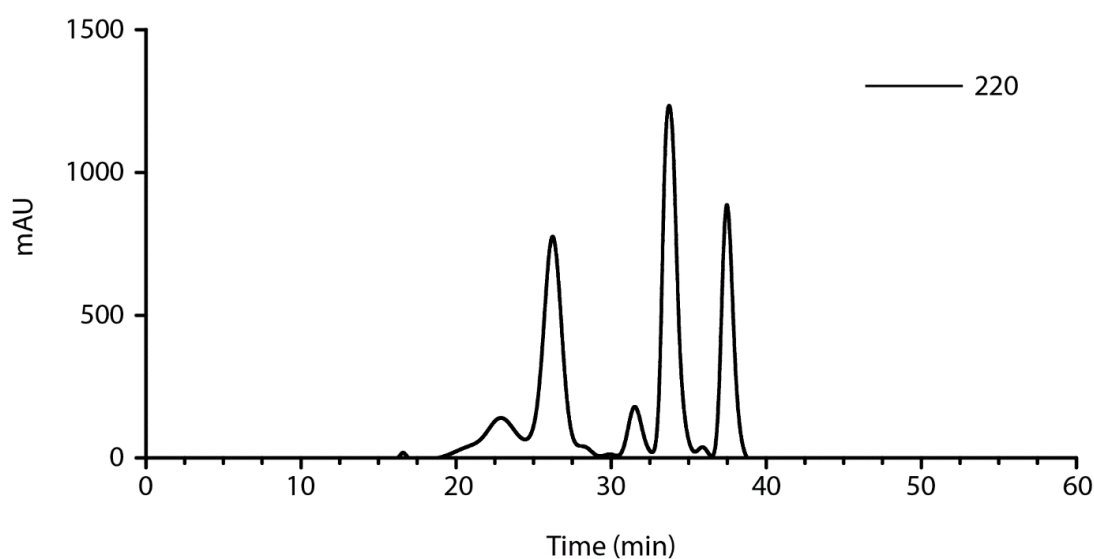


Figure S3. High molecular weight protein standard SEC elution profile. Thyroglobulin (660 kDa), Bovine Serum Albumin (66.5 kDa) and Myoglobin (16.7 kDa) obtained with a Superose 6 Increase 10/300 column at a flow of 0.5 ml/min.



Supplementary Tables

Table S1. Gene Ontology of proteins identified from NES-NDsEV. The analysis was performed with DAVID database using the total *homo sapiens* dataset as reference database. Proteins used for the analysis: 431. Significantly enriched 'Cellular component' GO terms are reported in the table with the relative count number and *p*-value FDR corrected.

CELLULAR COMPONENT		
Term	Count	FDR
Extracellular Exosome	311	$1,4 \times 10^{-161}$
Extracellular Space	146	$2,5 \times 10^{-58}$
Blood Microparticle	59	$1,2 \times 10^{-53}$
Extracellular Region	150	$8,2 \times 10^{-52}$
Extracellular Matrix	64	$5,2 \times 10^{-41}$
Focal Adhesion	61	$1,3 \times 10^{-30}$
Extracellular Vesicle	22	$4,0 \times 10^{-20}$
Melanosome	28	$5,3 \times 10^{-20}$
Plasma Membrane	168	$2,2 \times 10^{-14}$
Cell-Cell Adherens Junction	38	$2,4 \times 10^{-14}$
Basement Membrane	21	$2,4 \times 10^{-14}$
Platelet Alpha Granule Lumen	9	$3,40 \times 10^{-03}$
Vesicle	5	$8,60 \times 10^{-03}$
Myelin Sheath	4	$1,60 \times 10^{-02}$
Cell Surface	6	$3,30 \times 10^{-02}$

Table S2. Comparison of the identified proteins from NES-NDsEV with the Human Brain Proteome database (Human Proteome Atlas, <https://www.proteinatlas.org/humanproteome/brain/human+brain>). The table reports the proteins identified in common between the NES-NDsEV and the proteins annotated as elevated in the brain (2582 entries).

Gene	Protein description
<i>ARF3</i>	ADP ribosylation factor 3
<i>SLC44A1</i>	solute carrier family 44 member 1
<i>NCAN</i>	neurocan
<i>DIRAS2</i>	DIRAS family GTPase 2
<i>DBH</i>	dopamine beta-hydroxylase
<i>CELSR2</i>	cadherin EGF LAG seven-pass G-type receptor 2
<i>BASP1</i>	brain abundant membrane attached signal protein 1
<i>C1QTNF5</i>	C1q and tumor necrosis factor related protein 5
<i>ENPP2</i>	ectonucleotide pyrophosphatase/phosphodiesterase 2
<i>RAC3</i>	ras-related C3 botulinum toxin substrate 3 (rho family, GTP binding protein Rac3)
<i>NRCAM</i>	neuronal cell adhesion molecule
<i>EDIL3</i>	EGF like repeats and discoidin domains 3
<i>CHST6</i>	carbohydrate sulfotransferase 6
<i>METRN</i>	meteorin, glial cell differentiation regulator
<i>GPR37L1</i>	G protein-coupled receptor 37 like 1
<i>GABRG3</i>	gamma-aminobutyric acid type A receptor gamma3 subunit
<i>F5</i>	coagulation factor V
<i>BCAN</i>	brevican
<i>GAP43</i>	growth associated protein 43
<i>GPRC5B</i>	G protein-coupled receptor class C group 5 member B
<i>RAP2A</i>	RAP2A, member of RAS oncogene family
<i>CRISPLD1</i>	cysteine rich secretory protein LCCL domain containing 1
<i>COL4A5</i>	collagen type IV alpha 5 chain
<i>ALDOC</i>	aldolase, fructose-bisphosphate C
<i>TNIK</i>	TRAF2 and NCK interacting kinase
<i>LY6H</i>	lymphocyte antigen 6 complex, locus H
<i>PFN2</i>	profilin 2
<i>IGSF8</i>	immunoglobulin superfamily member 8
<i>GPM6A</i>	glycoprotein M6A
<i>TTYH2</i>	tweety family member 2
<i>CNP</i>	2',3'-cyclic nucleotide 3' phosphodiesterase
<i>TTYH1</i>	tweety family member 1
<i>LYPD1</i>	LY6/PLAUR domain containing 1

<i>SLC1A3</i>	solute carrier family 1 member 3
<i>CRMP1</i>	collapsin response mediator protein 1
<i>THY1</i>	Thy-1 cell surface antigen
<i>FSTL5</i>	follistatin like 5
<i>PTPRZ1</i>	protein tyrosine phosphatase, receptor type Z1
<i>TSPAN9</i>	tetraspanin 9
<i>MVB12B</i>	multivesicular body subunit 12B
<i>SPOCK1</i>	SPARC/osteonectin, cwcv and kazal like domains proteoglycan 1
<i>NCAM1</i>	neural cell adhesion molecule 1 or L1CAM
<i>CKB</i>	creatine kinase B
<i>GPM6B</i>	glycoprotein M6B
<i>BTBD17</i>	BTB domain containing 17
<i>BDNF</i>	brain derived neurotrophic factor
<i>ST8SIA1</i>	ST8 alpha-N-acetyl-neuraminide alpha-2,8-sialyltransferase 1
<i>PCDHGC3</i>	protocadherin gamma subfamily C, 3
<i>LSAMP</i>	limbic system-associated membrane protein
<i>HSPA2</i>	heat shock protein family A (Hsp70) member 2
<i>SMOC1</i>	SPARC related modular calcium binding 1
<i>PLP1</i>	proteolipid protein 1
<i>CSPG5</i>	chondroitin sulfate proteoglycan 5
<i>HRG</i>	histidine rich glycoprotein
<i>FOLR1</i>	folate receptor 1
<i>CAPS</i>	calcyphosine
<i>ITM2C</i>	integral membrane protein 2C

Table S3. Proteins identified from plasma-NDsEV of PD patients in common with the Top100 ExoCarta database. The table reports the proteins shared between the ExoCarta Top 100 database (www.exocarta.org) and the plasma-NDsEV with relative replicate's number of samples in which they have been identified.

Gene name	Description	# replicates
<i>ANXA2</i>	Annexin A2	6
<i>A2M</i>	Alpha-2-macroglobulin	6
<i>GAPDH</i>	Glyceraldehyde-3-phosphate dehydrogenase	6
<i>ALDOA</i>	Fructose-bisphosphate aldolase	6
<i>TPI1</i>	Triosephosphate isomerase	5
<i>HSPA5</i>	Endoplasmic reticulum chaperone BiP	5
<i>PRDX1</i>	Peroxiredoxin-1	5
<i>HSPA8</i>	Heat shock cognate 71 kDa protein	5
<i>PRDX2</i>	Peroxiredoxin-2	5
<i>ENO1</i>	Alpha-enolase	5
<i>PKM</i>	Erythrocyte band 7 integral membrane protein	5
<i>ANXA1</i>	Annexin A1	4
<i>AHCY</i>	Adenosylhomocysteinase	4
<i>STOM</i>	Erythrocyte band 7 integral membrane protein	4
<i>LGALS3BP</i>	Galectin-3-binding protein	4
<i>EEF1A1</i>	Elongation factor 1-alpha 1	4
<i>EEF2</i>	Elongation factor 2	3
<i>GNAI2</i>	Guanine nucleotide-binding protein G(i) subunit alpha-2	3
<i>FLOT1</i>	Flotillin-1	3
<i>TKT</i>	Transketolase	2
<i>CFL1</i>	Cofilin-1	2
<i>MYH9</i>	Myosin-9	2
<i>LDHA</i>	Flotillin-1 OS=Homo sapiens	2
<i>PFN1</i>	Profilin-1	2
<i>CDC42</i>	Cell division control protein 42 homolog	2
<i>CD9</i>	CD9 antigen	2
<i>TFRC</i>	Transferrin receptor protein 1	2
<i>FLNA</i>	Filamin-A	2
<i>PGK1</i>	Phosphoglycerate kinase 1	2
<i>MSN</i>	Moesin	1
<i>MFGE8</i>	Lactadherin	1
<i>THBS1</i>	Thrombospondin-1	1
<i>GDI2</i>	Rab GDP dissociation inhibitor bet	1
<i>YWHAZ</i>	14-3-3 protein zeta/delta OS=Homo sapien	1
<i>ITGB1</i>	Erythrocyte band 7 integral membrane protein	1

<i>RAB1A</i>	Ras-related protein Rab-1A	1
<i>VCP</i>	Transitional endoplasmic reticulum ATPase	1
<i>RAN</i>	GTP-binding nuclear protein Ran	1
<i>RAB7A</i>	Ras-related protein Rab-7a	1
<i>YWHAB</i>	14-3-3 protein beta/alpha	1
<i>ACTN4</i>	Alpha-actinin-4	1

Table S4. Gene Ontology of the proteins identified from plasma-NDsEV of PD patients. The analysis was performed with DAVID database using the total *homo sapiens* dataset as reference database. The analysis was performed on the 177 protein groups in common to all the 4 PD patients' samples. Significantly enriched 'Cellular component' GO terms are reported in the table with the relative count number and *p*-value FDR corrected.

CELLULAR COMPONENT		
Term	Count	FDR
Blood microparticle	74	$1,5 \times 10^{-112}$
Extracellular region	127	$1,9 \times 10^{-99}$
Extracellular exosome	140	$7,9 \times 10^{-88}$
Extracellular space	110	$4,6 \times 10^{-83}$
Platelet alpha granule lumen	22	$3,7 \times 10^{-28}$
High-density lipoprotein particle	13	$2,2 \times 10^{-18}$
Extracellular matrix	26	$2,4 \times 10^{-16}$
Very-low-density lipoprotein particle	11	$6,9 \times 10^{-15}$
Membrane attack complex	7	$4,9 \times 10^{-11}$
Spherical high-density lipoprotein particle	7	$1,6 \times 10^{-10}$
Chylomicron	8	$1,6 \times 10^{-10}$
Endocytic vesicle lumen	8	$4,9 \times 10^{-10}$
Platelet dense granule lumen	7	$1,4 \times 10^{-08}$
Fibrinogen complex	6	$6,4 \times 10^{-08}$
External side of plasma membrane	15	$6,4 \times 10^{-08}$

Table S5. Gene Ontology of the proteins identified from plasma-NDsEV of PD patients. The analysis was performed with DAVID database using the total *homo sapiens* dataset as reference database. The analysis was performed on the total number of identified proteins (630). Significantly enriched 'Cellular component' GO terms are reported in the table with the relative count number and *p*-value FDR corrected.

CELLULAR COMPONENT		
Term	Count	FDR
Extracellular Exosome	411	$1,2 \times 10^{-198}$
Blood Microparticle	102	$1,8 \times 10^{-112}$
Extracellular Region	251	$4,0 \times 10^{-109}$
Extracellular Space	230	$6,6 \times 10^{-107}$
Platelet Alpha Granule Lumen	37	$1,0 \times 10^{-38}$
Extracellular Matrix	67	$7,8 \times 10^{-35}$
High-Density Lipoprotein Particle	16	$3,2 \times 10^{-16}$
Focal Adhesion	49	$8,8 \times 10^{-14}$
Very-Low-Density Lipoprotein Particle	14	$1,1 \times 10^{-13}$
Cell-Cell Adherens Junction	43	$6,0 \times 10^{-13}$
Cytosol	182	$1,6 \times 10^{-12}$
Melanosome	24	$3,8 \times 10^{-12}$
Platelet Dense Granule Lumen	11	$3,3 \times 10^{-11}$
Chylomicron	11	$3,3 \times 10^{-11}$
External Side Of Plasma Membrane	32	$6,3 \times 10^{-11}$

Table S6. Proteins identified from plasma-NDsEV of PD patients belonging to the Gene Ontology Cellular component term 'Early Endosome'.

Early Endosome (GO:0005769)	
Gene name	Description
ANXA2	Annexin A2
APOB	Apolipoprotein B-100
APOA1	Apolipoprotein A-I
APOA5	Apolipoprotein A-V
APOC2	Apolipoprotein C-II
APOA4	Apolipoprotein A-IV
HSPD1	60 kDa heat shock protein, mitochondrial
RAB1A	Ras-related protein Rab-1A
APOE	Apolipoprotein E
EEA1	Early endosome antigen 1
PLA2G4E	Cytosolic phospholipase A2 epsilon
TFRC	Transferrin receptor protein 1

<i>FLOT1</i>	Flotillin-1
<i>LTF</i>	Lactotransferrin
<i>STX7</i>	Syntaxin-7
<i>APOA2</i>	Apolipoprotein A-II
<i>TF</i>	Serotransferrin
<i>B2M</i>	Beta-2-microglobulin
<i>APOC3</i>	Apolipoprotein C-III
<i>ANXA1</i>	Annexin A1

Table S7. Proteins identified from plasma-NDsEV of PD patients belonging to the Gene Ontology Cellular Component term 'Endosome'.

Endosome (GO:0005768)	
Gene name	Description
<i>RAN</i>	GTP-binding nuclear protein Ran
<i>HLA-DRB1</i>	HLA class II histocompatibility antigen, DRB1-15 beta chain
<i>STEAP3</i>	Metalloreductase STEAP3
<i>ANXA2</i>	Annexin A2
<i>CD14</i>	Monocyte differentiation antigen CD14
<i>HSPA8</i>	Heat shock cognate 71 kDa protein
<i>APOB</i>	Apolipoprotein B-100
<i>APOA1</i>	Apolipoprotein A-I
<i>CDH1</i>	Cadherin-1
<i>RAB11B</i>	Ras-related protein Rab-11B
<i>FLOT2</i>	Flotillin-2
<i>ATP6V0A1</i>	V-type proton ATPase 116 kDa subunit a isoform 1
<i>APOA5</i>	Apolipoprotein A-V
<i>CTSD</i>	Cathepsin D
<i>IFITM3</i>	Interferon-induced transmembrane protein 3
<i>ANTXR1</i>	Anthrax toxin receptor 1
<i>APOC2</i>	Apolipoprotein C-II
<i>APOA4</i>	Apolipoprotein A-IV
<i>HSPD1</i>	60 kDa heat shock protein, mitochondrial
<i>RAB1A</i>	Ras-related protein Rab-1A
<i>APOE</i>	Apolipoprotein E
<i>TPP1</i>	Tripeptidyl-peptidase 1
<i>GRN</i>	Progranulin
<i>EEA1</i>	Early endosome antigen 1
<i>HLA-DRA</i>	HLA class II histocompatibility antigen, DR alpha chain
<i>PLA2G4E</i>	Cytosolic phospholipase A2 epsilon
<i>TFRC</i>	Transferrin receptor protein 1

<i>LAMP1</i>	Lysosome-associated membrane glycoprotein 1
<i>RPS27A</i>	Ubiquitin-40S ribosomal protein S27a
<i>RAB7A</i>	Ras-related protein Rab-7a
<i>FLOT1</i>	Flotillin-1
<i>LTF</i>	Lactotransferrin
<i>HRG</i>	Histidine-rich glycoprotein
<i>TMBIM1</i>	Protein lifeguard 3
<i>STX7</i>	Syntaxin-7
<i>APOA2</i>	Apolipoprotein A-II
<i>TF</i>	Serotransferrin
<i>B2M</i>	Beta-2-microglobulin
<i>ITGB1</i>	Integrin beta-1
<i>PDCD6</i>	Programmed cell death protein 6
<i>APOC3</i>	Apolipoprotein C-III
<i>ANXA1</i>	Annexin A1
<i>RAN</i>	GTP-binding nuclear protein Ran
<i>HLA-DRB1</i>	HLA class II histocompatibility antigen, DRB1-15 beta chain