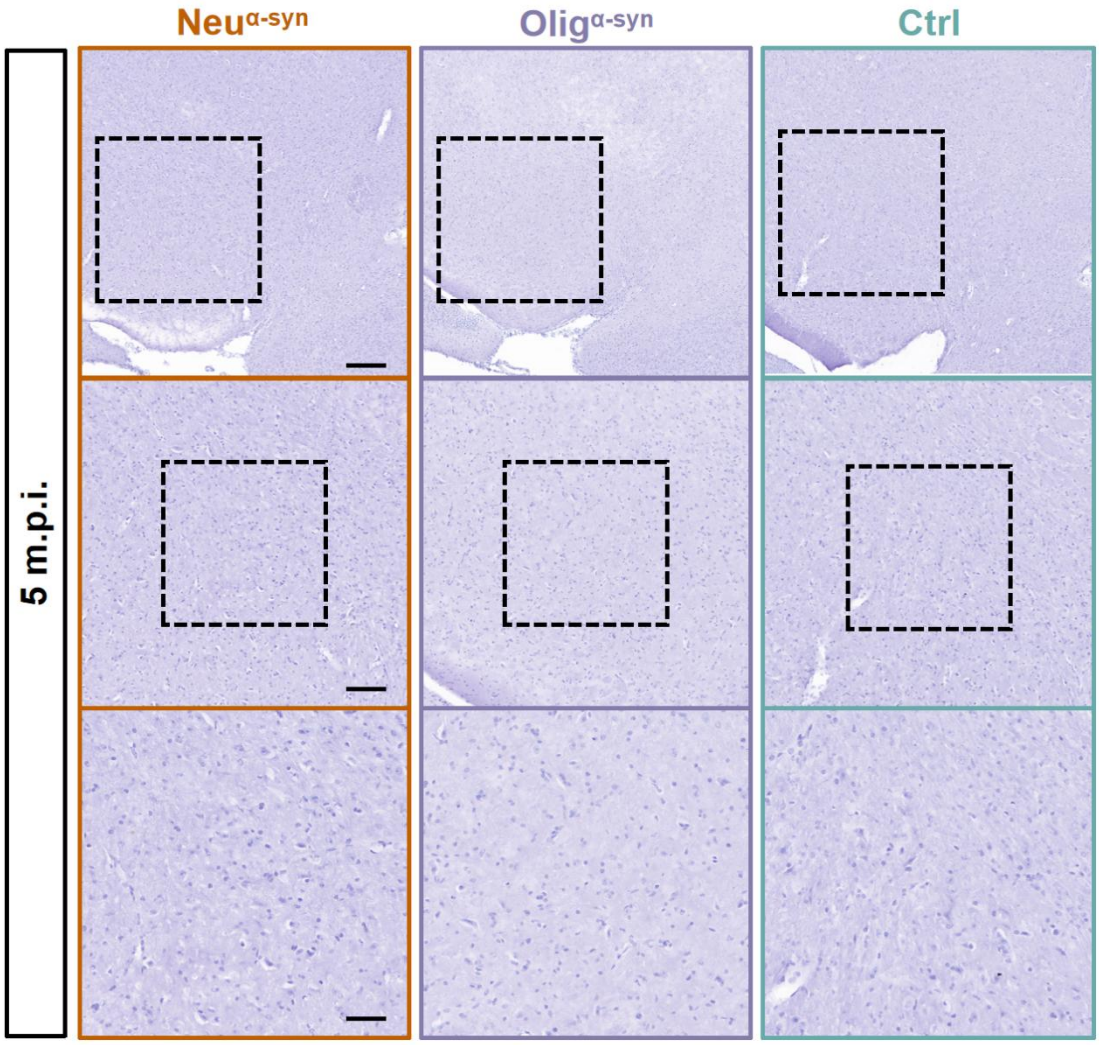
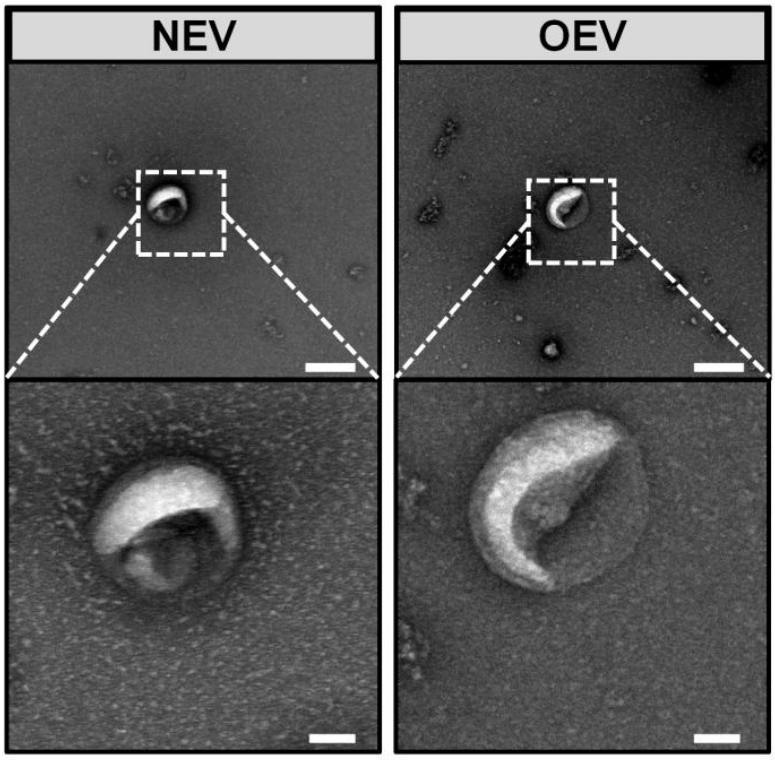
Extracellular vesicle α-synuclein seeds drive divergent phenoconversion from iRBD to Parkinsonism

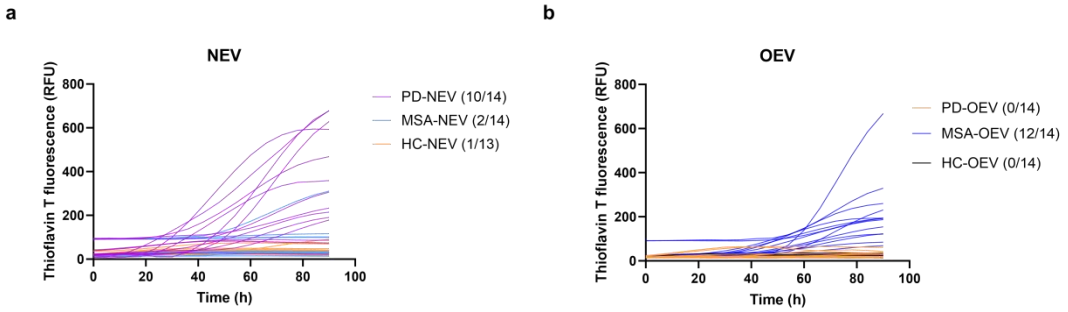
Supplementary Figures



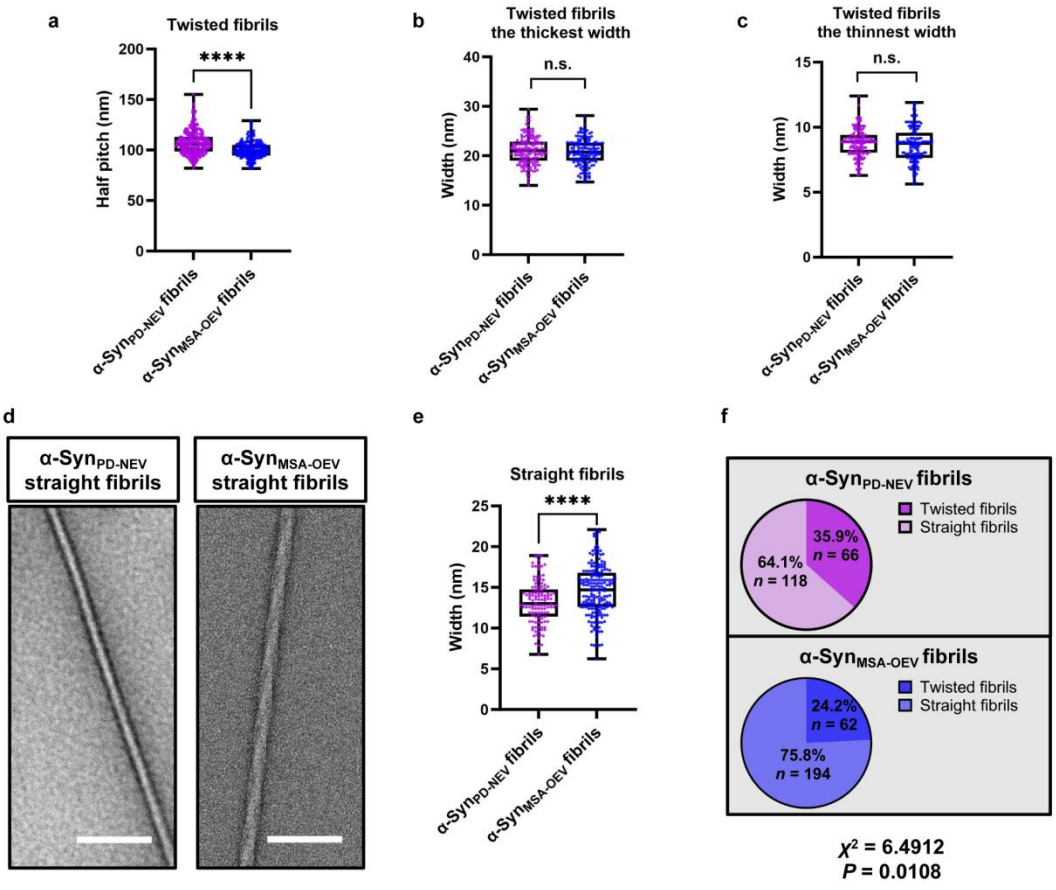
Supplementary Fig. 1 | No α-synucleinopathy was detected in substantia nigra of mice. Brain sections of substantia nigra were stained with pS129 α-syn antibody, and no pS129 α-syn puncta was detected. Scale bar = 200 μm (top), 100 μm (middle) and 50 μm (bottom).



Supplementary Fig. 2 | Transmission electron microscopic images of neuronal extracellular vesicles and oligodendroglial extracellular vesicles. Scale bar = 200 nm (top) and 50 nm (bottom).

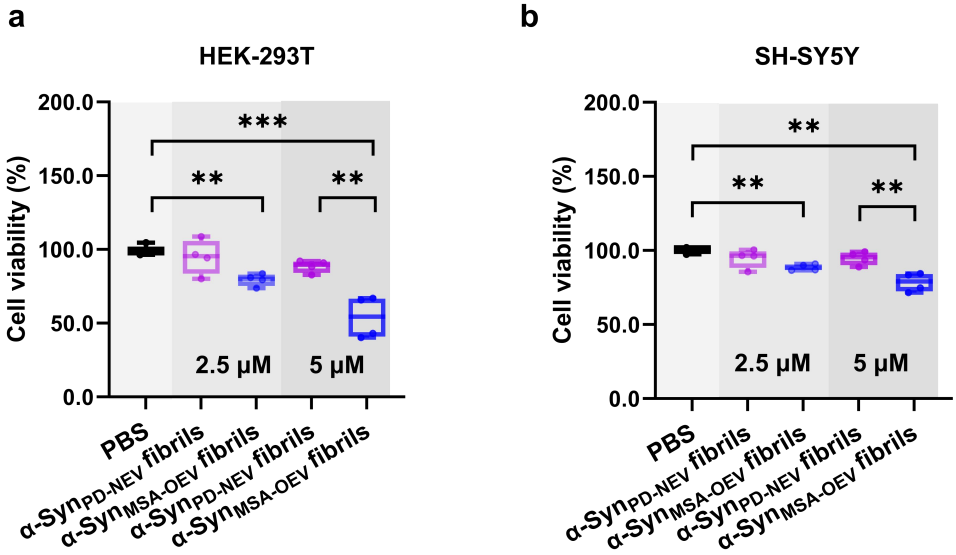


Supplementary Fig.3 | The kinetic curves of α-syn seeding activity in the presence of NEV and OEV samples from patients with PD, patients with MSA, and healthy controls in seed amplification assay. a-b The individual kinetic curves in the presence of NEV samples (**a**) and OEV samples (**b**) from all participants. Each curve shows the average of replicates of each individual from PD (*n* = 14), MSA (*n* = 14) and HC (*n* = 13) groups.

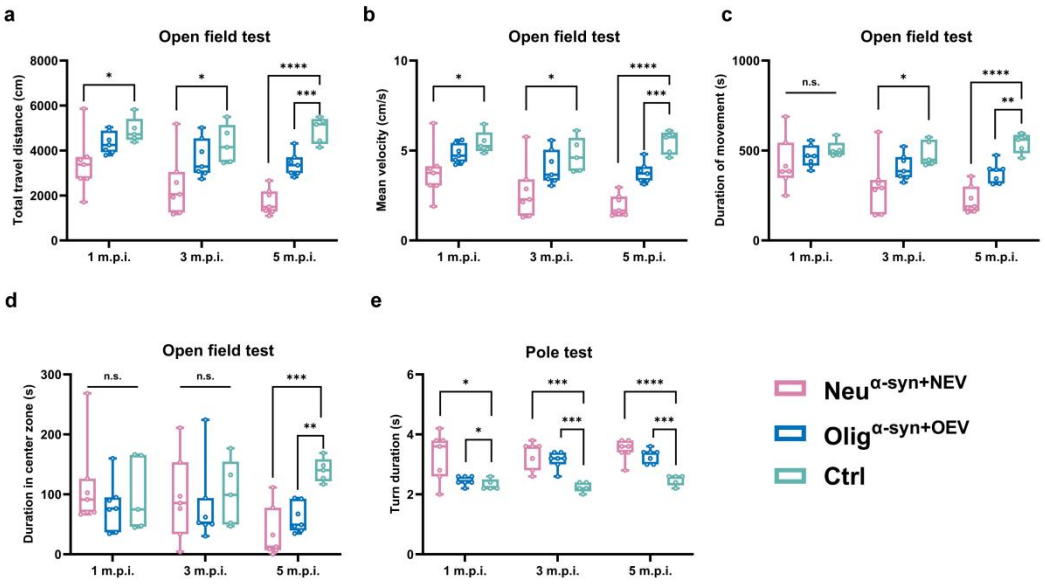


Supplementary Fig.4 | Structural characteristics of α-Syn$_{PD-NEV}$ and α-Syn$_{MSA-OEV}$ fibrils. a Comparison of half-pitch lengths between α-Syn$_{PD-NEV}$ twisted fibrils (n = 311) and α-Syn$_{MSA-OEV}$ twisted fibrils (n = 216). **b** Comparison of thickest widths between α-Syn$_{PD-NEV}$ twisted fibrils (n = 155) and α-Syn$_{MSA-OEV}$ twisted fibrils (n = 146). **c** Comparison of thinnest widths between α-Syn$_{PD-NEV}$ twisted fibrils (n = 159) and α-Syn$_{MSA-OEV}$ twisted fibrils (n = 146). **d** Representative negative-stained TEM images of α-Syn$_{PD-NEV}$ straight fibrils and α-Syn$_{MSA-OEV}$ straight fibrils. Scale bar = 100 nm. **e** Comparison of widths between α-Syn$_{PD-NEV}$ straight fibrils (n = 118) and α-Syn$_{MSA-OEV}$ straight fibrils (n = 194). **f** Pie chart showing the percentage of twisted and straight fibrils in α-Syn$_{PD-NEV}$ and α-Syn$_{MSA-OEV}$ fibril samples. Box-and-whisker plots show the minimums, maximums, medians, 25th, and 75th percentiles. Statistical

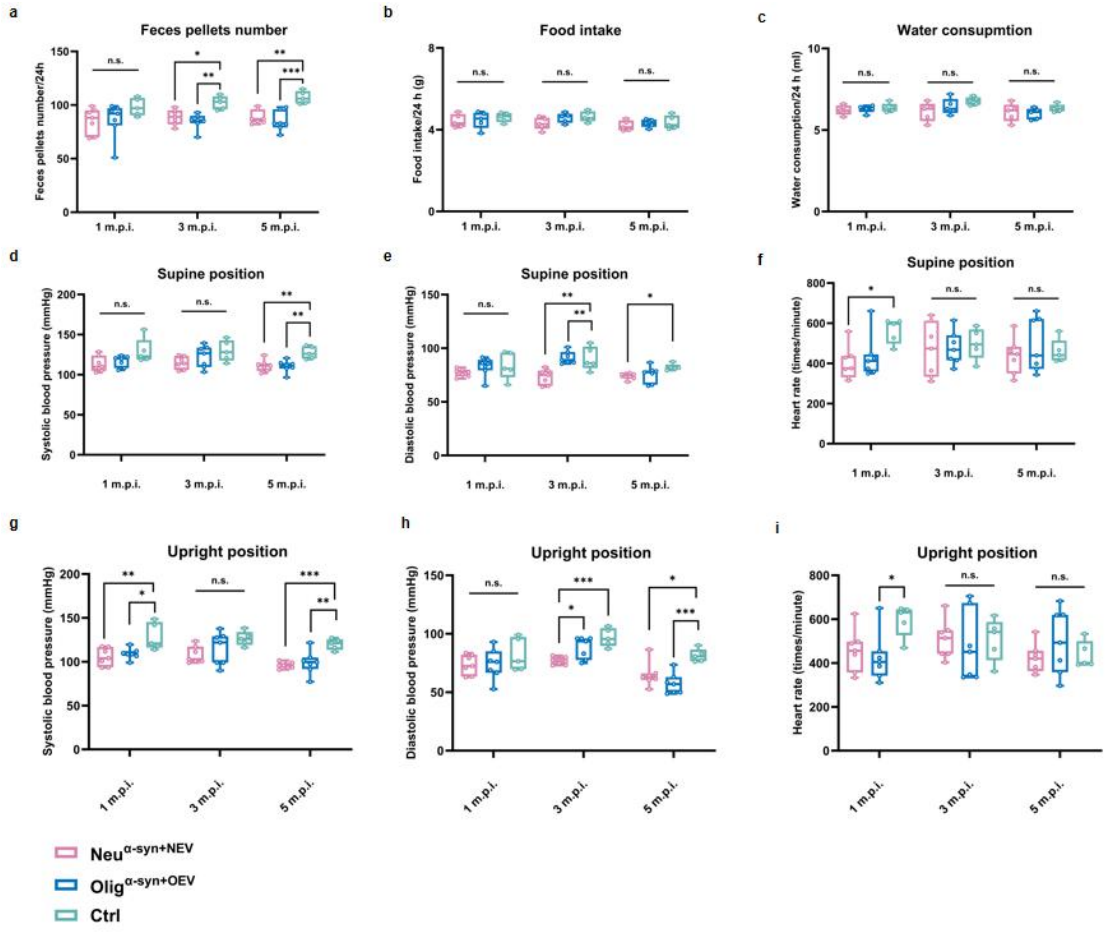
significance was determined by two-tailed Mann-Whitney test (**a**), two-tailed unpaired t test (**b**, **c**, **e**), and Chi-square test (**f**). n.s. = no significance. ****$P <$ 0.0001.



Supplementary Fig. 5 | Reduced cell viability induced by α-Syn$_{PD\text{-}NEV}$ and α-Syn$_{MSA\text{-}OEV}$ fibrils. **a** Quantification of cell viability of HEK-293T cells treated with 2.5 μM and 5 μM α-Syn$_{PD\text{-}NEV}$ and α-Syn$_{MSA\text{-}OEV}$ fibrils. **b** Quantification of cell viability of SHSY5Y cells treated with 2.5 μM and 5 μM α-Syn$_{PD\text{-}NEV}$ and α-Syn$_{MSA\text{-}OEV}$ fibrils. Box-and-whisker plots show the minimums, maximums, medians, 25th, and 75th percentiles. Statistical significance was determined by one-way ANOVA followed by Tukey's post-hoc test (HEK293T: PBS vs 2.5 μM, SHSY5Y: PBS vs 2.5 μM and 5 μM) or Brown-Forsythe ANOVA followed by Dunnett's T3 post-hoc test (HEK293T: PBS vs 5 μM). $n = 3$ for PBS, $n = 4$ for fibril treatments. Box-and-whisker plots show the minimums, maximums, medians, 25th, and 75th percentiles. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$.



Supplementary Fig. 6 | Performance in open field test and turn duration in pole test between mice groups at 1-, 3-, and 5 m.p.i.. a Comparison of total travel distance between mice groups. **b** Comparison of mean velocity between mice groups. **c** Comparison of duration of movement between mice groups. **d** Comparison of duration in center zone between mice groups. **e** Comparison of turn duration in pole test. Box-and-whisker plots show the minimums, maximums, medians, 25th, and 75th percentiles. Statistical significance was determined by one-way ANOVA followed by Tukey's post-hoc test. $n = 7$ for Neu$^{\alpha\text{-syn+NEV}}$ and Olig$^{\alpha\text{-syn+OEV}}$ groups and $n = 5$ for Ctrl group. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$.



Supplementary Fig.7 | Performance in 24h feces collection test and measurement of blood pressure and heart rate at supine and upright position between mice groups at 1-, 3-, and 5 m.p.i.. a Comparison of feces pellets number between mice groups in 24 feces collection test. **b** Comparison of food intake between mice groups in 24 feces collection test. **c** Comparison of water consumption between mice groups in 24 feces collection test. **d** Comparison of systolic blood pressure of mice in supine position between mice groups. **e** Comparison of diastolic blood pressure of mice in supine position between mice groups. **f** Comparison of heart rate of mice in supine position between mice groups. **g** Comparison of systolic blood pressure of mice in upright position between mice groups. **h** Comparison of diastolic blood pressure of

mice in upright position between mice groups. **i** Comparison of heart rate of mice in upright position between mice groups. Box-and-whisker plots show the minimums, maximums, medians, 25th, and 75th percentiles. Statistical significance was determined by one-way ANOVA followed by Tukey's post-hoc test. $n = 7$ for $Neu^{\alpha\text{-syn+NEV}}$ and $Olig^{\alpha\text{-syn+OEV}}$ groups and $n = 5$ for Ctrl group. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

Supplementary Tables

Supplementary Table 1 | The clinical parameters of each patient with Parkinson's disease, each patient with multiple system atrophy and each healthy control.

Table is provided as a single Excel file.

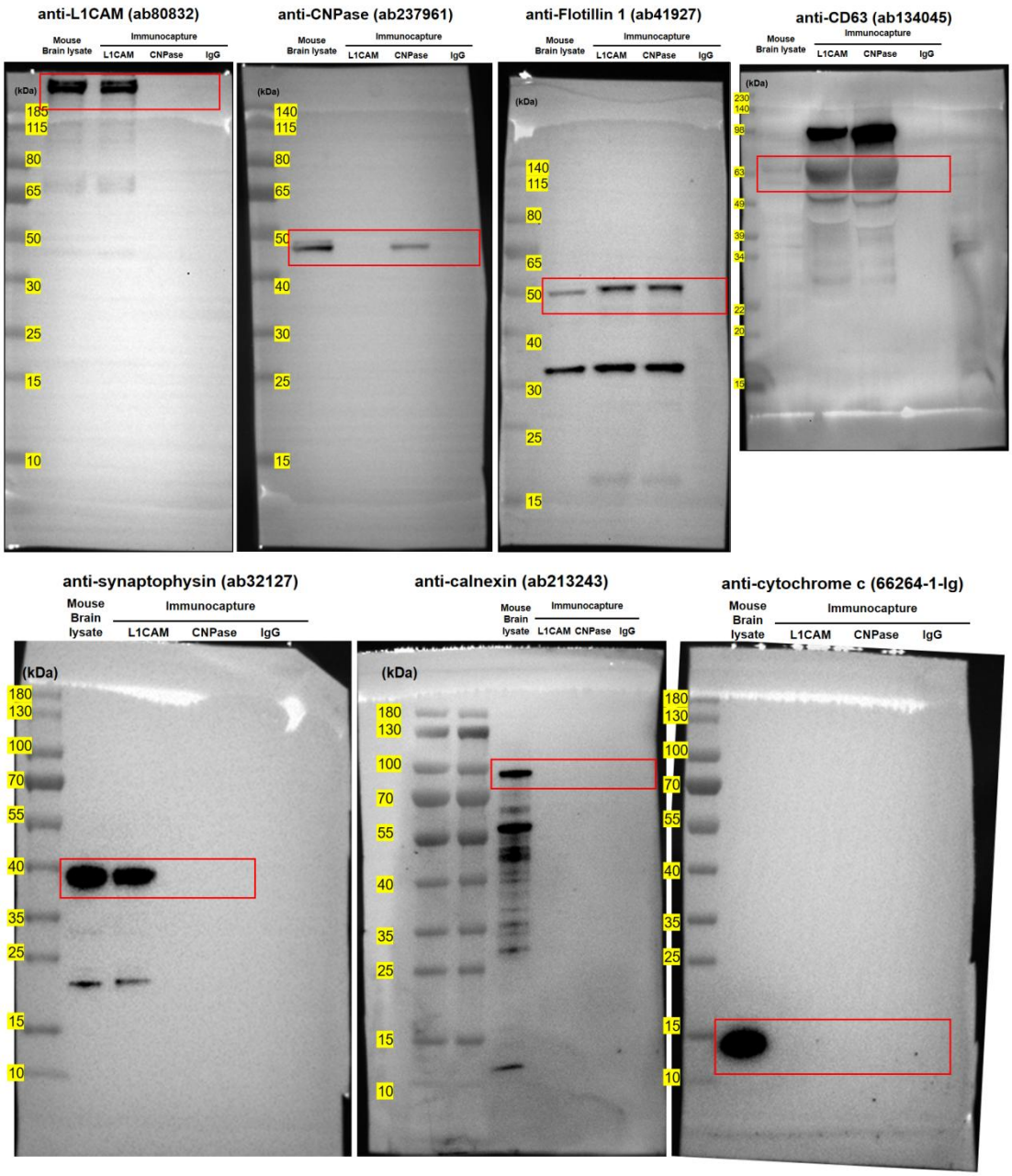
PD = Parkinson's disease; MSA = multiple system atrophy; HC = healthy controls; MSA-C = cerebellar-dominant multiple system atrophy; MSA-P = multiple system atrophy with predominant parkinsonism; M = Male. F = Female. MDS-UPDRS-III = Movement Disorders Society Unified Parkinson's disease Rating Scale Part III. UMSARS = Unified Multiple System Atrophy Rating Scale.

Supplementary Table 2 | The characteristics of patients with Parkinson's disease and patients with multiple system atrophy whose NEV or OEV were used for structural and biological experiments.

Sample ID	Diagnose	Sex	Age, years	Age at onset, years	Disease duration, years	MDS-UPDRS-III scores	UMSARS-II scores
PD10	PD	F	56	50	6	39	/
PD11	PD	M	64	60	4	29	/
PD12	PD	F	64	62	2	18	/
PD13	PD	M	77	62	15	56	/
MSA11	MSA-P	M	74	72	2	19	9
MSA12	MSA-C	F	59	58	1	31	13
MSA13	MSA-C	M	63	57	6	43	25
MSA14	MSA-P	F	63	57	6	37	23

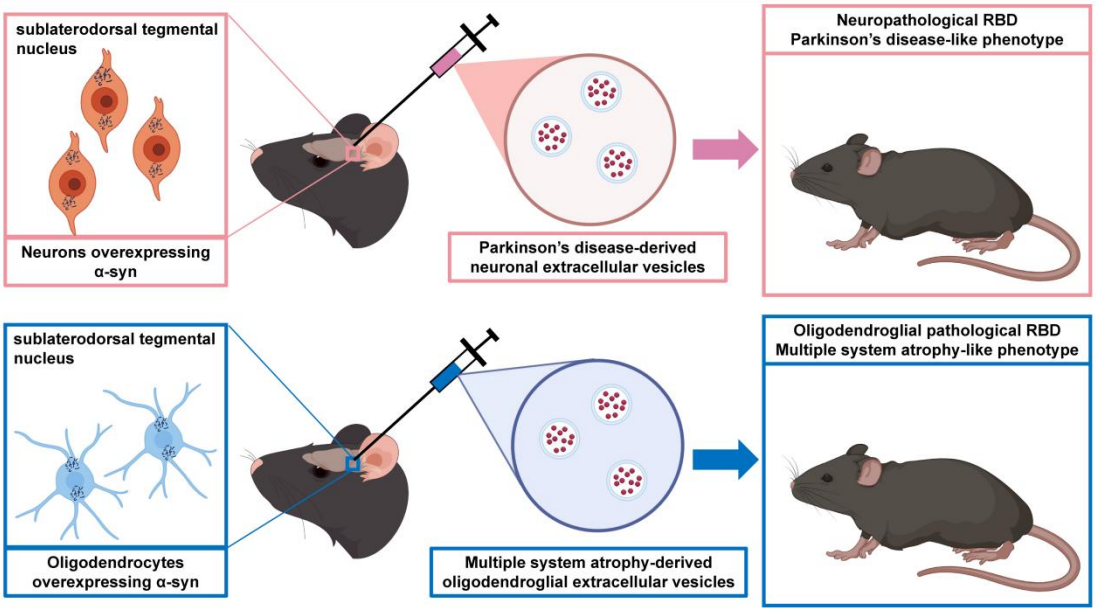
PD = Parkinson's disease; MSA = multiple system atrophy; HC = healthy controls; MSA-C = cerebellar-dominant multiple system atrophy; MSA-P = multiple system atrophy with predominant parkinsonism; M = Male. F = Female. MDS-UPDRS-III = Movement Disorders Society Unified Parkinson's disease Rating Scale Part III. UMSARS = Unified Multiple System Atrophy Rating Scale.

Uncropped gel blots



The molecular weight of prestained protein ladder were given in yellow boxes. Lanes from left to right: prestained protein ladder, mouse brain lysate (positive control), neuronal exosomes immunocaptured by L1CAM antibody, oligodendroglial exosomes immunocaptured by CNPase antibody, negative control (normal mouse IgG). The red squares indicate the cropped blot images in **Fig. 2c**.

Thumbnail


sublaterodorsal tegmental nucleus
Neurons overexpressing α-syn
Parkinson's disease-derived neuronal extracellular vesicles
Neuropathological RBD
Parkinson's disease-like phenotype
sublaterodorsal tegmental nucleus
Oligodendrocytes overexpressing α-syn
Multiple system atrophy-derived oligodendroglial extracellular vesicles
Oligodendroglial pathological RBD
Multiple system atrophy-like phenotype