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Supplemental Information

Exosomal DNA Aptamer Targeting α-Synuclein Aggregates Reduced Neuropathological Deficits in a Mouse Parkinson's Disease Model

Xiaoxi Ren, Yun Zhao, Fenqin Xue, Yan Zheng, Haixia Huang, Wei Wang, Yongchang Chang, Hui Yang, and Jianliang Zhang

SUPPLEMENTARY MATERIALS

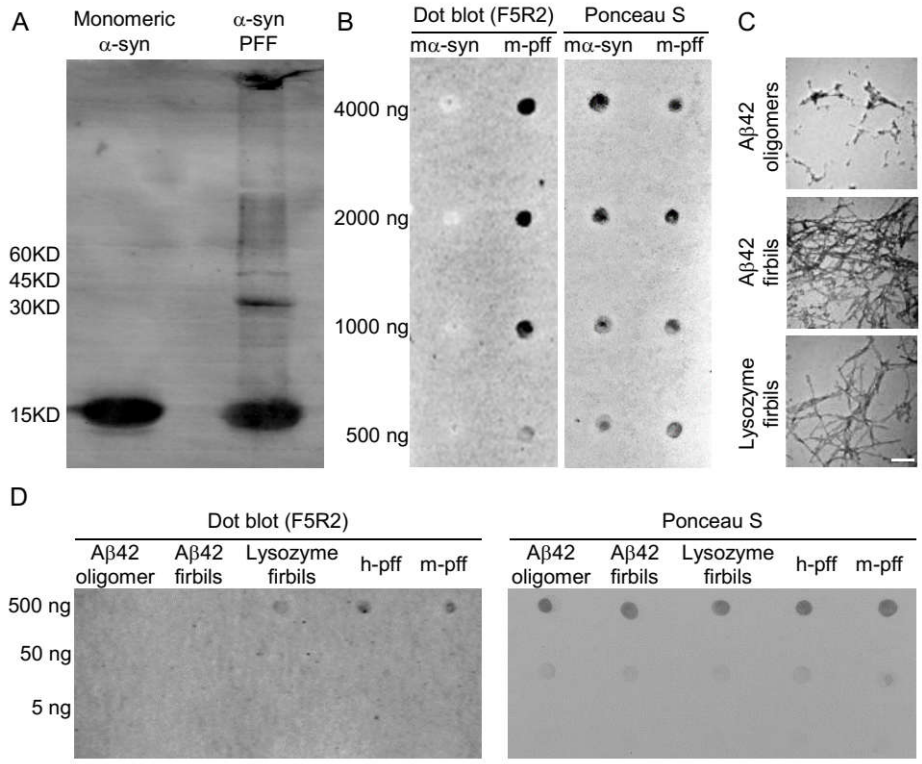

Figure S1. Analysis of the specificity of the aptamer F5R2. (**A**) The α-synuclein PFF was prepared in PBS with constant agitation. After the α-synuclein fibril (pellet fraction) was separated from the α-synuclein monomer (supernatant fraction) by ultracentrifuge, the proteins were detected by western blot with anti-α-synuclein (BD Biosciences, 1:2000). (**B**) Aptamer recognition capability assay by dot blotting. Monomeric and fibrillar mouse α- synuclein were spotted at 500 ng, 1000 ng, 2000 ng and 4000 ng and membranes were probed with FITC labeled aptamer F5R2. Blots were stained with Ponceau S after probing and washing to confirm protein retention. (**C**) Electron micrographs of Aβ42 oligomers, Aβ42 firbils and lysozyme firbils. Scale bar, 200 nm. (**D**) Reactivity of aptamer F5R2 with different amyloid fibrils. Aβ42 oligomers, Aβ42 firbils and lysozyme firbils were spotted (1 μL) onto NC membranes and probed with FITC-labeled aptamer F5R2. h-pff and m-pff were used as positive controls. Blots were stained with Ponceau S after probing and washing to confirm protein retention.

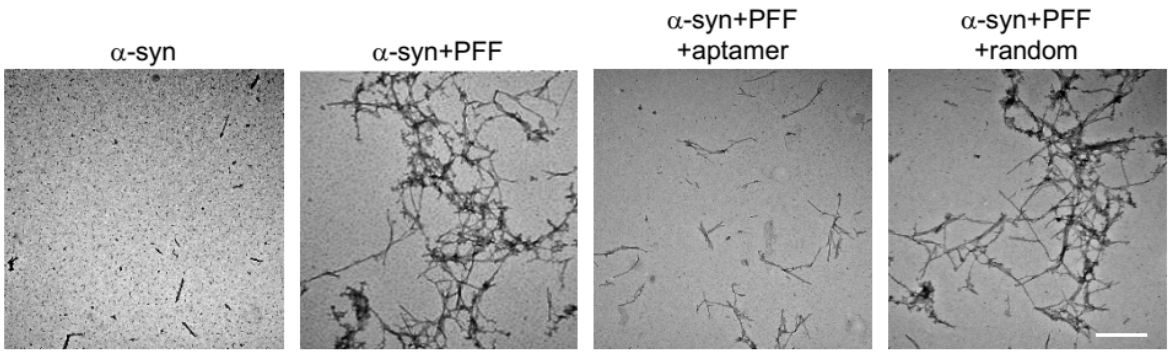

Figure S2. Aptamer F5R2 could inhibit PFF-induced aggregation procession *in vitro*. The human α-synuclein monomer (20 μM) was incubated with 10% PFF in the presence or absence of aptamer F5R2 (2 μM) at 37 °C under shaking at 1000 rpm. After shaking for 24 hours, amyloid formation was monitored by TEM. Sacle bar, 500 nm.

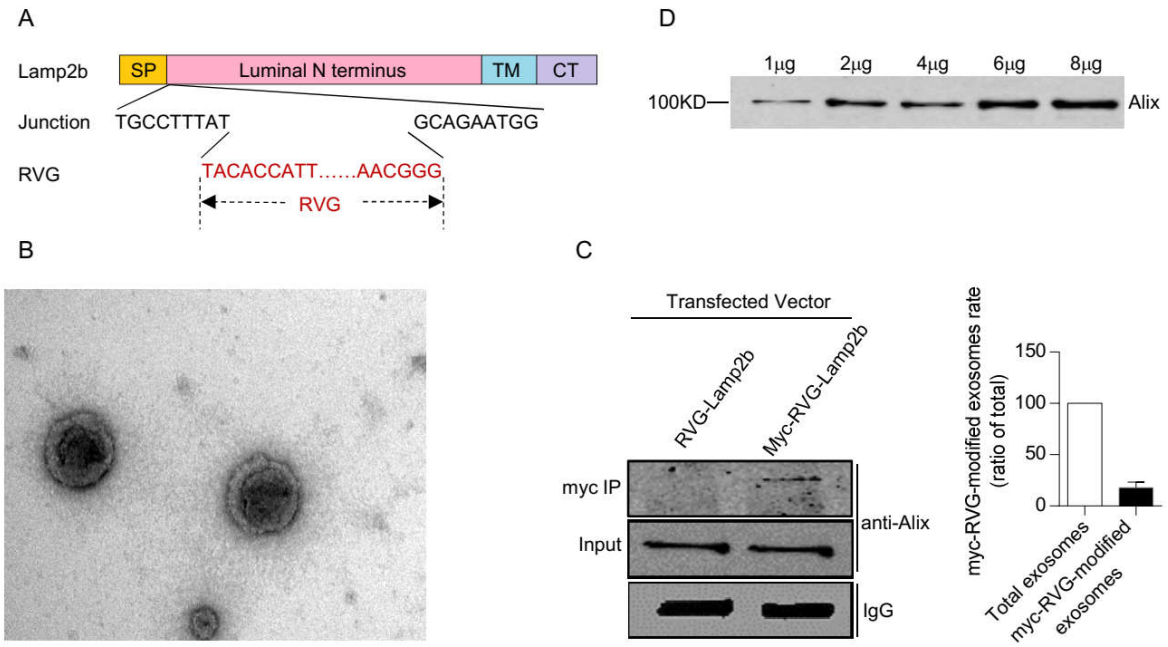

Figure S3. Schematic representation of RVG-Lamp2b cloning and characterization of the RVG-exosomes. (**A**) The RVG peptide was cloned into the N-terminus of Lamp2b. SP, signal peptide; TM, transmembrane domain; CT, C terminus. (**B**) The protein of Alix, marker of exosomes, was detected in the samples from the RVG-exosome lysates by western blotting. (**C**) The plasmids encoding myc-RVG-lamp2b were transfected into HEK 293Tcells for 48 h before exosomes were collected. Plasmids encoding RVG-lamp2b were used as negative control. The ratio of myc-RVG-positive exosomes to total ones was assessed by IP/Western blot analysis. Histograms show average densitometry values of Alix from immunoprecipitated and total fraction. (**D**) TEM image of the RVG-exosomes loaded with aptamers. Bar=200 nm.

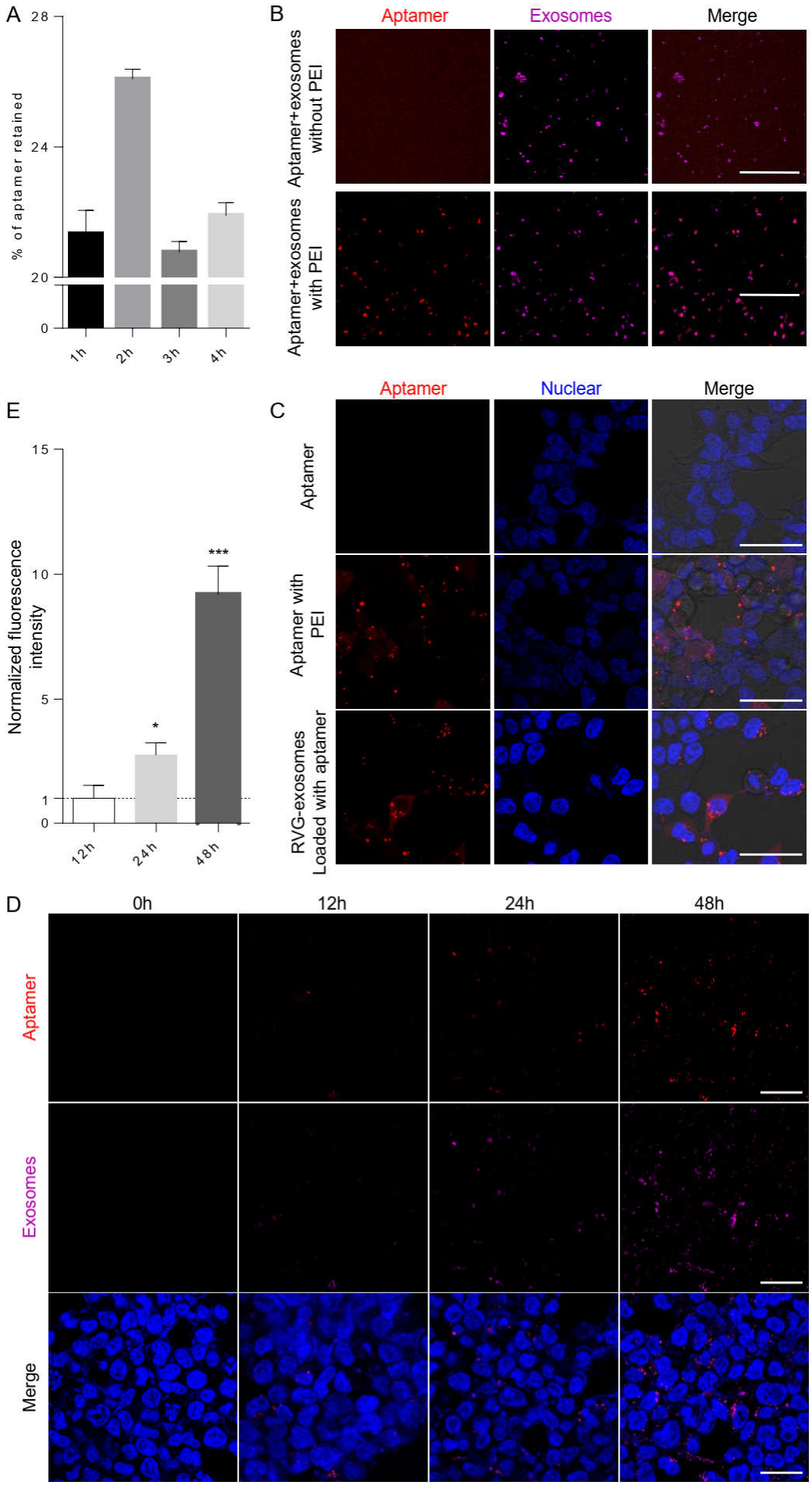
A
% of aptamer retained
28
24
20
0
1h
2h
3h
4h
B
Aptamer
Exosomes
Merge
Aptamer+exosomes without PEI
Aptamer+exosomes with PEI
E
Normalized fluorescence intensity
15
10
5
1
0
*
***
12h
24h
48h
C
Aptamer
Nuclear
Merge
Aptamer
Aptamer with PEI
RVG-exosomes Loaded with aptamer
D
0h
12h
24h
48h
Aptamer
Exosomes
Merge

Figure S4. Aptamer F5R2 was loaded into exosomes by polyethylenimine transfection. (**A**) RVG-exosomes stained with CellVue Claret were incubated with Alexa 594 labeled aptamer F5R2 for different times (1, 2, 3 and 4 hours) in the presence of polyethylenimine (PEI). After the exosomes were harvested with 100,000g for 1 hour, they were resuspended in the PBS and the aptamer fluorescence was assayed on the fluorescent plate reader. n=6. (**B**) After RVG-exosomes stained with CellVue Claret were incubated with Alexa 594 labeled aptamer F5R2 for 2 hours in the presence of polyethylenimine, the pelleted exosomes and aptamers were co-visualized by confocal microscopy, with non-transfection-treated group as negative control. Bar=50 μm. (**C**) Neuro2A cells were incubated with naked aptamers or the RVG-exosomes loaded with aptamers for 24h. The mixture of transfection reagent (PEI) and aptamers was used as a positive control. Confocal images was used to measure the delivery efficiency of different approaches. (**D**) The RVG-exosomes loaded with aptamers were incubated with Neuro2A cells for 0,12, 24 and 48 hours, and then the cells were washed by PBS for three times. Confocal micrograph was used to detect the fluorescence of Alexa594-labeled aptamers (Red) and exosomes (Purple). Bar=50 μm. (**E**) Quantitative analyses of fluorescence intensity of aptamers at 12, 24 and 48h. Values are presented as mean ± SD. One-way ANOVA followed by Tukey's post hoc test (n=3 per group), *$p<0.05$, ***$p < 0.001$ compared with the group that was treated for 12h.

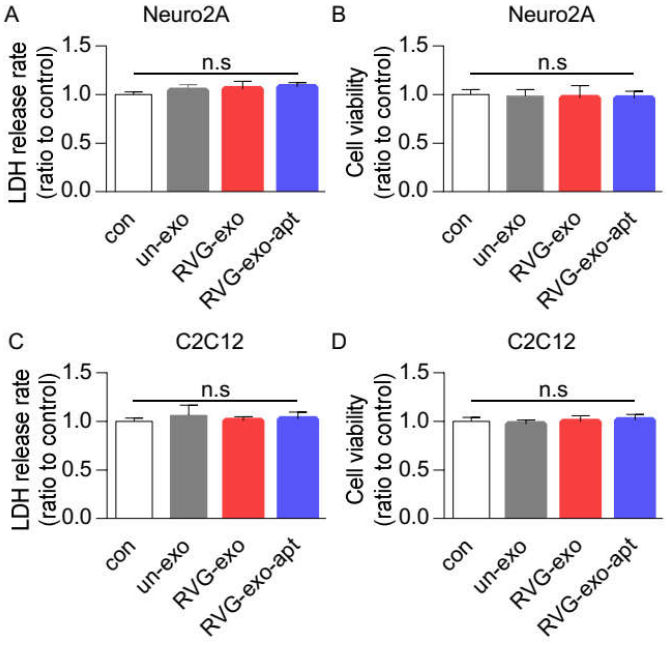

Figure S5. Treatment with aptamer-loaded exosomes was tolerated by cells. LDH assay (**A, C**) and MTT assay (**B, D**) were performed 24 hours after Neuro2A and C2C12 cells were incubated with naked aptamers (con), unmodified exosomes (un-exo), RVG-exosomes (RVG-exo) and RVG-exosomes loaded with aptamer F5R2 (RVG-exo-apt). Values are presented as mean ± SD. One-way ANOVA followed by Tukey's post hoc test (n=6 per group), n.s: not significant.

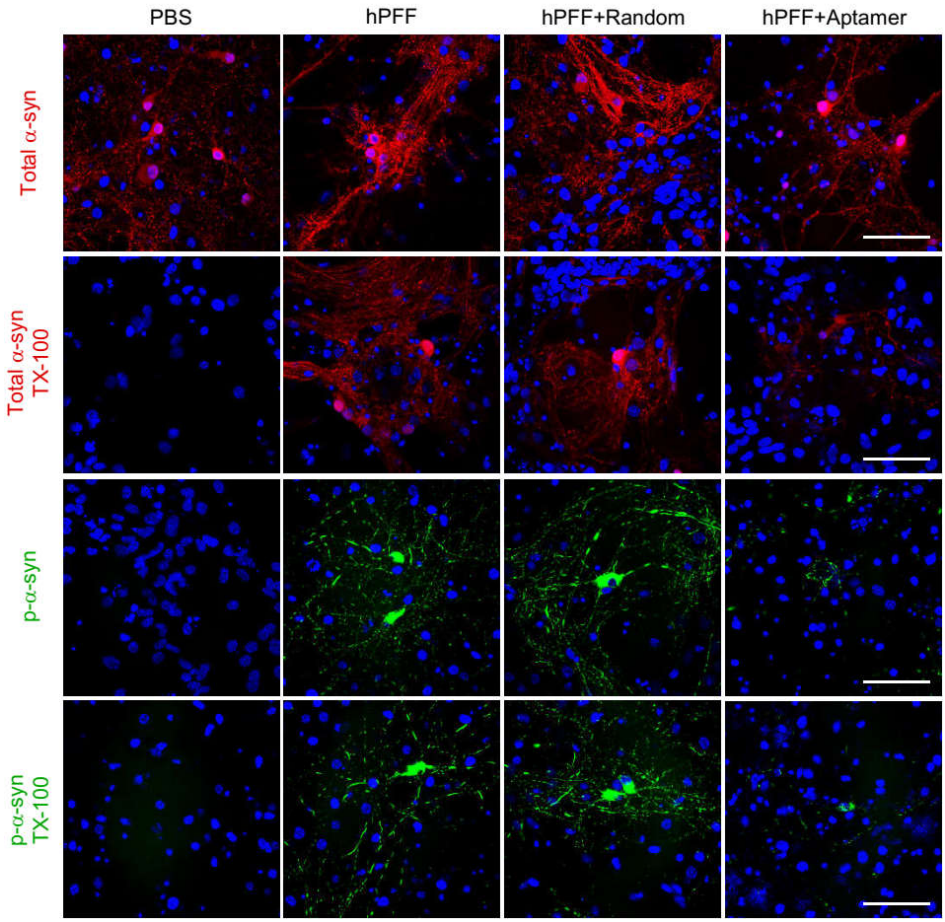

Figure S6. Confocal microscope images in Figure 3A were merged with DAPI blue to show cell density.

Scale bar, 25 mm.

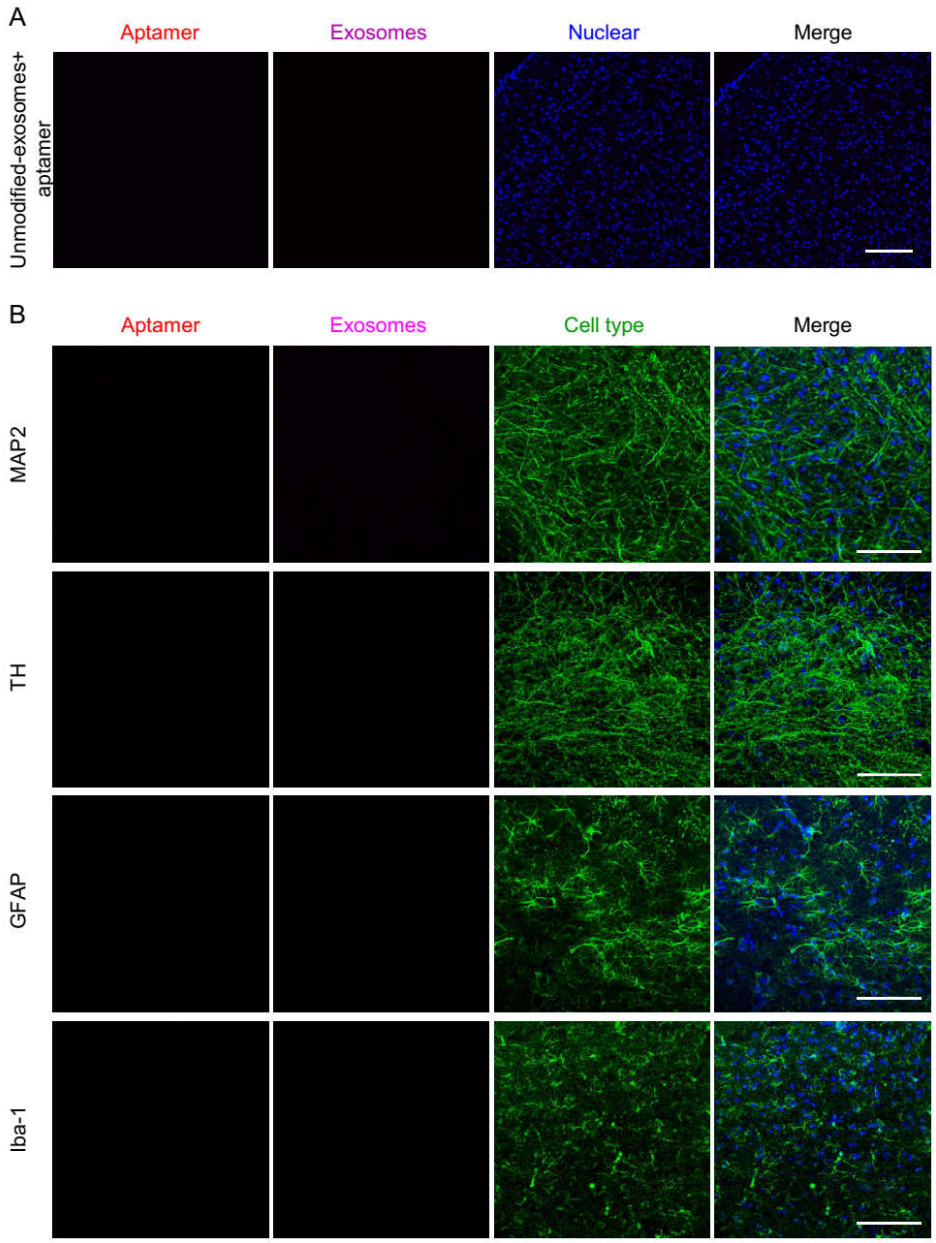

Figure S7. Unmodified exosomes could hardly deliver aptamers into the central nervous system in mice. Unmodified-exosomes were labeled, loaded and administered as described in Figure 5. Twenty-four hours later, mice were sacrificed, perfused, and brain slides were subjected for confocal examinations. Little, if any, of the red aptamer signals were detectable in the brain cortex (**A**) and midbrain (**B**). Brain slides were stained with antibodies (anti-MAP2, anti-TH, anti-GFAP and anti-Iba-1) to different cell types. Scale bar, 75 μm.

Supplementary Table 1. Plasm cytokine concentrations (pg/ml) in mice after aptamer treatment

	Control	aptamer-RVG exosomes	aptamer-exosomes	aptamer-PEI
IL-6	32.63(±2.06)	36.61（±2.59）	38.12(±1.98)	49.57(±1.85)**
IFN-α	14.75(±2.24)	15.60(±3.92)	15.84(±3.67)	18.30(±2.19)*
IP-10	46.79(±3.68)	48.98(±4.60)	50.40(±6.39)	54.53(±5.83)*
TNF-α	226.64(±22.78)	240.44(±16.11)	233.48(±33.75)	323.27(±46.07)*