YMTHE, Volume *30*

Supplemental Information

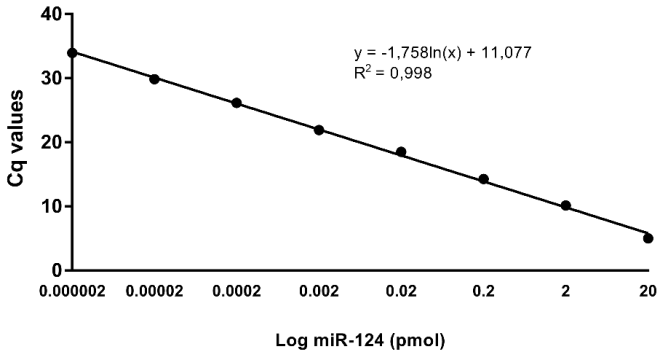
MicroRNA-124-3p-enriched small extracellular vesicles as a therapeutic approach for Parkinson's disease

Marta Esteves, Ricardo Abreu, Hugo Fernandes, Catarina Serra-Almeida, Patrícia A.T. Martins, Marta Barão, Ana Clara Cristóvão, Cláudia Saraiva, Raquel Ferreira, Lino Ferreira, and Liliana Bernardino

Supplementary material

Figures

A



miR-124-3p (pmol)	Average Cq
0,000002	33,94
0,00002	29,85
0,0002	26,16
0,002	21,92
0,02	18,51
0,2	14,29
2	10,13
20	5,02

Sample	Average Cq (3 µg sEV)	Estimated miR-124 quantity (pmol)
Native sEV	31,97	0,00001
miR-124-3p sEV	13,69	0,23

B

Sample	Estimated miR-124 copies number		
	3 µg sEV (6.90 x 10^9 particles)	**6 µg sEV (1.38 x 10^{10} particles)**	**10 µg sEV (2.30 x 10^{10} particles)**
Native sEV	6.022 x 106	1.2 x 10^7	2 x 10^7
miR-124-3p sEV	1.39 x 10^{11}	2.78 x 10^{11}	4.6 x 10^{11}

Figure S1. Copies number extrapolation of miR-124 in sEVs. (A) Calibration curve of miR-124-3p. Correlation analysis between Cq values and log amount of 10-fold serially diluted miR-124-3p (pmol). Each point represents the mean value of duplicate measurements. **(B)** miR-124 copy number extrapolation. Copies number of miR-124 endogenously present in native sEVs or loaded into miR-124-3p sEVs were extrapolated using (**A**) calibration curve of synthetic miR-124-3p. The resultant **(A)** value of miR-124 (pmol) was converted to miRNA molecules by multiplication through Avogadro's constant (≈ 6.022 x 10^{23} mol^{-1}). Abbreviations: pmol: picomol; miR-124-3p sEV: miR-124-3p-loaded small extracellular vesicles; Cq: quantification cycle.

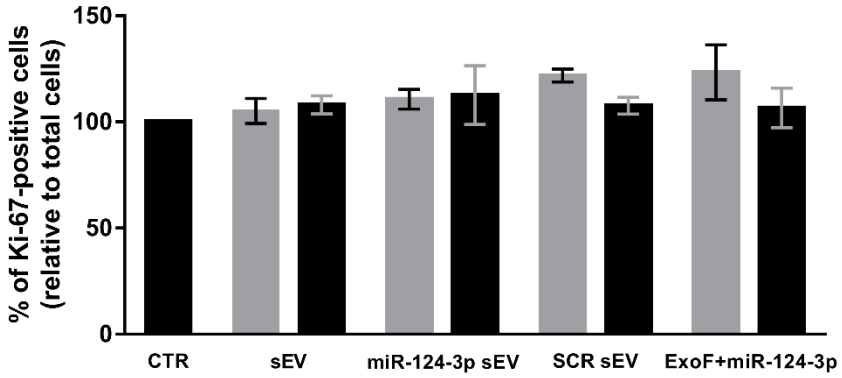


Figure S2. miR-124-3p sEVs do not affect total cell proliferation in SVZ cell cultures. Cell proliferation was evaluated by colocalization against Ki-67, 2 days after the SVZ NSCs treatment with 1.5x10^9 (gray bars) or 3x10^9 (black bars) particles/mL of sEVs, miR-124-3p SEVs, SCR sEVs or ExoF + miR-124-3p. Data are expressed as percentage of control (mean ± SEM; n = 3 - 5). Abbreviations: CTR: control; sEV: native small extracellular vesicles; miR-124-3p sEV: miR-124-3p-loaded small extracellular vesicles; SCR: scramble miR; ExoF: Exo-Fect™.

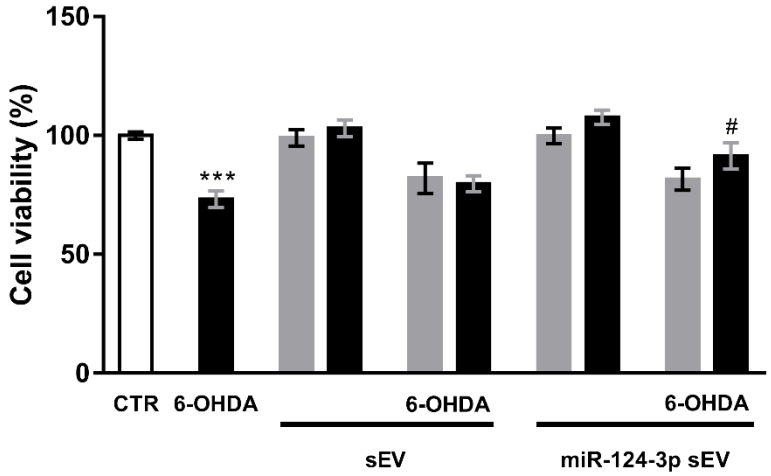


Figure S3. miR-124-3p sEVs promote neuroprotection against 6-OHDA-induced toxicity in N27 rat dopaminergic cells. MiR-124-3p sEVs counteract dopaminergic cell death induced by 6-OHDA. Cell viability was assessed in N27 rat dopaminergic neural cell line exposed to 50 µM 6-OHDA and $1.5x10^9$ (gray bars) or $3x10^9$ particles/mL (black bars) of sEVs or miR-124-3p SEVs for 24h, by the CCK-8 kit assay. Data are expressed as a percentage of control (mean ± SEM; n=3 in all experimental conditions except for sEVs + 6-OHDA at $3x10^9$ particles/mL: n = 2), from three independent experiments performed in triplicate. $^{***}p < 0.0001$ vs. control and $^{\#}p < 0.05$ vs. 6-OHDA-treated cells, using one-way ANOVA followed by Dunnett's multiple comparison test. Abbreviations: CTR: control; 6-OHDA: 6-hydroxydopamine; sEV: native small extracellular vesicles; miR-124-3p sEV: miR-124-3p-loaded small extracellular vesicles.