Supporting Information

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Small Extracellular Vesicles Secreted by Nigrostriatal Astrocytes Rescue Cell Death and Preserve Mitochondrial Function in Parkinson’s Disease

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Supporting Information

Figure S1. Characterization of AS primary cultures from VMB, STR and from VMB- and STR-depleted brain regions (ΔVS-AS), under basal and CCL3-treated conditions. A) Immunofluorescence (IF) images show the presence of AS (GFAP⁺ cells, in green) and microglial cells (Iba1⁺ cells, in red), with DAPI⁻ nuclei (in blue). Scale bars: 20 µm. B) IF images show the presence of proliferative AS (GFAP⁺/BrdU⁺ cells, in green and red respectively), with DAPI⁻ nuclei (in blue). Scale bars: 10 µm. C) Quantification of the staining in A: the number of GFAP⁺ and Iba1⁺ cells are normalized over total DAPI⁻ nuclei. Data are expressed as mean ± SD from n=4
(for VMB- and STR-AS) and n=3 (for ΔVS-AS) independent experiments, indicated with different symbols. D) Quantification of the staining in B: the number of BrdU+ cells are normalized over total DAPI+ nuclei, while GFAP+ cells are normalized over BrdU+ cells. Data are expressed as mean ± SD from n=4 (for VMB- and STR-AS) and n=3 (for ΔVS-AS) independent experiments, indicated with different symbols.

E) Analysis of cell viability and death. Data are expressed as mean over VMB-AS ± SD from n=3 independent experiments, indicated with different symbols.
Table S1. Diameter values of AS-EV samples.

| Diameter (nm) | VMB-AS-EVs | VMB-CCL3-AS-EVs | STR-AS-EVs | STR-CCL3-AS-EVs | ΔVS-AS-EVs | ΔVS-CCL3-AS-EVs |
|--------------|------------|-----------------|------------|----------------|-------------|-----------------|
| Minimum      | 28.28      | 22.12           | 24.19      | 24.25          | 18.5        | 23.7            |
| Maximum      | 290.8      | 440.1           | 280.3      | 340.9          | 142.5       | 192.7           |
| Median       | 63.5       | 59.5            | 64.9       | 59.6           | 53.3        | 54.0            |
| Mean         | 72.5       | 68.5            | 75         | 63.5           | 58.7        | 64              |
| Std. Deviation | 14.5      | 14.7            | 7          | 17.8           | 6.8         | 8.5             |
Figure S2. A) qPCR analyses of CCL3 receptors in AS, showing average ΔCt values for Ccr1 and Ccr5. Gusb was used as housekeeping gene. Data are presented as mean ± SD from n=3 independent replicates, indicated with different symbols. One-way ANOVA with Tukey’s multiple comparison test shows that ΔCt values for Ccr1 in ΔVS-AS are significantly higher compared to all the other groups (***p < 0.001); ΔCt values for Ccr5 in ΔVS-AS are significantly higher compared to all the other groups (#####p < 0.0001). B) Semithin sections stained with toluidine blue show differences in the membranes of STR-AS (CCL3 vs. basal) but not in VMB. C) SEM analysis shows that STR-AS bear more irregular membrane protrusions after CCL3 supplementation. Scale bars B: 50 µm, C: 10 µm, inserts: 1 µm.
Figure S3. PKH26-labelled AS-EVs internalization by differentiated and undifferentiated SH-SY5Y cells. A) Single plan confocal images show the uptake of both VMB-AS- and STR-AS-PKH26-labelled EVs by differentiated SH-SY5Y (white dotted squares were shown as max projections in Figure 3A). Scale bar 20 µm. B) 3D reconstruction from all z stacks (see Figure 3A). Scale bars 10 µm. C) IF (in red PKH26 labelled AS-EVs and in blue DAPI counterstained nuclei) and bright field (whole cells) images of differentiated SH-SY5Y upon treatment with PKH26-labeled EVs. EVs are distributed in cell bodies and also in neurites. On the right, PKH26
dye-only were administered to target cells. Scale bars: 20 μM. D) IFC analysis of undifferentiated SH-SY5Y cells treated with PKH26-AS-EVs at different time points. Data are expressed as fold change of the mean fluorescence intensity ± SD over CTRL (dashed line at y axis =1) from n=3 independent experiment, indicated with different symbols. One-way ANOVA with Tukey’s multiple comparison vs. CTRL. **p < 0.01, ****p < 0.0001, ns: not significant.
Figure S4. A) Dose response curve of H$_2$O$_2$ on differentiated SH-SY5Y cells at 24 h. B) Dose response curve of MPP$^+$ on differentiated SH-SY5Y cells at 24 h. C-D) Analysis of cell viability (C) and cell death (D) of differentiated SH-SY5Y neurons treated with CCL3 and challenged with H$_2$O$_2$, expressed as percentage (in C) or fold change (in D) over CTRL. Data are expressed as mean ± SD from n=3 independent replicates, indicated with different symbols. One-way ANOVA with Tukey’s multiple comparison $^*$p < 0.05, $^{**}$p < 0.01 vs. CTRL.
ns: not significant. E) IF staining for MAP2 (in green), c-Casp-3 (in red) and DAPI (in blue), on differentiated SH-SY5Y exposed to cont-EVs and treated with 35 µM H₂O₂. Scale bars: 50 µm. F) Quantification of the c-Casp-3 staining in E. The fluorescent intensity values were normalized over total DAPI+ nuclei. Data are expressed as mean ± SD over CTRL, set to 1 for comparison. G) Caspase 3/7 activities in undifferentiated SH-SY5Y exposed to AS-EVs (ratio 5:1) for 6 h and then treated with 35 µM H₂O₂ for 24 h. Data are expressed as mean ± SD over CTRL, set to 1 for comparison. One-way ANOVA with Tukey’s multiple comparison. In (F) ****p < 0.0001 (CTRL vs. H₂O₂ and vs. H₂O₂ + cont-EVs), ns: not significant. In (G) ***p < 0.001 (CTRL vs. H₂O₂), ns: not significant.
Figure S5 A-D) Analysis of O₂ flows correspondent to the main respiratory states ROUTINE and OXPHOS achieved upon different experimental conditions and/or EV treatment in differentiated (A-B) or undifferentiated (C-D) SH-SY5Y cells. MPP⁺ did not affected respiration in any condition tested. All data are expressed as flux control ratio, as percentage of the maximal respiratory capacity. E-F) Analysis of net and coupling ROUTINE achieved upon different experimental conditions and/or EVs, ACM or SNT treatment in differentiated SH-SY5Y. In this case, MPP⁺ promoted a general and significative decrease of both net and coupling respirations.
However, no effect was observed upon EVs, ACM or SNT treatment. Data are expressed as a flux control ratio, as percentage of specific reference states maximal and basal respiratory capacity for net and coupling respiration, respectively. G-H) Analysis of net and coupling ROUTINE achieved upon different experimental conditions and/or EVs treatment in undifferentiated SH-SY5Y. As for differentiated cells, MPP° promoted a reduction of both parameters which is not restored by EVs. Data are expressed as a flux control ratio, as percentage of specific reference states maximal and basal respiratory capacity for net and coupling respiration, respectively. In (A-H) data are expressed as mean ± SD. One-way ANOVA with Tukey’s multiple comparison was performed, with *p <0.05, **p<0.01 and ****p<0.001 (CTRL vs. MPP°), ns: not significant.