**SUPPLEMENTARY MATERIAL**

***Immune profiling of plasma-derived extracellular vesicles***

***identifies Parkinson’s disease***

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**Table e-1 List of 37 EV-surface antigens**

|  |  |  |
| --- | --- | --- |
| **EV-surface marker description** | | **Role** |
| CD1c | APC cells surface glycoprotein | Antigen-presenting protein |
| CD2 | T and NK cell surface antigen | Mediator of adhesion between T-cells and other cell types |
| CD3 | T cells surface glycoprotein | Mediator of signal transduction |
| CD4 | T cells transmembrane glycoprotein | Co-receptor for MHC class II molecule |
| CD8 | T cells transmembrane glycoprotein | Co-receptor for MHC class I molecule |
| CD9 | Tetraspanin super-family – EV-surface protein | Regulator of cell adhesion |
| CD11c | Integrin alpha-X | Receptor for fibrinogen |
| CD14 | Monocyte differentiation antigen | Co-receptor for bacterial lipopolysaccharide |
| CD19 | B-lymphocyte antigen | Co-receptor for the B-cell antigen receptor complex (BCR) |
| CD20 | B-lymphocyte antigen | Regulation of cellular calcium influx necessary for the development, differentiation, and activation of B cells |
| CD24 | Signal Transducer | Modulator of B-cell activation responses |
| CD25 | Interleukin-2 receptor subunit alpha | Marker for immune cell activation |
| CD29 | Integrin beta-1 | Extracellular matrix component |
| CD31 | Platelet endothelial cell adhesion molecule | Regulator of leukocyte trans endothelial migration (TEM) |
| CD40 | Costimulatory surface molecule | Co-stimulator of T and B cells |
| CD41b | Integrin alpha-IIb | Receptor for fibronectin, fibrinogen, plasminogen, prothrombin, thrombospondin and vitronectin |
| CD42a | Platelet glycoprotein 9 | Mediator of platelet adhesion to blood vessels |
| CD44 | Cell-surface receptor | Regulator of activation, recirculation and homing of T cells |
| CD45 | Receptor-type tyrosine-protein phosphatase C | Positive regulator of T-cell coactivation |
| CD49e | Integrin alpha-5 | Receptor for fibronectin and fibrinogen |
| CD56 | Neural Cell Adhesion Molecule 1 | Cell adhesion molecule involved in neuron-neuron adhesion, neurite fasciculation, outgrowth of neurites |
| CD62P | P-selectin | Mediator of interaction between activated endothelial cells or platelets with leukocytes |
| CD63 | Tetraspanin super-family – EV-surface protein | Modulator of signal transduction |
| CD69 | Early activation antigen | Signal transmitting receptor in lymphocytes, natural killer cells, and platelets |
| CD81 | Tetraspanin super-family – EV-surface protein | Modulator of signal transduction |
| CD86 | T-lymphocyte activation antigen | Co-stimulator of T cells proliferation and interleukin-2 production |
| CD105 | Endoglin | Vascular endothelium glycoprotein that regulates angiogenesis |
| CD133/1 | Prominin-1 | Regulator of cell differentiation, proliferation and apoptosis |
| CD142 | Tissue factor | Coagulation regulator |
| CD146 | Melanoma Cell Adhesion Molecule | Cell adhesion molecule |
| CD209 | C-type lectin receptor | Pathogen-recognition receptor |
| CD326 | Epithelial cell adhesion molecule | Cell adhesion regulator |
| HLA-ABC | Major Histocompatibility Complex class I | Immune response regulator |
| HLA-DRDPDQ | Major Histocompatibility Complex class II | Immune response regulator |
| MCSP | Melanoma-associated Chondroitin Sulfate Proteoglycan | Regulator of cell proliferation and migration |
| ROR1 | Neurotrophic Tyrosine Kinase, receptor-related 1 | Neurite growth modulation in central nervous system |
| SSEA-4 | Stage-Specific Embryonic Antigen-4 | Marker of bone-marrow derived very small embryonic-like stem cells |

Description and role for each EV-surface marker included in flow cytometry multiplex analysis

**Figure e-1 MACSPlex Exosome Assay: with and without ultracentrifugation**

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Comparison between samples (n=5) with EV enrichment (W) by ultracentrifugation vs. without EV enrichment (W/O). The heatmap reports the nMFI (purple = low nMFI; yellow = high nMFI) for the 37 EV-surface markers evaluated by flow cytometry and median values for the two protocols.

**Figure e-2. Technical validation of the MACSPlex Exosome Assay**

**Immagine che contiene disegnando

Descrizione generata automaticamente**

(A) Comparison of triplicates of sample A vs. sample B analyzed the same day (delta mean:0.4; st.dev.:4.5); (B) Comparison of duplicates of sample C, D, and E analyzed in 2 consecutive days (delta mean:0.5; st.dev.:1.9). (C) Comparison of samples from the same patient taken at day 1, day 2 and day 60 (delta mean:0.7; st.dev.:5.6). The heatmap reports the nMFI (purple = low nMFI; yellow = high nMFI) for the 37 EV-surface markers evaluated by flow cytometry.

**Table e-2 Clinical diagnosis of atypical parkinsonisms**

|  |  |  |  |
| --- | --- | --- | --- |
| **Subject n°** | **Diagnosis MSA** | **Subject n°** | **Diagnosis AP-Tau** |
| #14 | Prob. MSA-C | #31 | Prob. PSP-RS |
| #17 | Prob. MSA-C | #32 | Poss. CBD |
| #22 | Prob. MSA-C | #40 | Poss. CBD |
| #26 | Prob. MSA-P | #42 | Prob. PSP-RS |
| #35 | Prob. MSA-C | #43 | Prob. PSP-RS |
| #55 | Prob. MSA-P | #57 | Poss. CBD |
| #59 | Prob. MSA-P | #58 | Prob. PSP-F |
| #60 | Prob. MSA-P | #63 | Prob. PSP-RS |
|  |  | #64 | Prob. PSP-F |

Clinical diagnosis of probable (prob.) Multisystem atrophy (MSA) according to the second consensus diagnostic criteria for MSA11; clinical diagnosis of prob. Progressive Supranuclear Palsy (PSP) according to the Movement Disorder Society criteria12; clinical diagnosis of possible (poss.) Corticobasal degeneration (CBD) according to the criteria for the diagnosis of CBD13.

MSA-P= MSA with predominant parkinsonism; MSA-C= MSA with predominant cerebellar ataxia; PSP-RS= PSP with Richardson's syndrome; PSP-F= PSP with predominant frontal presentation.

**Table e-3 Nanoparticle characterization**

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Variable** | **HC**  [n=19] | **PD**  [n=27] | **AP** | | **Overall *P*-value** | ***Pairwise Comparisons*** | | | | | |
| **MSA**  [n=8] | **AP-Tau**  [n=9] | HC  *vs.*  PD | HC  *vs.*  MSA | HC  *vs.*  AP-Tau | PD  *vs.*  MSA | PD  *vs.*  AP-Tau | MSA  *vs.*  AP-Tau |
| Diameter (nm) | 132  [123-138] | 136  [107-144] | 140  [123-154] | 133  [127-152] | 0.866 | - | - | - | - | - | - |
| Nanoparticle concentration (N/ml)  [all nanoparticles\*E12] | 1.04  [0.54-1.28] | 2.38  [1.60-3.52] | 1.11  [0.77-3.52] | 0.66  [0.30-1.57] | **<0.001** | **0.001** | 1.000 | 1.000 | 0.497 | **0.001** | 0.731 |
| Nanoparticle concentration (N/ml) [30-150nm \*E12] | 0.75  [0.45-1.06] | 1.99  [1.21-2.92] | 0.67  [0.56-2.48] | 0.35  [0.20-1.23] | **<0.001** | **0.002** | 1.000 | 1.000 | 0.503 | **0.002** | 0.898 |
| Nanoparticle concentration (N/ml) [151-500nm \*E12] | 0.21  [0.17-0.38] | 0.59  [0.34-0.99] | 0.43  [0.20-1.05] | 0.19  [0.09-0.37] | **<0.001** | **0.001** | 0.811 | 1.000 | 1.000 | **0.005** | 0.554 |
| MFI CD9-CD63-CD81 | 5.58  [3.01-12.96] | 10.14  [5.99-18.15] | 7.66  [5.43-13.44] | 5.86  [5.07-9.98] | **0.048** | **0.023** | 0.178 | 0.721 | 0.328 | **0.037** | 0.897 |

Nanoparticle concentration and diameters at Nanoparticle tracking analysis (NTA) is reported for subjects with PD, MSA, AP-Tau and HC. Mean Median Fluorescence Intensity (MFI) of CD9, CD63, and CD81 after flow cytometry analysis reported. Data are expressed as median and interquartile range. *P-*values < 0.05 were considered significant and shown in bold.

**Table e-4 Normalized Median Fluorescence Intensity of all 37 EV-surface markers**

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Variable** | **HC**  [n=19] | **PD**  [n=27**]** | **AP** | | **Overall *P*-value** | ***Pairwise Comparisons*** | | | | | |
| **MSA**  [n=8] | **AP-Tau**  [n=9] | HC  *vs.*  PD | HC  *vs.*  MSA | HC  *vs.*  AP-Tau | PD  *vs.*  MSA | PD  *vs.*  AP-Tau | MSA  *vs.*  AP-Tau |
| CD1c | 9.68  [2.00-16.53] | 20.98  [8.03-48.27] | 11.12  [5.96-40.66] | 6.74  [2.10-22.00] | **0.030** | **0.035** | 1.000 | 1.000 | 1.000 | 0.285 | 1.000 |
| CD2 | 0.00  [0.00-4.00] | 11.14  [0.88-20.85] | 9.31  [4.33-19.34] | 0.00  [0.00-3.31] | **0.001** | **0.003** | **0.045** | 1.000 | 1.000 | **0.023** | **0.049** |
| CD3 | 5.71  [1.05-10.23] | 10.57  [1.26-23.51] | 19.67  [0.44-46.10] | 6.05  [0.06-16.37] | 0.541 | - | - | - | - | - | - |
| CD4 | 7.86  [4.36-17.23] | 20.93  [8.52-48.47] | 23.88  [8.42-73.66] | 10.90  [7.86-27.00] | **0.032** | **0.033** | **0.045** | 1.000 | 1.000 | 1.000 | 1.000 |
| CD8 | 33.56  [16.25-52.11] | 52.69  [26.20-87.12] | 37.13  [24.01-48.27] | 30.08  [21.12-70.96] | 0.121 | - | - | - | - | - | - |
| CD9 | 52.51  [27.61-81.31] | 61.16  [17.17-111.35] | 45.28  [19.49-90.88] | 38.84  [23.97-82.04] | 0.985 | - | - | - | - | - | - |
| CD11c | 3.83  [0.00-7.35] | 16.70  [8.75-57.46] | 18.45  [11.43-22.40] | 7.50  [1.40-30.91] | **0.011** | **0.007** | **0.041** | 0.786 | 1.000 | 1.000 | 1.000 |
| CD14 | 9.17  [0.00-23.15] | 10.11  [0.00-60.33] | 23.31  [0.00-67.23] | 2.64  [0.00-13.46] | 0.704 | - | - | - | - | - | - |
| CD19 | 6.78  [1.54-25.38] | 28.40  [13.40-60.07] | 32.43  [14.75-63.63] | 8.61  [4.96-36.81] | **0.006** | **0.015** | **0.032** | 1.000 | 1.000 | 0.453 | **0.047** |
| CD20 | 12.05  [3.56-15.67] | 16.15  [4.26-30.0] | 7.13  [4.73-12.98] | 12.68  [1.69-42.99] | 0.643 | - | - | - | - | - | - |
| CD24 | 3.60  [0.00-22.31] | 18.09  [2.62-39.36] | 26.74  [14.34-57.92] | 6.62  [0.92-20.77] | 0.151 | - | - | - | - | - | - |
| CD25 | 1.91  [0.00-11.14] | 9.85  [4.00-18.59] | 14.84  [1.23-19.82] | 7.07  [4.64-26.73] | **0.039** | **0.037** | 0.705 | **0.044** | 1.000 | 1.000 | 1.000 |
| CD29 | 10.79  [5.34-15.41] | 25.94  [7.90-72.83] | 39.76  [19.30-62.70] | 11.78  [4.29-44.97] | **0.042** | 0.491 | **0.032** | 1.000 | 1.000 | 1.000 | 1.000 |
| CD31 | 10.68  [4.07-14.87] | 17.63  [6.62-32.38] | 20.65  [11.53-40.63] | 18.84  [10.33-67.37] | **0.045** | **0.046** | **0.039** | **0.046** | 1.000 | 1.000 | 1.000 |
| CD40 | 4.59  [0.00-14.23] | 25.22  [7.99-52.59] | 28.37  [11.64-60.37] | 20.69  [6.47-36.87] | **0.004** | **0.010** | **0.019** | **0.22** | 1.000 | 1.000 | 1.000 |
| CD41b | 22.88  [8.42-30.99] | 44.98  [28.49-96.75] | 27.76  [9.80-79.51] | 35.24  [11.65-78.90] | **0.010** | **0.005** | 1.000 | 0.604 | 1.000 | 1.000 | 1.000 |
| CD42a | 33.59  [14.38-55.63] | 59.81  [43.07-100.37] | 91.11  [25.01-309.05] | 89.88  [43.42-133.55] | **0.043** | **0.019** | **0.034** | **0.028** | 1.000 | 1.000 | 1.000 |
| CD44 | 12.15  [0.00-21.87] | 12.99  [5.27-27.83] | 24.74  [9.79-35.84] | 17.58  [0.00-35.23] | 0.491 | - | - | - | - | - | - |
| CD45 | 6.17  [1.72-16.74] | 20.49  [10.84-49.29] | 13.01  [2.40-77.20] | 11.73  [6.38-33.73] | **0.026** | **0.015** | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 |
| CD49e | 0.85  [0.00-2.19] | 10.25  [0.00-24.00] | 4.05  [0.75-6.63] | 13.14  [0.00-31.64] | 0.174 | - | - | - | - | - | - |
| CD56 | 0.00  [0.00-13.74] | 10.11  [0.00-27.23] | 30.25  [2.61-63.85] | 10.75  [3.32-20.44] | 0.139 | - | - | - | - | - | - |
| CD62P | 29.73  [11.90-56.62] | 58.13  [27.22-102.25] | 91.37  [36.33-215.78] | 49.14  [12.28-95.54] | **0.029** | **0.026** | **0.029** | 1.000 | 1.000 | 1.000 | 0.731 |
| CD63 | 50.63  [35.79-74.73] | 70.83  [47.01-84.90] | 69.43  [42.14-98.56] | 50.38  [26.95-60.64] | 0.155 | - | - | - | - | - | - |
| CD69 | 13.01  [5.17-46.78] | 44.53  [12.53-58.58] | 18.44  [5.12-31.18] | 15.67  [4.434-44.57] | 0.154 | - | - | - | - | - | - |
| CD81 | 188.98  [139.75-216.13] | 195.49  [151.51-239.15] | 168.14  [127.31- 206.35] | 202.95  [160.40-232.28] | 0.663 | - | - | - | - | - | - |
| CD86 | 6.57  [0.00-13.56] | 10.00  [0.53-22.94] | 12.32  [0.32-20.26] | 3.36  [0.71-33.90] | 0.674 | - | - | - | - | - | - |
| CD105 | 8.81  [0.00-23.86] | 21.00  [4.00-39.43] | 8.61  [0.89-37.22] | 10.61  [0.00-42.70] | 0.292 | - | - | - | - | - | - |
| CD133/1 | 12.89  [1.87-27.09] | 26.01  [10.20-45.10] | 20.96  [11.27-34.65] | 8.35  [6.60-36.56] | 0.147 | - | - | - | - | - | - |
| CD142 | 4.57  [0.36-12.34] | 9.47  [0.77-23.00] | 11.10  [0.35-49.58] | 4.36  [0.94-39.38] | 0.769 | - | - | - | - | - | - |
| CD146 | 1.48  [0.00-4.72] | 8.19  [2.97-17.37] | 9.20  [5.13-25.84] | 3.64  [0.15-11.74] | **0.013** | **0.024** | **0.036** | 1.000 | 1.000 | 1.000 | 0.886 |
| CD209 | 0.00  [0.00-6.12] | 13.22  [3.05-47.50] | 15.60  [2.79-37.48] | 5.47  [0.00-25.67] | **0.007** | **0.004** | **0.028** | 1.000 | 1.000 | 0.991 | 1.000 |
| CD326 | 4.53  [0.00-7.32] | 10.54  [1.71-22.65] | 11.99  [1.59-30.18] | 0.92  [0.00-17.51] | 0.079 | - | - | - | - | - | - |
| HLA-ABC | 11.35  [0.00-21.52] | 26.93  [12.75-44.79] | 30.32  [10.53-99.92] | 43.02  [4.91-52.47] | **0.036** | **0.029** | **0.045** | 0.604 | 1.000 | 1.000 | 1.000 |
| HLA-DRDPDQ | 39.64  [11.0-64.68] | 45.19  [24.31-73.84] | 56.51  [33.90-121.73] | 58.74  [3.05-76.74] | 0.238 | - | - | - | - | - | - |
| MCSP | 0.00  [0.00-5.79] | 8.23  [5.62-21.38] | 0.09  [0.00-27.99] | 5.91  [1.07-22.06] | **0.002** | **0.001** | 1.000 | 0.237 | 0.375 | 1.000 | 1.000 |
| ROR1 | 13.44  [4.19-31.93] | 31.74  [5.59-49.11] | 28.19  [12.55-78.87] | 8.67  [5.44-46.69] | 0.316 | - | - | - | - | - | - |
| SSEA-4 | 12.00  [0.00-18.37] | 27.04  [10.77-52.05] | 28.81  [12.01-45.69] | 9.50  [0.54-68.85] | 0.079 | - | - | - | - | - | - |

Median Fluorescence Intensity (MFI) normalized for mean MFI of CD9, CD63, and CD81 (nMFI) of all EV-surface markers analyzed by flow cytometry in patients with PD, MSA and AP-Tau, compared to HC. Data are expressed as median and interquartile range. *P-*values < 0.05 were considered significant and shown in bold.

**Table e-5. Network topological analysis**

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Gene name** | **Protein names** | **PD *vs.* HC** | | | **MSA *vs.* HC** | | | **AP-Tau *vs.* HC** | | |
| **Betweenness** | **Bridging** | **Centroid** | **Betweenness** | **Bridging** | **Centroid** | **Betweenness** | **Bridging** | **Centroid** |
| ITGB2 | Integrin beta-2 | 4200 | 36.4 | -732 | 5190 | 28 | -729 | - | - | - |
| C1QBP | Complement component 1 Q subcomponent-binding protein | 2822 | 28.9 | -724 | 2576 | 28.5 | -750 | 463 | 18.6 | -127 |
| CALR | Calreticulin | 2262 | 26.8 | -711 | 1891 | 22 | -724 | - | - | - |
| EZR | Ezrin | 1584 | 20.7 | -707 | 1571 | 19.1 | -729 | - | - | - |
| NCK2 | Cytoplasmic protein NCK2 | 1496 | 18.6 | -749 | - | - | - | - | - | - |
| SP1 | Transcription factor Sp1 | 1271 | 15.4 | -720 | 1107 | 17.9 | -766 | - | - | - |
| CLU | Clusterin | 1252 | 32.3 | -766 | - | - | - | - | - | - |
| HLA-E | HLA class I histocompatibility antigen, alpha chain E | 1194 | 22.4 | -757 | - | - | - | - | - | - |
| MSN | Moesin | 1170 | 22.2 | -722 | 1328 | 20.7 | -737 | - | - | - |
| B2M | Beta-2-microglobulin | - | - | - | 5207 | 15.8 | -724 | - | - | - |
| LYN | Tyrosine-protein kinase Lyn | - | - | - | 3610 | 15.1 | -668 | - | - | - |
| CEACAM1 | Carcinoembryonic antigen-related cell adhesion molecule 1 | - | - | - | 1594 | 70.2 | -775 | - | - | - |
| ITGA2 | Integrin alpha-2 | - | - | - | 1305 | 18.4 | -781 | - | - | - |
| LGALS3BP | Galectin-3-binding protein | - | - | - | 1303 | 30.7 | -751 | - | - | - |
| DYNLL1 | Dynein light chain 1, cytoplasmic | - | - | - | 1157 | 17.9 | -797 | - | - | - |
| SLC3A2 | 4F2 cell-surface antigen heavy chain | - | - | - | 1081 | 15.2 | -735 | - | - | - |
| PDCD6IP | Programmed cell death 6-interacting protein | - | - | - | 1075 | 24.6 | -779 | - | - | - |
| UBC | Polyubiquitin-C | - | - | - | - | - | - | 10051 | 5.4 | 71 |
| PIK3R1 | Phosphatidylinositol 3-kinase regulatory subunit alpha | - | - | - | - | - | - | 1095 | 8.2 | -85 |
| FN1 | Fibronectin | - | - | - | - | - | - | 606 | 7.6 | -112 |
| YWHAZ | 14-3-3 protein zeta/delta | - | - | - | - | - | - | 440 | 6.3 | -106 |
| STAT3 | Signal transducer and activator of transcription 3 | - | - | - | - | - | - | 425 | 4.8 | -96 |
| FLNA | Filamin-A | - | - | - | - | - | - | 416 | 7.7 | -111 |
| NFKB1 | Nuclear factor NF-kappa-B p105 subunit | - | - | - | - | - | - | 414 | 4.2 | -101 |
| GP1BB | Platelet glycoprotein Ib beta chain | - | - | - | - | - | - | 334 | 10.2 | -125 |
| VWF | von Willebrand factor | - | - | - | - | - | - | 295 | 13.7 | -128 |
| HSPA4 | Heat shock 70 kDa protein 4 | - | - | - | - | - | - | 223 | 5.5 | -128 |
| VIM | Vimentin | - | - | - | - | - | - | 190 | 7.1 | -127 |
| PTPN6 | Tyrosine-protein phosphatase non-receptor type 6 | - | - | - | - | - | - | - | - | - |
| ABL1 | Tyrosine-protein kinase ABL1 | - | - | - | - | - | - | - | - | - |
| ITK | Tyrosine-protein kinase ITK/TSK | - | - | - | - | - | - | - | - | - |
| NCK1 | Cytoplasmic protein NCK1 | - | - | - | - | - | - | - | - | - |
| AKT1 | RAC-alpha serine/threonine-protein kinase | - | - | - | - | - | - | - | - | - |
| LCK | Tyrosine-protein kinase Lck | - | - | - | - | - | - | - | - | - |
| FYN | Tyrosine-protein kinase Fyn | - | - | - | - | - | - | - | - | - |
| **Average network values** | | **1001** | **15.0** | **-767** | **1062** | **14.8** | **-798** | **179** | **4.3** | **-133** |

Hubs and bottlenecks selected by the topological evaluation of PPI (Protein-Protein Interaction) sub-networks reconstructed considering the first neighbors of each differentially expressed EV-surface marker protein, per comparison PD *vs.* HC; MSA *vs.* HC; AP-Tau *vs.* HC. Betweenness, Bridging and Centroid were calculated and only nodes with all values above the average derived from the whole network were retained; EV-surface marker proteins were removed from this list.

**Table e-6 Functional evaluation by DAVID database**

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **ID Term** | **Term** | **PD** *vs.* **HC** | | | **MSA** *vs.* **HC** | | | **AP-Tau** *vs*. **HC** | | |
| **Count** | ***P*-value** | **Bonferroni** | **Count** | ***P*-value** | **Bonferroni** | **Count** | ***P*-value** | **Bonferroni** |
| **Immune system** | |  |  |  |  |  |  |  |  |  |
| hsa04640 | Hematopoietic cell lineage | 40 | 2.5E-20 | 6.7E-18 | 44 | 2.5E-22 | 6.5E-20 | 16 | 2.8E-10 | 4.9E-08 |
| hsa04612 | Antigen processing and presentation | 36 | 8.0E-19 | 2.1E-16 | 36 | 3.7E-17 | 9.7E-15 | - | - | - |
| hsa04672 | Intestinal immune network for IgA production | 16 | 2.8E-06 | 7.4E-04 | 19 | 7.1E-08 | 1.9E-05 | - | - | - |
| hsa04670 | Leukocyte trans-endothelial migration | 29 | 1.1E-07 | 2.8E-05 | 42 | 4.9E-15 | 1.3E-12 | 11 | 1.5E-04 | 2.6E-02 |
| hsa04660 | T cell receptor signaling pathway | 46 | 2.3E-23 | 6.0E-21 | 46 | 3.3E-21 | 8.5E-19 | 29 | 1.3E-24 | 2.4E-22 |
| hsa04611 | Platelet activation | 37 | 3.1E-11 | 8.1E-09 | 36 | 3.6E-09 | 9.5E-07 | 20 | 2.0E-11 | 3.6E-09 |
| hsa04062 | Chemokine signaling pathway | 39 | 9.5E-08 | 2.5E-05 | 40 | 7.4E-07 | 1.9E-04 | 24 | 4.7E-12 | 8.2E-10 |
| hsa04620 | Toll-like receptor signaling pathway | 27 | 2.6E-07 | 6.9E-05 | 29 | 2.1E-07 | 5.5E-05 | 19 | 5.2E-12 | 9.2E-10 |
| hsa04610 | Complement and coagulation cascades | 20 | 1.7E-06 | 4.4E-04 | 21 | 2.4E-06 | 6.4E-04 | 11 | 1.6E-06 | 2.8E-04 |
| hsa04622 | RIG-I-like receptor signaling pathway | 18 | 3.6E-05 | 9.5E-03 | 18 | 1.6E-04 | 4.2E-02 | 14 | 1.7E-09 | 3.1E-07 |
| hsa04621 | NOD-like receptor signaling pathway | 16 | 3.0E-05 | 7.9E-03 | 16 | 1.2E-04 | 3.1E-02 | 12 | 1.8E-08 | 3.1E-06 |
| hsa04664 | Fc epsilon RI signaling pathway | 24 | 1.7E-09 | 4.5E-07 | 26 | 5.5E-10 | 1.4E-07 | 15 | 9.3E-11 | 1.6E-08 |
| hsa04662 | B cell receptor signaling pathway | 30 | 1.8E-14 | 4.7E-12 | 32 | 6.5E-15 | 1.7E-12 | 17 | 5.4E-13 | 9.5E-11 |
| hsa04666 | Fc gamma R-mediated phagocytosis | 23 | 6.6E-07 | 1.7E-04 | 28 | 3.5E-09 | 9.2E-07 | 14 | 1.8E-08 | 3.1E-06 |
| hsa04650 | Natural killer cell mediated cytotoxicity | 38 | 7.8E-13 | 2.1E-10 | 40 | 1.3E-12 | 3.4E-10 | 16 | 3.4E-08 | 6.0E-06 |
| **Signal transduction** | |  |  |  |  |  |  |  |  |  |
| hsa04012 | ErbB signaling pathway | 30 | 1.9E-11 | 4.9E-09 | 32 | 1.2E-11 | 3.0E-09 | 16 | 2.8E-10 | 4.9E-08 |
| hsa04066 | HIF-1 signaling pathway | 22 | 2.5E-05 | 6.6E-03 | 29 | 2.0E-08 | 5.3E-06 | 9 | 9.3E-04 | 1.5E-01 |
| hsa04015 | Rap1 signaling pathway | 33 | 4.4E-04 | 1.1E-01 | 42 | 2.7E-06 | 7.1E-04 |  |  |  |
| hsa04151 | PI3K-Akt signaling pathway | 47 | 6.3E-04 | 1.5E-01 | 90 | 1.6E-20 | 4.3E-18 | 29 | 5.1E-10 | 8.9E-08 |
| hsa04064 | NF-kappa B signaling pathway | 28 | 5.9E-10 | 1.6E-07 | 30 | 3.5E-10 | 9.0E-08 | 20 | 1.0E-14 | 1.8E-12 |
| hsa04668 | TNF signaling pathway | 29 | 2.0E-08 | 5.3E-06 | 30 | 6.9E-08 | 1.8E-05 | 22 | 3.6E-15 | 6.3E-13 |
| hsa04014 | Ras signaling pathway | - | - | - | 41 | 4.1E-05 | 1.1E-02 | 18 | 5.1E-06 | 8.9E-04 |
| hsa04010 | MAPK signaling pathway | - | - | - | 40 | 9.6E-04 | 2.2E-01 | 19 | 5.8E-06 | 1.0E-03 |
| hsa04370 | VEGF signaling pathway | - | - | - | 17 | 9.5E-05 | 2.4E-02 | - | - | - |
| hsa04630 | Jak-STAT signaling pathway | 26 | 2.8E-04 | 7.1E-02 | - | - | - | 22 | 1.9E-12 | 3.4E-10 |
| hsa04071 | Sphingolipid signaling pathway | 25 | 3.3E-05 | 8.6E-03 | - | - | - | 18 | 4.2E-10 | 7.3E-08 |
| hsa04068 | FoxO signaling pathway | - | - | - | - | - | - | 11 | 5.3E-04 | 8.8E-02 |
| **Endocrine system** | |  |  |  |  |  |  |  |  |  |
| hsa04915 | Estrogen signaling pathway | 22 | 4.1E-05 | 1.1E-02 | 25 | 8.0E-06 | 2.1E-03 | - | - | - |
| hsa04917 | Prolactin signaling pathway | 24 | 4.5E-09 | 1.2E-06 | 20 | 1.6E-05 | 4.1E-03 | 20 | 1.6E-16 | 2.0E-14 |
| hsa04910 | Insulin signaling pathway | 25 | 3.2E-04 | 8.2E-02 | - | - | - | 15 | 1.1E-06 | 2.0E-04 |
| hsa04920 | Adipocytokine signaling pathway | - | - | - | - | - | - | 12 | 2.0E-07 | 3.5E-05 |
| hsa04919 | Thyroid hormone signaling pathway | - | - | - | - | - | - | 11 | 1.5E-04 | 2.6E-02 |
| **Signaling molecules and interaction cellular**  **community, cell motility** | | | |  |  |  |  |  |  |  |
| hsa04514 | Cell adhesion molecules (CAMs) | 42 | 2.7E-13 | 7.1E-11 | 51 | 6.6E-18 | 1.7E-15 | 12 | 2.0E-04 | 3.4E-02 |
| hsa04510 | Focal adhesion | 42 | 6.3E-08 | 1.7E-05 | 86 | 6.1E-36 | 1.6E-33 | 24 | 4.0E-11 | 7.0E-09 |
| hsa04512 | ECM-receptor interaction | 18 | 6.1E-04 | 1.5E-01 | 50 | 9.5E-29 | 2.5E-26 | 12 | 1.9E-06 | 3.3E-04 |
| hsa04810 | Regulation of actin cytoskeleton | 35 | 8.8E-05 | 2.3E-02 | 59 | 5.9E-15 | 1.5E-12 | 14 | 4.9E-04 | 8.2E-02 |
| hsa04520 | Adherens junction |  |  |  | 19 | 5.7E-05 | 1.5E-02 | 8 | 7.2E-04 | 1.2E-01 |
| **Excretory system** | |  |  |  |  |  |  |  |  |  |
| hsa04961 | Endocrine and other factor-regulated calcium reabsorption | 15 | 8.4E-06 | 2.2E-03 | 15 | 3.3E-05 | 8.6E-03 | - | - | - |
| hsa04960 | Aldosterone-regulated sodium reabsorption | 11 | 9.3E-04 | 2.2E-01 | - | - | - | - | - | - |
| **Folding, sorting and degradation** | |  |  |  |  |  |  |  |  |  |
| hsa03050 | Proteasome | 17 | 1.7E-07 | 4.4E-05 | 17 | 8.7E-07 | 2.3E-04 | - | - | - |
| hsa04141 | Protein processing in endoplasmic reticulum | 42 | 1.3E-10 | 3.3E-08 | 44 | 4.0E-10 | 1.1E-07 | - | - | - |
| **Transport and catabolism** | |  |  |  |  |  |  |  |  |  |
| hsa04145 | Phagosome | 41 | 8.9E-12 | 2.3E-09 | 48 | 1.4E-14 | 3.7E-12 | - | - | - |
| hsa04144 | Endocytosis | 47 | 3.7E-08 | 9.8E-06 | 49 | 2.0E-07 | 5.2E-05 | - | - | - |
| **Other KEGG categories** | |  |  |  |  |  |  |  |  |  |
| hsa04380 | Osteoclast differentiation | 39 | 1.8E-12 | 4.7E-10 | 37 | 1.2E-09 | 3.1E-07 | 24 | 2.1E-15 | 3.7E-13 |
| hsa04722 | Neurotrophin signaling pathway | 32 | 4.8E-09 | 1.3E-06 | 32 | 8.2E-08 | 2.1E-05 | 19 | 4.6E-11 | 8.1E-09 |
| hsa04210 | Apoptosis | 19 | 1.4E-06 | 3.6E-04 | 19 | 7.6E-06 | 2.0E-03 | 12 | 5.4E-08 | 9.6E-06 |

KEGG pathways enriched by considering the first neighbors of each differentially expressed EV-surface marker protein per comparison PD *vs.* HC; MSA *vs.* HC; AP-Tau *vs.* HC; data were filtered using a gene count > 5 and a *P*-value <0.001.

**Table e-7 Correlation between clinical scales and EV-surface markers’ fluorescence**

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Pearson’s R**  *P*-value | | **Age (years)** | **Disease Duration (years)** | **H &Y** | **MDS-UPDRS** | **BDI-II** | **MMSE** | **MoCA** | **Olfactory test** | **RBD** | **LEDD** |
| PD | CD1c | 0.037  0.856 | -0.059  0.770 | 0.194  0.331 | 0.073  0.729 | 0.126  0.549 | -0.186  0.364 | -0.236  0.246 | 0.335  0.313 | -0.401  **0.042** | -0.009  0.967 |
| CD25 | 0.049  0.809 | 0.054  0.790 | 0.403  **0.037** | 0.253  0.223 | -0.195  0.350 | -0.531  **0.005** | -0.636  **<0.001** | -0.265  0.432 | -0.109  0.596 | 0.178  0.395 |
| CD31 | -0.123  0.542 | 0.358  0.067 | -0.201  0.315 | 0.297  0.149 | -0.044  0.835 | -0.473  **0.015** | -0.372  0.061 | -0.350  0.271 | 0.080  0.697 | 0.235  0.259 |
| CD40 | 0.160  0.426 | -0.152  0.450 | 0.072  0.722 | -0.014  0.947 | -0.204  0.328 | -0.201  0.324 | -0.449  **0.021** | -0.044  0.898 | -0.206  0.312 | -0.178  0.393 |
| CD62P | 0.192  0.338 | 0.204  0.308 | 0.185  0.356 | 0.316  0.124 | 0.505  **0.010** | -0.160  0.435 | -0.045  0.827 | 0.366  0.268 | 0.175  0.393 | 0.342  0.094 |
| CD146 | 0.264  0.183 | 0.259  0.192 | 0.117  0.560 | 0.285  0.167 | -0.034  0.874 | -0.566  **0.003** | -0.386  0.051 | -0.328  0.324 | 0.104  0.613 | 0.342  0.094 |
| HLA-ABC | 0.177  0.377 | 0.186  0.353 | 0.103  0.609 | 0.380  0.061 | 0.290  0.159 | -0.446  **0.022** | -0.425  **0.030** | 0.072  0.833 | -0.171  0.405 | 0.241  0.246 |
| Nanoparticle concentration | -0.270  0.173 | 0.382  **0.049** | -0.281  0.155 | -0.024  0.907 | -0.352  0.084 | 0.094  0.647 | 0.068  0.741 | 0.143  0.675 | -0.232  0.255 | 0.238  0.251 |
| MFI CD9-CD63-CD81 | -0.314  0.111 | 0.166  0.407 | -0.194  0.333 | 0.296  0.151 | 0.132  0.528 | -0.012  0.952 | -0.024  0.909 | -0.468  0.147 | 0.314  0.118 | 0.141  0.503 |
| MSA | CD31 | 0.125  0.768 | 0.011  0.979 | -0.789  **0.035** | -0.406  0.462 | -0.369  0.472 | -0.358  0.430 | 0.107  0.840 | 0.427  0.398 | -0.058  0.892 | 0.309  0.800 |
| Nanoparticle  concentration | 0.449  0.265 | 0.731  **0.039** | -0.150  0.749 | 0.533  0.615 | -0.079  0.882 | -0.260  0.574 | -0.312  0.548 | -0.440  0.383 | -0.633  0.092 | 0.170  0.891 |
| MFI CD9-CD63-CD81 | 0.038  0.929 | -0.085  0.844 | -0.191  0.681 | 0.319  0.634 | 0.004  0.994 | -0.662  0.105 | -0.893  **0.016** | -0.188  0.722 | -0.152  0.720 | 0.231  0.497 |
| AP-Tau | Nanoparticle  concentration | 0.619  0.075 | -0.780  0.130 | -0.178  0.646 | -0.118  0.925 | 0.261  0.532 | 0.449  0.313 | 0.563  0.323 | 0.253  0.546 | -0.159  0.684 | -0.412  0.358 |
| MFI CD9-CD63-CD81 | 0.358  0.344 | -0.569  0.110 | -0.330  0.386 | 0.882  0.313 | 0.422  0.297 | 0.274  0.553 | 0.329  0.589 | 0.244  0.561 | -0.014  0.972 | -0.688  0.087 |

Correlations between clinical scales and a) normalized MFI (nMFI) for differentially expressed EV-surface markers, b) CD9-CD63-CD81 mean Median Fluorescence Intensity (MFI) and c) nanoparticle concentration, between HC and pathological groups (PD; MSA; AP-Tau). Pearson’s R coefficient (above) and *P*-value (below) are reported for each correlation. The correlation was considered strong for Pearson’s R between |1.0| and |0.5|, moderate between |0.5| and |0.3|, weak between |0.3| and |0.1|. The correlation was direct for R>0, or inverse for R<0. 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 Daily Dose). *P-*values < 0.05 were considered significant and shown in bold. Not significant correlations were not reported.

**Figure e-3 Univariate analysis of EV-surface markers**

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Logistic regression analysis was performed to assess the odd ratio (OR) and 95% confidence intervals (95% CI) for EV-surface markers differentially expressed between HC and each pathological group (PD; MSA; AP-Tau). An OR greater than 1 indicates an increased likelihood of diagnosis (PD, MSA, AP-Tau); *P-*values < 0.05 were considered significant. Not significant associations were not reported.

**Table e-8 ROC curve analysis of each EV-surface markers**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **Marker** | **Asymptotical significance** | **AUC (95% CI)** | **Sensitivity (%)** | **Specificity (%)** |
|
| PD  *vs.* HC | CD1c | 0.005 | 0.743 (0.601-0.884) | 63.0 | 78.9 |
| CD4 | 0.011 | 0.723 (0.573-0.874) | 70.4 | 68.4 |
| CD11c | 0.002 | 0.775 (0.635-0.915) | 81.5 | 78.9 |
| CD19 | 0.003 | 0.758 (0.613-0.903) | 77.7 | 73.7 |
| CD25 | 0.007 | 0.737 (0.580-0.893) | 96.3 | 52.6 |
| CD31 | 0.051 | 0.671 (0.513-0.828) | 59.3 | 78.9 |
| CD40 | 0.003 | 0.760 (0.623-0.898) | 55.5 | 100.0 |
| CD42a | 0.015 | 0.713 (0.561-0.865) | 70.4 | 73.7 |
| CD45 | 0.002 | 0.770 (0.628-0.912) | 100.0 | 52.6 |
| CD62P | 0.013 | 0.717 (0.569-0.866) | 74.1 | 63.2 |
| CD146 | 0.005 | 0.744 (0.602-0.885) | 74.1 | 73.7 |
| CD209 | 0.001 | 0.793 (0.654-0.933) | 100.0 | 52.6 |
| HLA-ABC | 0.009 | 0.727 (0.579-0.876) | 85.2 | 57.9 |
| MSA  *vs.* HC | CD2 | 0.019 | 0.789 (0.559-1.000) | 87.5 | 73.7 |
| CD4 | 0.042 | 0.734 (0.536-0.949) | 75.0 | 68.4 |
| CD11c | 0.036 | 0.760 (0.545-0.975) | 87.5 | 78.9 |
| CD31 | 0.039 | 0.744 (0.534-0.976) | 75.0 | 78.9 |
| CD42a | 0.048 | 0.723 (0.467-0.964) | 50.0 | 100.0 |
| CD62P | 0.021 | 0.786 (0.543-1.000) | 75.0 | 94.7 |
| CD146 | 0.014 | 0.806 (0.613-0.999) | 87.5 | 73.7 |
| CD209 | 0.034 | 0.763 (0.567-0.959) | 75.0 | 78.9 |
| HLA-ABC | 0.026 | 0.776 (0.582-0.971) | 62.5 | 84.2 |
| AP-Tau  *vs.* HC | CD25 | 0.041 | 0.743 (0.560-0.925) | 100.0 | 47.4 |

ROC curve analysis of differentially expressed EV-surface markers in patients with PD, MSA and AP-Tau, compared to healthy controls (HC). The table reports AUC (Area Under the Curve), 95% CI (95% confidence interval), asymptotical significance (*vs.* referral line), sensitivity and specificity. *P-*values < 0.05 were considered significant.

**Table e-9 Demographic data and clinical scores of the validation cohort**

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Variable** | **HC**  [n=20] | **PD**  [n=10] | **AP** | | **Overall *P*-value** | ***Pairwise Comparisons*** | | | | | |
| **MSA**  [n=5] | **AP-Tau**  [n=5] | HC  vs.  PD | HC  vs.  MSA | HC  vs.  AP-Tau | PD  vs.  MSA | PD  vs.  AP-Tau | MSA  vs.  AP-Tau |
| Age (years) | 61 ± 9.9 | 67 ± 9.3 | 65 ± 6.4 | 72 ± 13.0 | 0.108 | - | - | - | - | - | - |
| Sex (ref. male) | 8 (40.0%) | 5 (50.0%) | 2 (40.0%) | 2 (40.0%) | 0.959 | - | - | - | - | - | - |
| Disease duration (years) | - | 5.5  [4.0-10.0] | 7.0  [3.5-8.5] | 5.0  [3.5-7.0] | 0.545 | - | - | - | - | - | - |
| H&Y | - | 2.0  [2.0-3.0] | 5.0  [3.0-5.0] | 4.0  [2.5-4.8] | **0.004** | - | - | - | **0.010** | 0.112 | 1.000 |
| MDS-UPDRS | - | 25.00  [14.5-29.0] | 26.5  [24.0-28.0] | 30.5  [19.8-81.5] | 0.227 | - | - | - | - | - | - |
| BDI-II | - | 5.5  [4.0-10.5] | 10.0  [3.5-16.5] | 16.0  [11.8-19.0] | 0.258 | - | - | - | - | - | - |
| MMSE | - | 30.0  [29.0-30.0] | 29.0  [28.0-29.5] | 27.0  [16.3-28.0] | **0.012** | - | - | - | 0.668 | **0.016** | 0.845 |
| MoCA | - | 26.0  [20.0-28.3] | 26.0  [22.5-26.5] | 19.0  [7.8-22.8] | 0.083 | - | - | - | - | - | - |
| Olfactory test | - | 7.5  [4.8-9.3] | 9.0  [5.5-10.5] | 5.0  [4.8-5.0] | 0.565 | - | - | - | - | - | - |
| RBD | - | 5.0  [2.8-6.5] | 1.0  [0.0-3.5] | 1.5  [0.3-6.5] | 0.072 | - | - | - | - | - | - |
| LEDD | - | 481.3  [100.0-1009.3] | 123.0  [0.0-187.5] | 100.0  [93.8-162.5] | 0.087 | - | - | - | - | - | - |

Demographic and clinical characteristics of patient enrolled in the validation cohort: 20 healthy controls (HC), 10 patients with PD, 5 with MSA and 5 with AP-Tau. 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 Behaviour Disorder scale), LEDD (Levodopa Equivalent Daily Dose).