Supporting Information

Synaptic vesicles-inspired nanoparticle with spatiotemporally controlled release ability as "nanoguard" for synergistic treatment of synucleinopathies.

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Experimental Section

Materials: 4-Cyano-4-(ethylsulfanylcarbothioyl)sulfanylpentanoic acid (CEP), propylene sulfide (PS), tetraphenylphosphonium chloride (TPPCl 98%), 1-methyl-2-pyrrolidinone (NMP, 99.5%), 2-(dimethylamino)-ethyl acrylate (DMAEA, 98%), 4-cyano-4-(dodecylsulfanylthiocarbonyl) sulfanylpentanoic acid (CTA), 4-(bromomethyl)phenylboronic acid (98%), 2,2'-dicyano-2,2'-azopropane (AIBN), cholesterol were purchased from J&K Scientific Ltd. Co (Shanghai, China). Benzyl bromide (99%), sodium oleate (95%), oleic acid (90%) and 1-octadecene (95%) were purchased from Alfa Aesar. PEGylated lipid 1,2-distearoyl-sn-glycero-3-phosphoethanolamine N-[methoxy(polyethylene glycol)-2000] (DSPE-PEG2000), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[maleimide (polyethylene glycol)-2000] (DSPE-PEG-Mal), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), 1,2-dipalmitoyl-sn-glycero-3-phospho-L-serine (DPPS) were purchased from AVT. Co (Shanghai, China). Rapamycin (RAPA) was obtained from

Melonepharma (Dalian, China). RVG29-Cys (YTIWMPENPRPGTPCDIFTNSRGKRASNGC) and TMS-syb (RKKILGVICAIILIIIIVYKKK) were synthesized and purchased from GL Biochem Ltd. Co (Shanghai, China). 4',6-Diamidino-2-phenylindole dihydrochloride (DAPI), 3-(4,5-dimethylthiazol-2-thiazolyl)-2,5-diphenyltetrazolium bromide (MTT) and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) were obtained from Sigma Aldrich. SiRNA against nSMase2 (sense strand, 5'-sense sequence CCAAAGAAUUGUUGGGUACAUCUU-3', antisense sequence 5'-GAUGUACCCAAVAAUUCUUUGGUU-3') were synthesized by GenePharma company (Suzhou, China). Anti-NSMase2 was purchased from Abcam. All the reagents were used as received without further purification. High-purity water (Milli-Q Integral) with a conductivity of 18 MΩ cm^{-1} was used for the preparation of all aqueous solutions.

Synthesis and purification of poly(propylene sulfide) (PPS) polymers. Poly(propylene sulfide) (PPS) was synthesized using the reversible addition-fragmentation chain transfer (RAFT) chain transfer agent 4-cyano-4-(ethylsulfanylcarbothioyl) sulfanylpentanoic acid (CEP) through the thioacyl group transfer (TAGT) polymerization method. Briefly, PS (0.15 mL, 1.9 mmol), CEP (25 mg, 0.095 mmol), TPPCl (3.56 mg, 0.013 mmol), and NMP (2 mL) were placed into a clean and dry schlenk flask. The system was degassed by three freeze-pump-thaw cycles and recharged with nitrogen. The schlenk flask was immersed in an oil bath at 60 °C for 24 h, and afterwards, cooled in a liquid nitrogen bath. The crude polymerization mixture was precipitated twice from NMP into hexane, which is nonsolvent for PPS. The resulting polymer was dissolved into chloroform and precipitated into tenfold excess of cold methanol to better remove the TPPCl catalyst. The product was dried at 60 °C under vacuum to yield a yellow viscous oil PPS. ^{1}H NMR (Bruker 600 MHz) was carried out to characterize the obtained products. The molecular weight (Mw) of PPS was calculated by gel permeation chromatography (GPC) (Mw=2938 g/mol, PDI=1.52).

Synthesis and purification of PPS-PDMAEA and C-PDMAEA polymers. PPS-PDMAEA polymers were synthesized by RAFT polymerization. PPS (100mg 0.04mmol), 2-(dimethylamino)ethyl acrylate (DMAEA, 686.4 mg) and AIBN (6 mg) were dissolved in N,N-Dimethylformamide (DMF), and then added into a clean and dry schlenk flask. The system was degassed by three freeze-pump-thaw cycles and recharged with nitrogen. The reaction mixture was stirred for 24 h at 60 °C. The resulting liquid was dissolved in dichloromethane (DCM), and precipitated in cold n-hexane to a yellow viscous oil PPS-PDMAEA. ^{1}H NMR was carried out to characterize the obtained products.

C-PDMAEA polymers were synthesized by RAFT polymerization. Briefly, 4-cyano-4-(dodecylsulfanylthiocarbonyl)sulfanylpentanoic acid (CTA, 13.3 mg), DMAEA (686.4 mg) and AIBN (6 mg) were dissolved in DMF, and then added into a clean and dry schlenk flask. The system was degassed by three freeze-pump-thaw cycles and recharged with nitrogen. The reaction mixture was stirred for 24 h at 60 °C. The resulting liquid was dissolved in DCM, and precipitated in cold n-hexane to obtain the final product C-PDMAEA. ^{1}H NMR was carried out to characterize the obtained products.

Synthesis and purification of PPB, PPBn, CPB and CPBn polymers. PPS-PDMAEA (200mg) were reacted with 4-(bromomethyl)phenylboronic acid (327 mg, 1.53 mmol) and benzyl bromide (260mg, 1.53 mmol) in 15 mL DMF at room temperature for 24 h, respectively. The resulting solutions were dialyzed overnight against deionized water using a Cellu SepH1-membrane (MWCO 7000) and then lyophilized to obtain the final product PPS-PDMAEA-B (PPB) and PPS-PDMAEA-Bn (PPBn). C-PDMAEA (200 mg) were reacted with 4-(bromomethyl)phenylboronic acid (368 mg, 1.72 mmol) and benzyl bromide (292 mg, 1.72 mmol) in 15 mL DMF at room temperature for 24 h, respectively. The resulting solutions were dialyzed overnight against deionized water using a Cellu SepH1-membrane (MWCO 7000) and then lyophilized to obtain the final product C-PDMAEA-B (CPB) and C-PDMAEA-Bn (CPBn). ^{1}H NMR was carried out to characterize the obtained products.

ROS-responsiveness of four polymers. PPB, PPBn, CPB and CPBn polymers were dissolved in 10 mM H_2O_2 solution (H_2O_2 diluted in D_2O) at a concentration of 5 mg mL^{-1} at 37 °C, respectively. At given time intervals (3 h, 12 h), 200 μL of the solution was detected by ^1H NMR (600 MHz, D_2O).

Synthesis of superparamagnetic iron oxide nanoparticles (SPIONs). Briefly, $FeCl_3 \cdot 6H_2O$ (10.8 g) and sodium oleate (36.5 g) were dissolved in a mixture solvent composed of ethanol (80 mL), distilled water (60 mL) and hexane (140 mL). The resulting solution was heated to 70°C and kept at that temperature for 4 h. Then the upper organic layer containing the iron-oleate complex was washed three times with 30 mL distilled water in a separating funnel. Hexane was removed *via* evaporation, resulting in iron-oleate complex in a waxy solid form after washing. Then 3.6 g of the iron-oleate complex synthesized as described above and 0.57 g of oleic acid were dissolved in 20 g of 1-octadeceneat room temperature. The reaction mixture was heated to 320 °C with a constant heating rate of 3.3 °C min^{-1}, and kept at that temperature for 30 min. Then the initial transparent solution became turbid and brownish black. The resulting solution was cooled to room temperature, and ethanol was added to the solution to precipitate the oleic acid coated iron oxide nanoparticles. The nanoparticles were separated by centrifugation (5000 rpm. for 10 min×3 times) to yield a dark-brown precipitate. Finally, the product was stored as a solution of known concentration in hexane in the fridge. The size and morphology of the SPIONs were observed by transmission electron microscope (TEM, JEM-2100 electron microscope) operating at an acceleration voltage of 200 kV.

Synthesis and purification of DSPE-PEG-Mal polymers. DSPE-PEG2000-Mal (70 mg) and RVG29-Cys (50 mg) were added to the HEPES buffer solution (0.01 M, pH = 7.0). The reaction mixture was stirred gently at room temperature for 48 h. The resulting solutions were dialyzed overnight against deionized water using a Cellu SepH1-membrane (MWCO 7000) and lyophilized to obtain the final product DSPE-PEG2000-RVG29. Matrix assisted laser

desorption ionization time of flight mass spectrometry (MALDI-TOFMS) (AB Sciex) was carried out to characterize the obtained product.

Preparation and Characterization of Liposome(PPB/RAPA/SPIONs@siRNA) Nanoparticles (Lipo/PPB) NPs. Lipo/PPB NPs were prepared by a thin-film dispersion method as reported before.[1] Specifically, POPC, DPPS, cholesterol, DSPE-PEG2000 and DSPE-PEG-Mal with a molar ratio of 3:1:1.2:0.8:0.2 were used to construct the shell-liposomes. After dissolving in chloroform, the organic phase was removed at 55 °C on a rotary evaporator to obtain a thin lipid film, followed by incubation overnight under vacuum to remove residual solvents. The lipid films were subsequently hydrated in 5% glucose aqueous solution of PPB core after sonication at 37 °C for 30 min, the Lipo/PPB NPs solution was dialysis in 5% glucose aqueous solution to remove organic reagent. The size and zeta potential of NPs were determined by DLS measurement.

Preparation and Characterization of RVG-TMS Liposome (PPB/RAPA/SPIONs@sinSMase2) Nanoparticles (RT-PPB NPs). RVG29 was subsequently added to the above Lipo/PPB NPs solution and incubated at room temperature for 48 h. The reaction mixture was dialysis in 5% glucose aqueous solution to remove free peptides. TMS-syb was subsequently added to the above solution and sonicated at 37 °C for 30 min, then the mixture was dialysis in 5% glucose aqueous solution to remove free peptides. The size and zeta potential of NPs were determined by DLS measurement. The size and morphology of the final NPs were observed by TEM (JEM-2100 electron microscope) operating at an acceleration voltage of 200 kV. The magnetic property was calculated by measuring the change in the spin-spin relaxation rate (R_2) per unit iron concentration with a 1.5-T Magnetic Resonance Analyzer (Bruker, minispec mq60).

Evaluation of TMS-syb Conjugation Efficiency and TMS-syb Density on Nanoparticle Surface. Aliquots of RT-PPB NPs or R-PPB NPs were dissolved in 5% glucose, and the

concentration of TMS-syb peptide was determined using a BCA assay. The conjugation efficiency (CE) of TMS-syb was calculated as:

$$CE\ (\%) = \frac{amount\ of\ conjugated\ TMS - syb}{total\ amount\ of\ peptide\ added} \times 100\%$$

The number (n) of incorporated TMS-syb peptide per nanoparticle was calculated using the following equation:

$$n = \frac{M_{TMS-syb} \times CE(\%)}{N_{NPs}} \times N_A$$

Where $M_{TMS\text{-}syb}$ was the initial molar concentration of TMS-syb, N_{NPs} was the number concentration of NPs measured by Nanoparticle Tracking Analysis (NTA), N_A was Avogadro's constant.

Fluorescence Resonance Energy Transfer (FRET) measurements. First, we constructed PPB core with FAM-siRNA, and RT-PPB NPs with FAM-siRNA and DSPE-PEG-RhoB. Then, fluorescence measurements were performed using a fluorescence spectrometer. PPB core with FAM-siRNA, and RT-PPB NPs with FAM-siRNA and DSPE-PEG-RhoB were respectively measured at an excitation wavelength of 490 nm with a slit width of 5 nm, integration time of 0.1 s, and increment of 0.5 nm and with an emission scan from 500 to 650 nm. FRET efficiency (E) and the distance between the donor and acceptor (R) were calculated as following equations:

$$E = 1 - \frac{F_{DA}}{F_D}$$

$$R = R_0 \sqrt[6]{\frac{1}{E} - 1}$$

Where F_{DA} is the intensity in the presence of the acceptor, F_D is the intensity in the absence of the acceptor, and R_0 is the Förster distance at 50% transfer efficiency. For FAM-RhoB, R_0 is 55 nm.[2]

Reactive Oxygen Species (ROS)-Responsiveness siRNA Release of Core Nanoparticles. In vitro release of RAPA: 2 mL of four core (2 mg mL^{-1}) in dialysis bag (MWCO 3500) were incubated in 50 mL of 0.5% Tween-80 with or without H_2O_2 (0.5 mM) at 37 °C under horizontal

shaking (150 rpm). At predetermined time intervals, 100 μL of the medium was removed and the same volume of fresh solution was supplemented. The concentration of RAPA was detected using HPLC, which equipped with a UV detector and C18 column at 30 °C (Agilent, Japan). For the HPLC analysis, the mobile phase was a mixture of acetonitrile/water (80:20, v/v) at a flow rate of 0.8 mL/min. The concentration of RAPA was determined at the retention time of 10.058 min at 277 nm.

In vitro release of siRNA: Specifically, 1 μL of H_2O_2 solution (0, 2.5 mM, 5 mM, 7.5mM, and 10 mM, respectively) were added into four solution to form a reaction system with a final volume of 10 μL. Naked siRNA and siRNA incubated with 1 mM H_2O_2 solution were used as controls. Those solutions were then subject to the electrophoresis as described above after incubation for 3 h at 37 °C.

The Size Changes after Incubation with H_2O_2: The four core, PPB core, CPB core, PPBn core, CPBn core, were incubated in 0.5 mM H_2O_2 for 3 h, then the diameter changes were measured DLS. Data are presented as the means ± SD (n = 3).

Cell culture and cytotoxicity. SH-SY5Y cells were purchased from ATCC and the α-synuclein over expression SH-SY5Y cell line was prepared. Briefly, the full-length cDNA of SNCA gene was amplified by PCR and was constructed into lentiviral vector pLV-mCherry-N at EcoRV restriction site. The lentiviral vector pLV-SNCA-mCherry was overexpressed. SH-SY5Y cells were infected with the lentiviral vector pLV-SNCA-mCherry and the corresponding empty vector, respectively, and then the SNCA gene stable overexpression cell line (α-syn-mCherry over-expression SH-SY5Y) was screened by puromycin. The SH-SY5Y and α-syn-mCherry over-expression SH-SY5Y ($^{\alpha\text{-syn-mCherry}}$SH-SY5Y) were cultured in DMEM medium (10% fetal bovine serum). MTT method was used to measure the cytotoxicity of NPs with sinSMase2 concentration of 1 μg mL^{-1} and RAPA concentration of 100 nM. In detail, SH-SY5Y cells were cultured in 96 well plates, in each well of which 5000-10000 cells were cultured. Then each NPs were incubated with SH-SY5Y cells for 24 h, and after that 20 μL MTT (5 mg

mL^{-1} in PBS) was added and then incubated for 2 h. At last, the culture in each well were poured and 100 μL DMSO were added and were shacked at low speed for 10 min to fully dissolve the crystals. The absorbance was measured at 490 nm using a Tecan microplate reader (Tecan, Switzerland).

Alamar blue assay. SH-SY5Y cells were cultured in 96 well plates, in each well of which 5000-10000 cells were cultured. SH-SY5Y cells were treated for 24 h. The alamar blue reagent was then added and incubated for 4 h. Fluorescence spectrophotometry was used for detection. The excitation wavelength was between 530-560 nm, and the emission wavelength was 590 nm.

Transportation across the in Vitro BBB and Cellular Uptake of NPs. To establish the in vitro BBB models for examining permeability of the nanoparticles across the BBB, bEnd.3 cells were seeded in the upper chambers (pore size: 0.4 μm) at 2×10^5 cells/insert. The medium was changed every 2 days. After 6 days, the integrity of the barrier was confirmed by detecting the transendothelial electrical resistance (TEER) by an epithelial volt-ohmmeter (Millicel-RES, Millipore, USA). The integrity of bEnd.3 monolayer was demonstrated by the TEER value of 200 Ω cm^{-2}. Monolayers with TEER over 200 Ω cm^{-2} were used for further experiments. After adding RT-PPB NPs or T-PPB NPs containing 1 μg FITC-siRNA in the upper culture medium for 12 h, the medium from bottom sides of the BBB model was collected. We measured the NP concentration in the medium by determining the fluorescence intensity, based on the analytical curves of each fluorescent particles with a fluorescent microplate reader (SpectraMax M5, Molecular Devices, CA, USA) with the excitation wavelength of 488 nm and emission wavelength of 525 nm. The transportation capacity was evaluated by permeability coefficient (P), which is calculated by following equation:

$$P = \frac{C_A}{t} \times \frac{1}{A} \times \frac{V}{C_L}$$

C_A: bottom chamber concentration; C_L: top chamber concentration; t: time; A: surface area of

membrane (for 24 transwell is 0.33 cm^2); *V*: medium volume in bottom side.

To establish the in vitro BBB models for analyzing the internalization of NPs in SH-SY5Y cells across the in vitro BBB, bEnd.3 cells were seeded in the upper chambers (pore size: 0.4 μm) at 2×10^5 cells/insert. The medium was changed every 2 days. After 6 days, SH-SY5Y cells were seeded on the lower chamber. After co-incubation for 24 h, the integrity of the barrier was confirmed by detecting the TEER. Monolayers with TEER over 200 Ω cm^{-2} were used for further experiments. Formulations with 1 μg Cy5-siRNA were added into upper inserts. The mean fluorescence intensity of Cy5 in SH-SY5Y cells were assessed using BD Caliburflow cytometry (BD Co., USA), after incubation for 0.5 h, 2 h, 4 h, 6 h, 8 h, and 12 h, respectively.

The Cellular Uptake of NPs in diverse cells. 2×10^5 BV2, HSC, SH-SY5Y cells were co-cultured with RT-PPB NPs (1 μg Cy5-siRNA) for 3 h, respectively. The mean fluorescence intensity of Cy5 was assessed using BD Caliburflow cytometry (BD Co., USA).

The Cellular Uptake of NPs by Membrane Fusion. 2×10^5 SH-SY5Y cells were seeded in 35 mm Petridishes (Cellvis) for 24 h, and then incubated with RT-PPB NPs or R-PPB NPs labeled FITC-liposome for 3 h at 37 °C. The cells were washed three times with PBS and stained with CellMask™ Deep Red plasma membrane stain for 10 min. The cells were then fixed with 4% paraformaldehyde for 15 min at 37 °C. Finally, the nucleus was stained with DAPI for 15 min at 37 °C. The fluorescence images were taken by CLSM (Zeiss Co., Germany).

The Cellular Uptake of NPs by Avoiding Endosomes Trapping. 2×10^5 SH-SY5Y cells were seeded in 35 mm Petridishes (Cellvis) for 24 h, and then incubated with RT-PPB NPs or T-PPB NPs with Cy5-siRNA for 3 h at 37 °C. The cells were washed three times with PBS followed by staining with LysoTracker Red DND-99 for 45 min at 37 °C. The cells were then washed three times with PBS and fixed with 4% paraformaldehyde for 15 min at 37 °C. Finally, the nucleus was stained with DAPI for 15 min at 37 °C. The fluorescence images were taken by CLSM (Zeiss Co., Germany). Additionally, 2×10^5 SH-SY5Y cells were seeded in 35 mm Petridishes (Cellvis) for 24 h, and then incubated with RT-PPB NPs or T-PPB NPs with Cy5-

siRNA for 3 h at 37 °C, respectively. The cells were washed three times with PBS followed by staining with Calcein for 30 min at 37 °C. The cells were then washed three times with PBS and fixed with 4% paraformaldehyde for 15 min at 37 °C. Finally, the nucleus was stained with DAPI for 15 min at 37 °C. The fluorescence images were taken by CLSM (Zeiss Co., Germany).

Endocytosis inhibition test. To inhibit specific endocytosis pathways, SH-SY5Y cells were preincubated with endocytic inhibitors including chlorpromazine (10 mg/mL), genistein (200 mg/mL), methyl-β-cyclodextrin (mβCD, 50 mM), dynasore (80 mM), and wortmannin (50 nM) for 30 min prior to the addition of Cy5-labeled NPs and throughout the 3 h uptake experiment at 37 °C. The mean fluorescence intensity of Cy5 in SH-SY5Y cells was assessed using BD Caliburflow cytometry (BD Co., USA).

ROS level in SH-SY5Y cells and $^{\alpha\text{-syn-mCherry}}$**SH-SY5Y cells**. SH-SY5Y cells and $^{\alpha\text{-syn-mCherry}}$SH-SY5Y cells were incubated for 24 h, and labeled with 10 μM DCFH-DA dye in culture medium at 37 °C for 20 min. After being washed three times with PBS, the fluorescence images were taken by CLSM (Zeiss Co., Germany).

The intracellular ROS-responsive release of siRNA. 2×10^5 SH-SY5Y cells and $^{\alpha\text{-syn-mCherry}}$SH-SY5Y cells were seeded in 35 mm Petridishes (Cellvis) for 24 h, respectively. The core of RT-PPB NPs encapsulated FITC and adsorbed Cy5-siRNA. Then SH-SY5Y and $^{\alpha\text{-syn-mCherry}}$SH-SY5Y cells were respectively incubated with RT-PPB NPs containing FITC and Cy5-siRNA at 37 °C for 3 h. The cells were then washed three times with PBS and fixed with 4% paraformaldehyde for 15 min at 37 °C. Finally, the nucleus was stained with DAPI for 15 min at 37 °C. The fluorescence images were taken by CLSM (Zeiss Co., Germany).

The expression of nSMase2 in SH-SY5Y cells. The SH-SY5Y cells were treated with 1×PBS and different formulations at 37 °C for 48 h. The concentration of siRNA was 3 μg/well in 6-well plates. The expression of nSMase2 was detected using western blot. Briefly, cells were lysed in 100 μL of lysate including PMSF and were shacked well on ice. After 14000 rpm centrifugation, the supernatant was taken for protein quantification BCA kit and boiled for 10

min at 95 °C. Considering the specificity of the primary antibody, only one protein was exposed at a time during the western blot experiment. The incubation with the target protein (nSMase2) antibody was first carried out and the corresponding image was obtained. Specifically, the proteins were firstly resolved by SDS-polyacrylamide gel electrophoresis (SDS-PAGE), secondly transferred to polyvinylidene fluoride membranes, thirdly blocked in 5% non-fat powdered milk in PBS-T (0.5% Tween-20) and at last incubated with nSMase2 antibody (primary antibody from Abcam) and then with secondary antibody (from Abcam). At last, enhanced chemiluminescence reagent (ECL) was used for protein bands detected by X-ray film and the image of nSMase2 was obtained. Then the membrane was regenerated by stripping buffer and incubated with the internal reference protein (β-actin) antibody. Western bands were scanned and normalized to the internal control β-actin. The western blot experiments in the article were all in accordance with the above operations.

The Expression of nSMase2 in $^{\alpha\text{-syn-mCherry}}$SH-SY5Y Cells. The $^{\alpha\text{-syn-mCherry}}$SH-SY5Y cells were treated with 1×PBS and different formulations at 37 °C for 48 h. The concentration of siRNA was 3 μg/well in 6-well plates. The expression of nSMase2 was detected using western blot. Briefly, cells were lysed in 100 μL of lysate including PMSF and were shacked well on ice. After 14000 rpm centrifugation, the supernatant was taken for protein quantification BCA kit and boiled for 10 min at 95 °C. The proteins were firstly resolved by SDS-PAGE, secondly transferred to polyvinylidene fluoride membranes, thirdly blocked in 5% non-fat powdered milk in PBS-T (0.5% Tween-20) and at last incubated with nSMase2 antibodies (primary antibody from Abcam) and then with secondary antibody (from Abcam). At last, ECL was used for protein bands detected by X-ray film. Western bands were scanned and normalized to the internal control β-actin.

Isolation and Characterization of Exosomes. 3×10^6 $^{\alpha\text{-syn-mCherry}}$SH-SY5Y cells were seeded in 100 mm cell culture dishes at 37 °C for 24 h. Then the mediums were removed and replaced with 10 mL fresh culture medium containing different formulations. After 12 h incubation, the

cells were rinsed three times with 1×PBS to ensure that interfering substances such as exosomes and nanoparticles were removed from the medium. Then $^{\alpha\text{-syn-mCherry}}$SH-SY5Y cells were continually cultured in medium supplemented with 10% exosome-depleted FBS for 48 h. Next, mediums were collected and subjected to centrifugation at 500 g for 10 min followed by centrifugation at 3000 g for 20 min at 4 °C to remove debris and dead cells. The mediums were further centrifuged at 10000 g for 30 min at 4 °C. The supernatants were passed through a 0.22 um filter. Then the supernatants were transferred to an ultrafiltration centrifuge tube (100 kDa) and centrifugated at 5000 g for 30 min to concentrate. The concentrated mediums were transferred to a new tube and add 0.2 volumes of the Exosome Isolation Reagent (for cell culture). Then the medium/reagent mixture was mixed by pipetting up and down until there was a homogenous solution with cloudy appearance and incubated overnight at 4 °C. Then the sample was centrifuged at 1500 g for 30 min and the supernatant was aspirated and discarded. Exosomes were contained in the pellet at the bottom of the tube and resuspended with sterile 1×PBS. The size and spherical morphology of exosomes were confirming with TEM and Nanoparticle Tracking Analysis (NTA).

The Expression of α-syn-mCherry in $^{\alpha\text{-syn-mCherry}}$SH-SY5Y Cells. The $^{\alpha\text{-syn-mCherry}}$SH-SY5Y cells were cultured and incubated with different formulations for 48 h at 37 °C. The cells were then washed three times with sterile 1×PBS and fixed with 4% paraformaldehyde for 15 min at 37 °C. Finally, the nucleus was stained with DAPI for 15 min at 37 °C. CLSM (Zeiss Co., Germany) was used to detect α-syn-mCherry (587 nm, 610 nm).

The Construction of Transwell Model and Expression of α-syn-mCherry in SH-SY5Y Cells. We constructed the transwell system according to the steps below (Fig. 6a). 1-1) The donor cells ($^{\alpha\text{-syn-mCherry}}$SH-SY5Y) were seeded on a polycarbonate membrane with 0.4 μm pore size for 24 h, and were treated with PBS and different formulations at 37 °C for 12 h. 1-2) Meanwhile, SH-SY5Y cells were seeded in 6-well plates for 24 h. 2) The $^{\alpha\text{-syn-mCherry}}$SH-SY5Y cells and SH-SY5Y cells were rinsed three times with 1×PBS and continually cultured in

medium supplemented with 10% exosome-depleted FBS. The $^{\alpha\text{-syn-mCherry}}$SH-SY5Y cells were placed on top of recipient SH-SY5Y cells, and subsequently co-cultured for 24 h at 37 °C. 3) Removal of the donor $^{\alpha\text{-syn-mCherry}}$SH-SY5Y cells. 4) The exosomes were extracted from cell culture medium according to the method above. The exosomes were repeatedly frozen and thawed, and the supernatants were obtained by centrifugation and used for ELISA assays. The SH-SY5Y cells also were repeatedly frozen and thawed to destroy the cells and release the intracellular components, then the supernatants were obtained by centrifugation and used for ELISA assays. Besides, the SH-SY5Y cells were then washed three times with 1×PBS and fixed with 4% paraformaldehyde for 15 min at 37 °C. Finally, the nucleus was stained with DAPI for 15 min at 37 °C. CLSM (Zeiss Co., Germany) was used to detect α-syn-mCherry (587 nm, 610 nm).

The Construction of MPTP-Induced PD Model Mice. All animal protocols in this work were approved by the Committee on the Ethics of Animal Experiments of the Institute of Process Engineering at the Chinese Academy of Sciences (approval ID: IPEAECA2020041). All animal experiments were performed in compliance with the policy of the Institute of Process Engineering at the Chinese Academy of Sciences on animal use and ethics. The 8-week-old male C57BL/6 mice were treated intraperitoneally with MPTP (30 mg kg^{-1}) for 7 consecutive days. PD model mice were randomly grouped (n=5). The open field test and pole test were utilized to investigate the change in exercise behaviour, and immunofluorescence staining and immunohistochemistry were used to evaluate the pathological changes.

In Vivo Biodistribution of NPs. To investigate the BBB permeability and neuron targeting ability, FITC was loaded in the hydrophobic region of the drug core of NPs and FITC-labeled RT-PPB NPs and T-PPB NPs were administrated intravenously to PD mice. After 12 h of treatment, the mice were sacrificed and the organs (brain, heart, liver, spleen, lung and kidney) were excised. The biodistribution of mice were detected *via* a Kadak in vivo imaging system. Besides, RT-PPB NPs and T-PPB NPs were administrated intravenously to PD mice (RAPA 3

mg kg^{-1}). After treatment for 12 h, mice were sacrificed and the brain was rapidly excised and methanol was added to the tissue. The samples were grinded, vortexed and the solution was immediately centrifuged at 14000 rpm for 10 min. The concentration of RAPA in the supernatant was measured by LC-MS. A HPLC system (Shimadzu, Kyoto, JPN) coupled to an AB Sciex 4000 triple quadrupole mass spectrometer (CA, USA) equipped with a Turbo Ionspray source was used for LC-MS analysis. Besides, Cy5-labeled RT-PPB NPs and T-PPB NPs were administrated intravenously to PD mice. With treatment for 12 h, the brains were sectioned and stained dopaminergic neurons for TH antibody.

In Vivo MRI of the NPs. The PD mice were treated with PBS, T-PPB NPs, and RT-PPB NPs *via* the tail vein for 12 h. During *in vivo* imaging, the PD mice were anesthetized with 2.5% isoflurane in 75% N_2 plus 22.5% O_2 and the isoflurane was reduced to 1% for maintenance of anesthesia. MRI studies of this line of mice were conducted on the 7 Tesla MRI system. The imaging parameters were: 100 μm isotropic spatial resolution; TR = 119.33 ms, TE = 4.99 ms, FA = 20^o.

Drug Treatment of PD Mice and Behavior Experiments. PD model mice were randomly grouped (n=5) and treated with different formulations (1 mg kg^{-1} siRNA and 3 mg kg^{-1} RAPA) by *i.v.* injection twice a week.[10] Mice were monitored for 5 weeks and evaluated behavior weekly. The apparatus was 50 × 50 × 40 cm. A mouse was placed in open field instrument, and its activities were recorded during the subsequent 5 min and then assessed. We then placed the mice upright on a 50 cm high pole and recorded the time they took for the entire journey to assess the movement coordination of the mice.

NP Effects on MPTP Treatment in Vivo. After the open field test and pole test, mice were anesthetized, then sacrificed. Western blot and quantified results of nSMase2 in the brain of mice with different treatment were obtained from 3 animals for each group. The brain tissues were dounce-homogenized in RIPA buffer, followed by centrifuging at 14000 g for 30 min at

4 °C. And the supernatants were collected and the nSMase2 levels in brain were analyzed by western blot. The α-syn aggregates levels of brain tissue in all treatment groups were determined by ELISA assays (n=5). Besides, the whole brains were harvested and fixed in 4% paraformaldehyde, embedded in paraffin, and sectioned at 4 μm. The immunofluorescence staining of α-syn at brain SN region and tyrosine hydroxylase (TH) for dopaminergic neurons to detect the α-syn changes (n=5). The brains were sectioned and stained for microglia by using Iba1 antibody. And immunohistochemistry of the TH-positive neurons to further detect the change of dopaminergic neurons (n=5). And heart, lungs, liver, kidneys, spleen and brain organs were fixed immediately in 4% PFA, then paraffnized and sectioned for hematoxylin and eosin staining.

Statistical Analysis. All data were expressed as mean ± SD. The significance was analyzed by the unpaired Student's t test by using GraphPad Prism version 6.0 software.

Supplementary Figure

Table S1 The abbreviation of formulations prepared in the experiments

Full name	Abbreviation
PPB/RAPA/SPIONs@sinSMase2	PPB Core
CPB/RAPA/SPIONs@sinSMase2	CPB Core
PPBn/RAPA/SPIONs@sinSMase2	PPBn Core
CPBn/RAPA/SPIONs@sinSMase2	CPBn Core
Liposome(PPB/RAPA/SPIONs@sinSMase2)	Lipo/PPB
RVG-TMS-Liposome(PPB/RAPA/SPIONs@sinSMase2)	RT-PPB
RVG-TMS-Liposome(PPB/RAPA/SPIONs@siNonsense)	RT-PPB(siNC)
RVG-Liposome(PPB/RAPA/SPIONs@sinSMase2)	R-PPB

TMS-Liposome(PPB/RAPA/SPIONs@sinSMase2)	T-PPB
RVG-TMS-Liposome(CPBn/RAPA/SPIONs@sinSMase2)	RT-CPBn

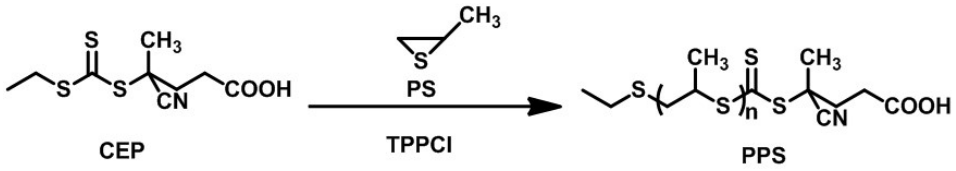

Fig. S1 Synthesis route of PPS.

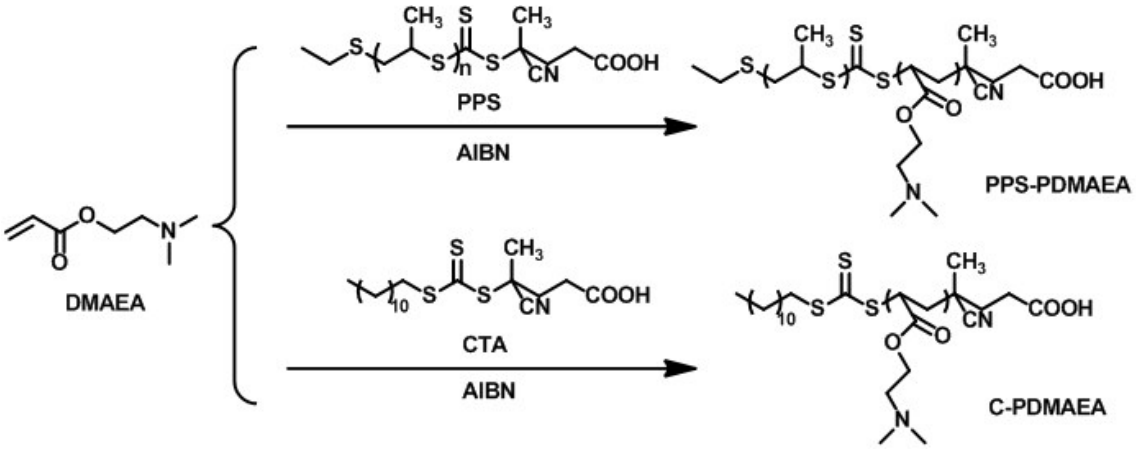

Fig. S2 Synthesis route of PPS-PDMAEA and C-PDMAEA.

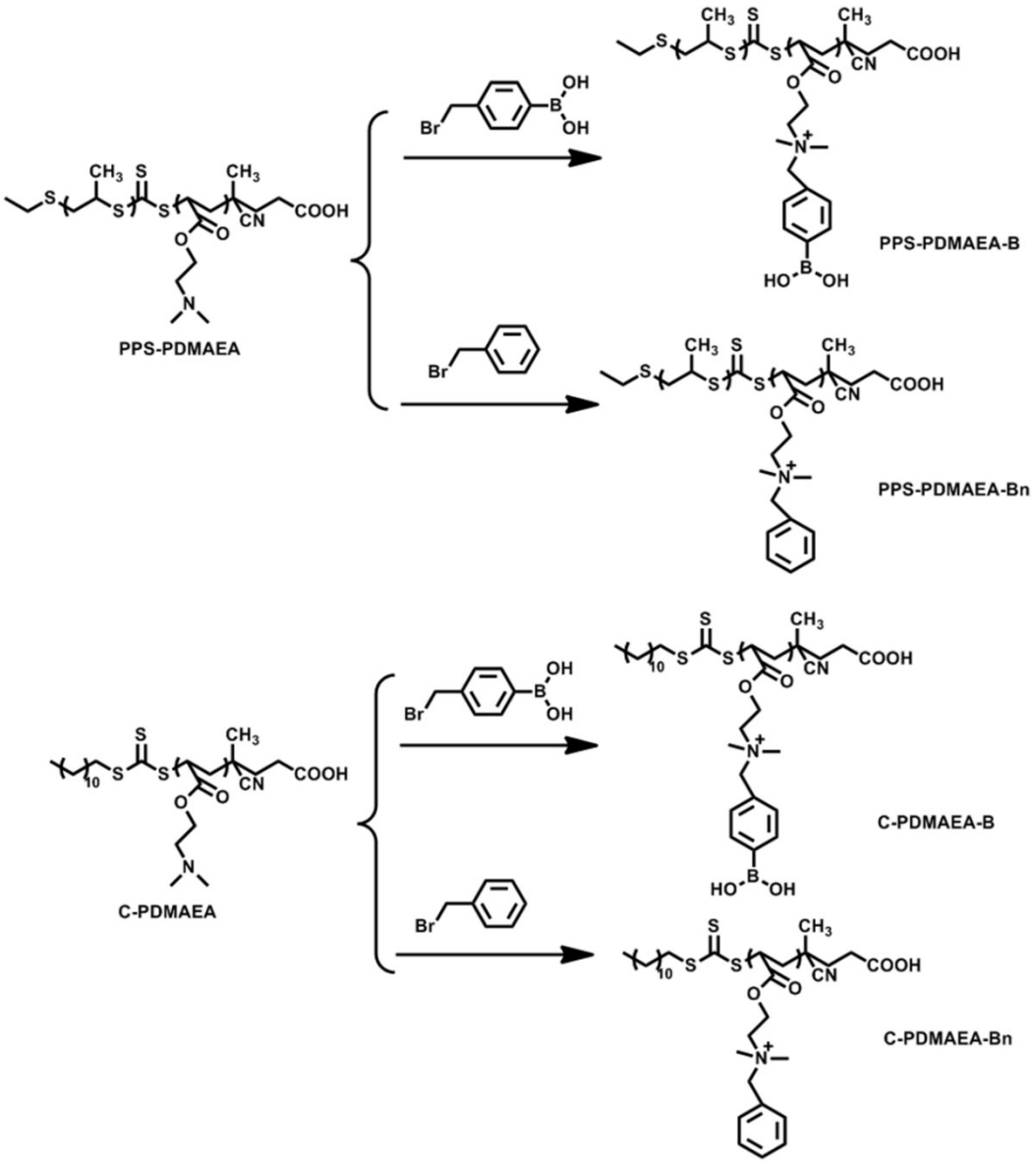

Fig. S3 Synthesis route of PPS-PDMAEA-B (PPB), PPS-PDMAEA-Bn (PPBn), C-PDMAEA-B (CPB) and C-PDMAEA-Bn (CPBn).

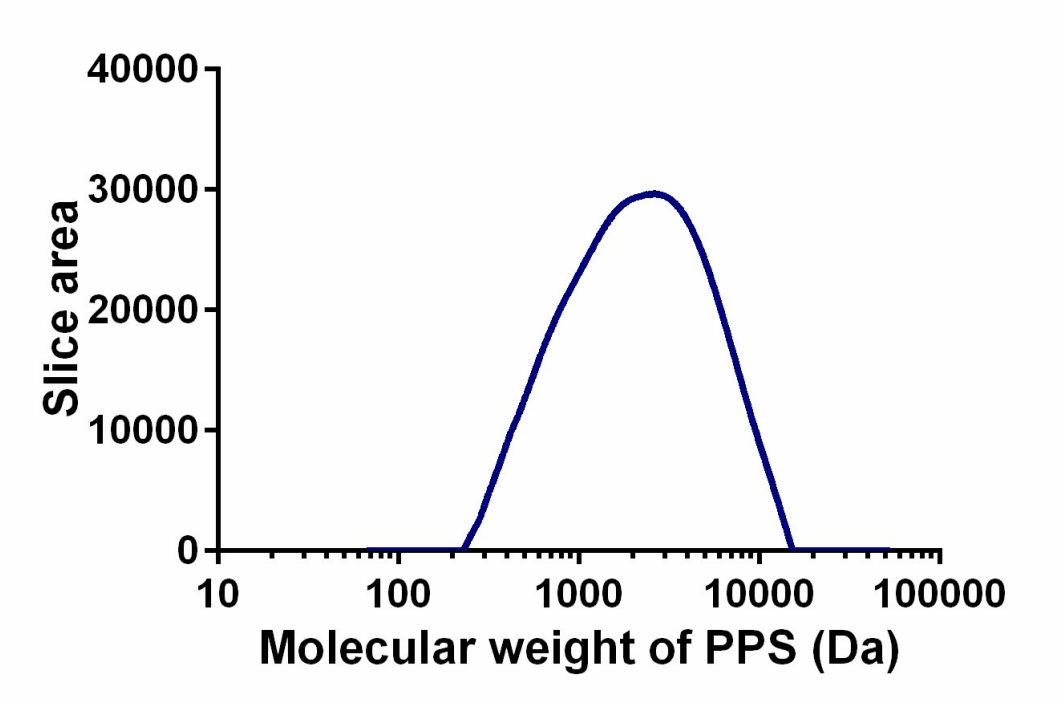

Fig. S4 The gel permeation chromatography of PPS polymer (Mw = 2938 Da, Mw/Mn = 1.52)

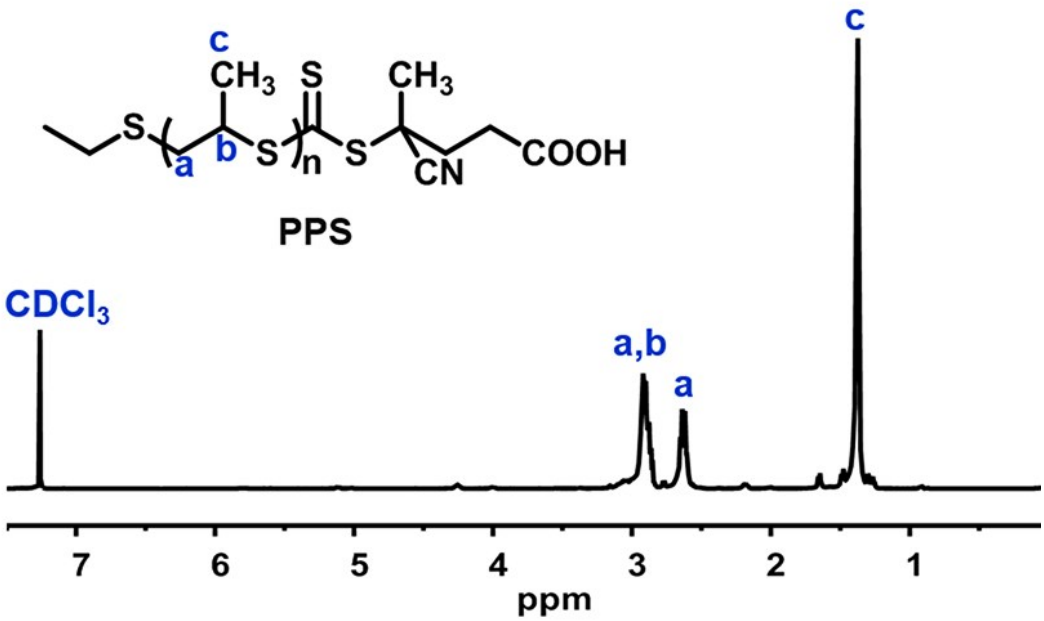

Fig. S5 ^{1}H NMR spectra of PPS.

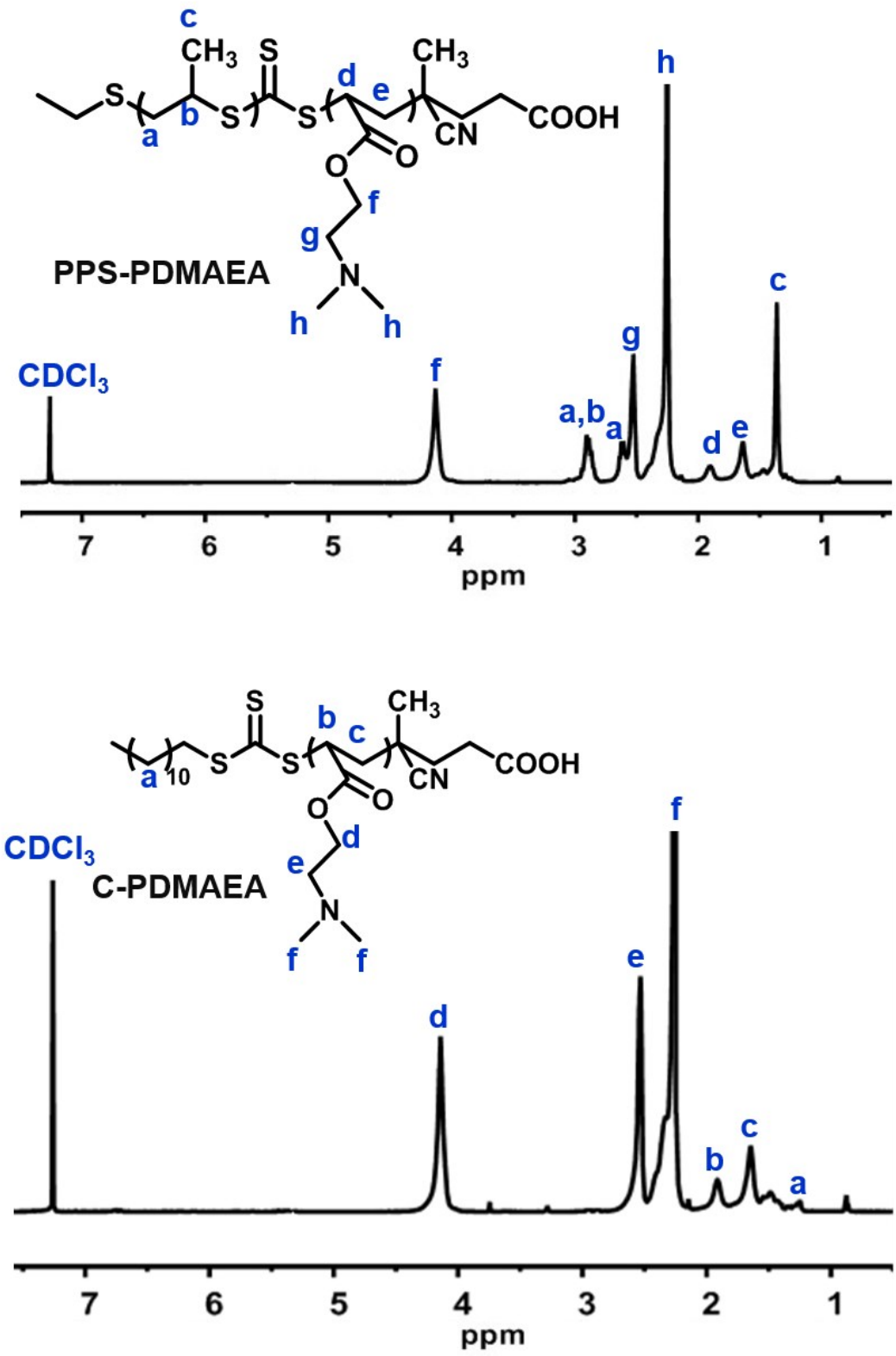

Fig. S6 ^{1}H NMR spectra of PPS-PDMAEA and C-PDMAEA.

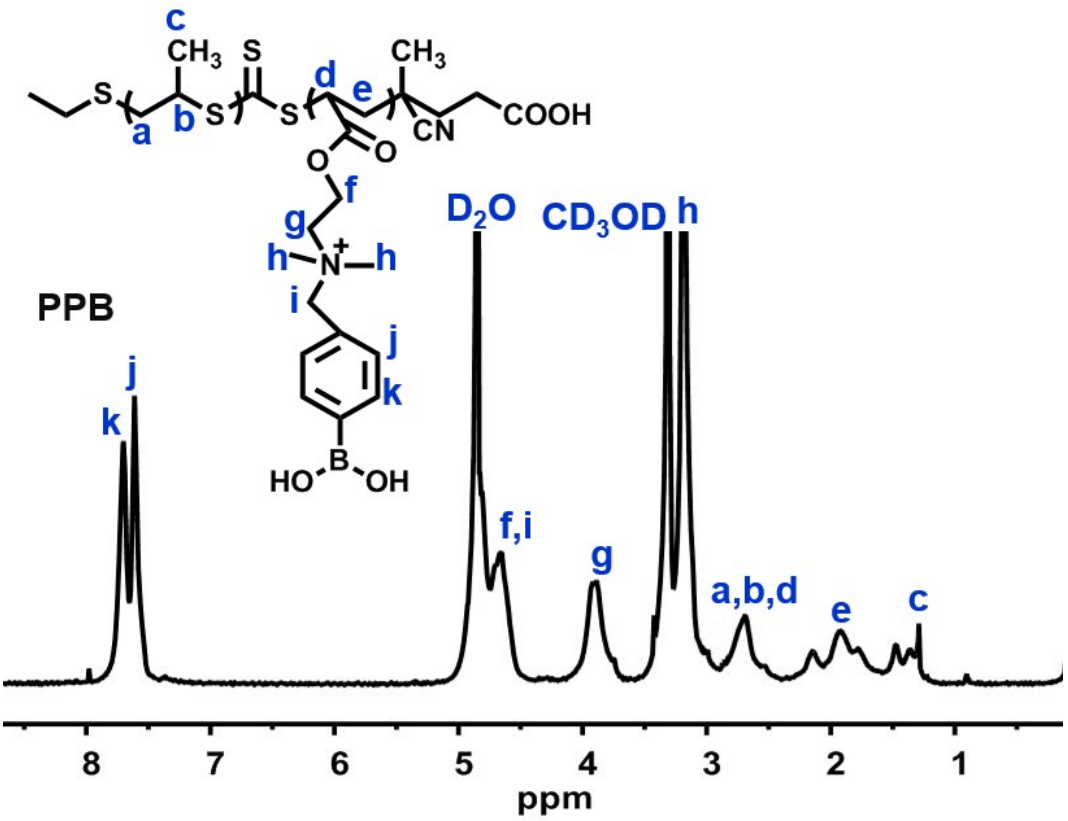

Fig. S7 ^{1}H NMR spectra of PPB.

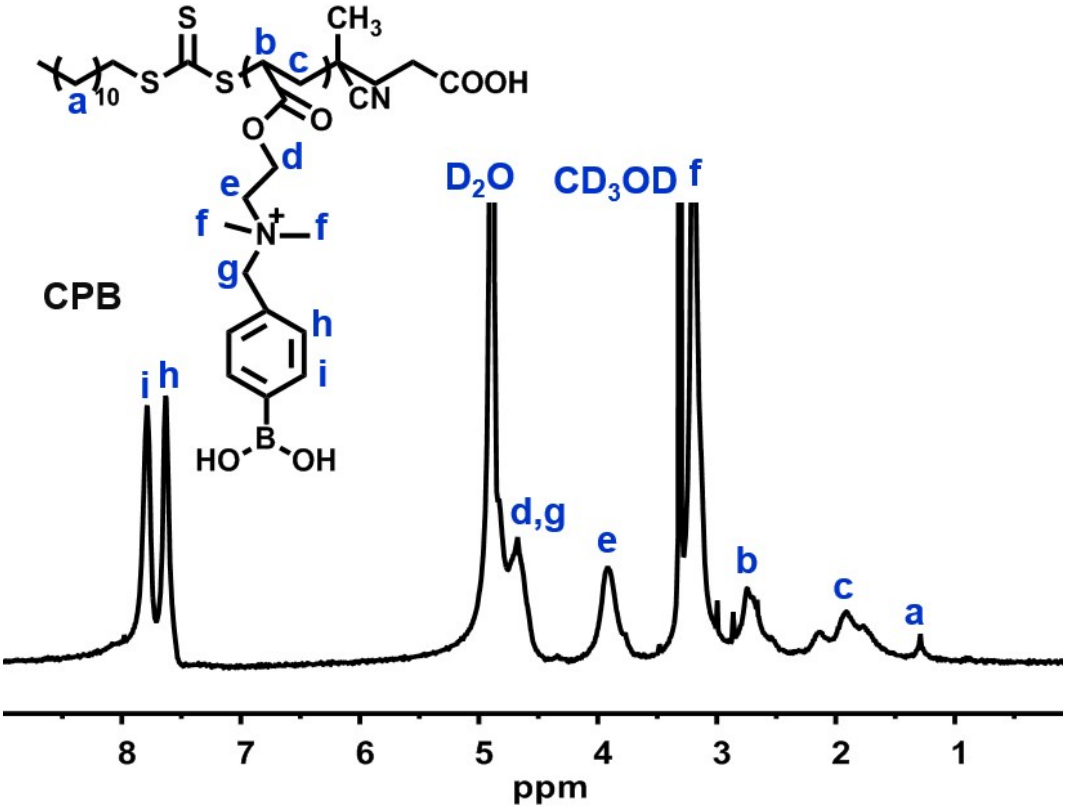

Fig. S8 ^{1}H NMR spectra of CPB.

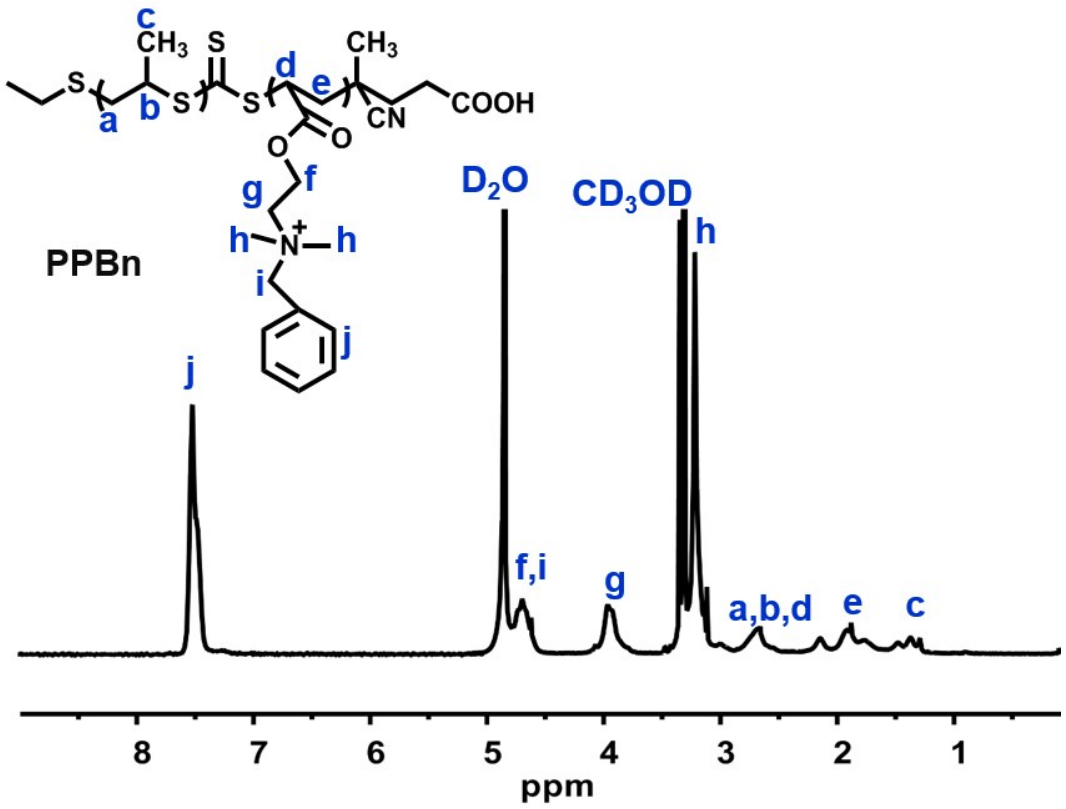

Fig. S9 ^{1}H NMR spectra of PPBn.

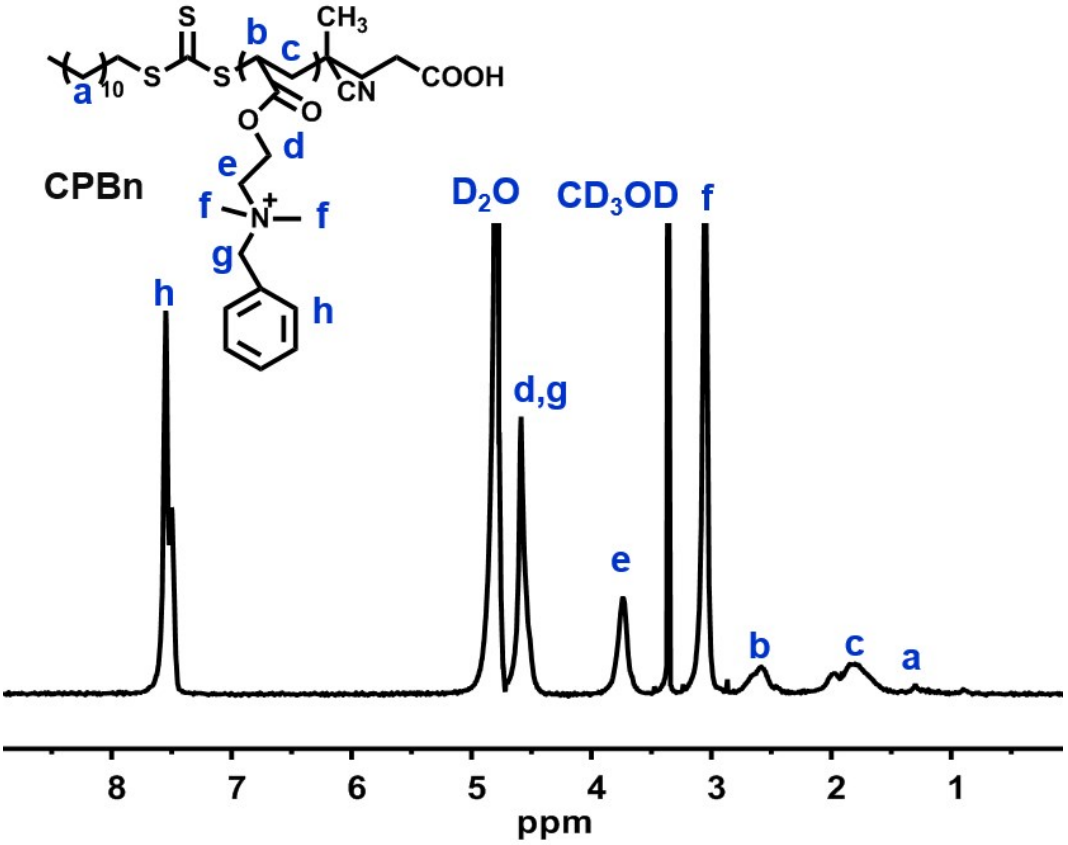

Fig. S10 ^{1}H NMR spectra of CPBn.

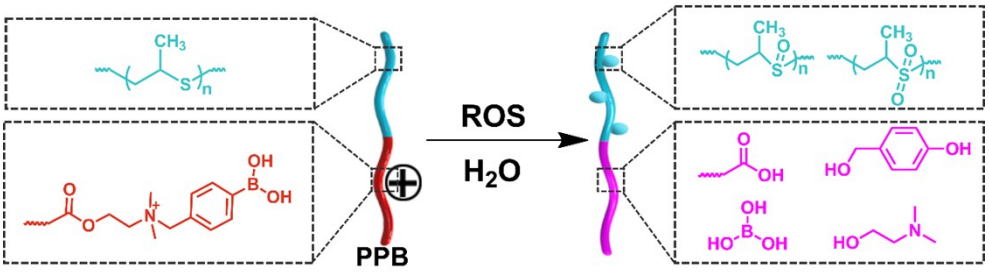

Fig. S11 The proposal chemical structure changes of PPB after being treated with H_2O_2.

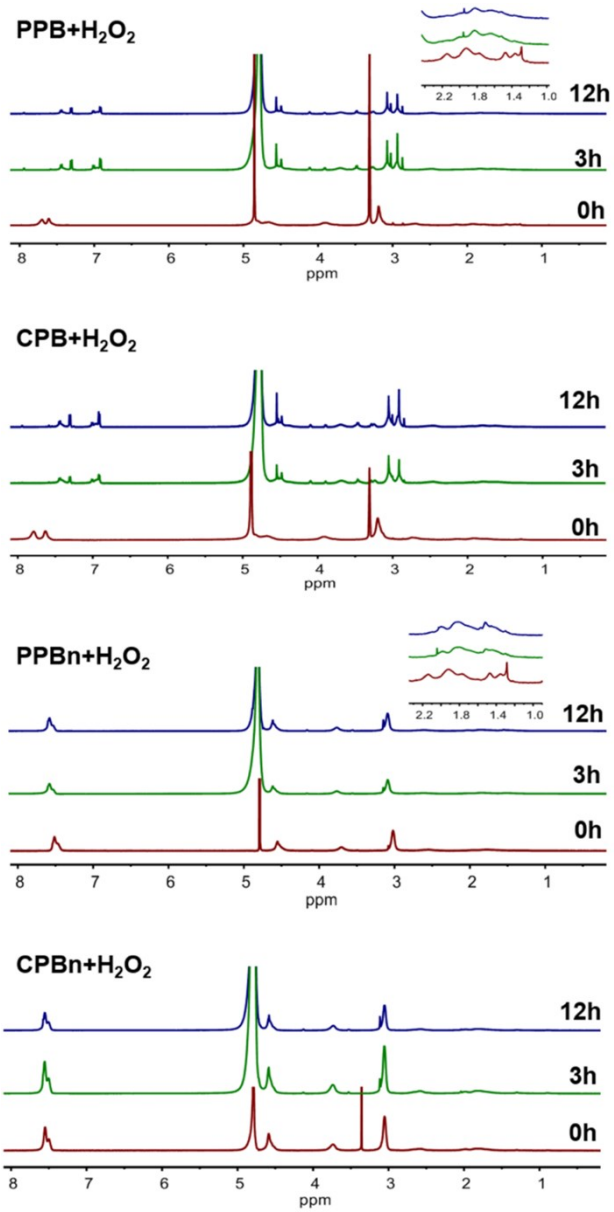

Fig. S12 ^{1}H NMR spectra of PPB, CPB, PPBn, and CPBn polymers (5 mg mL^{-1}) after being treated with H_2O_2 (10 mM) for 3 h and 12 h, respectively.

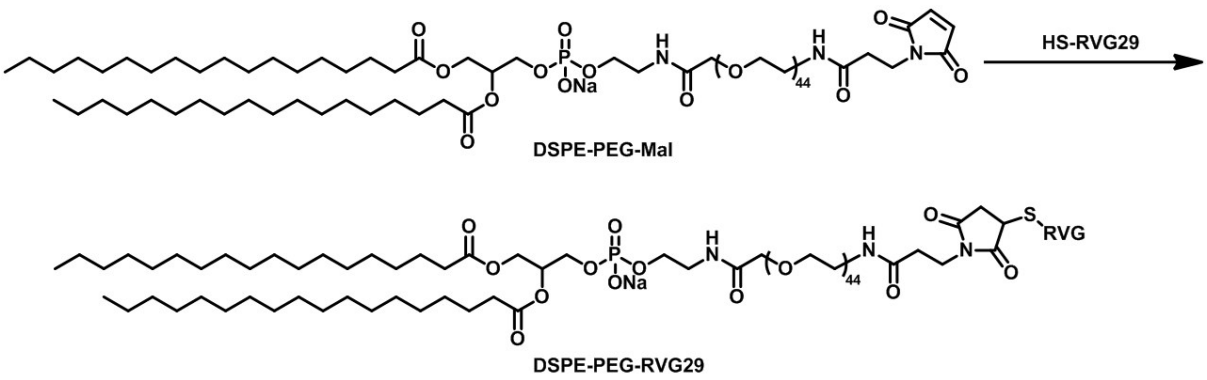

Fig. S13 Synthesis route of DSPE-PEG-RVG29.

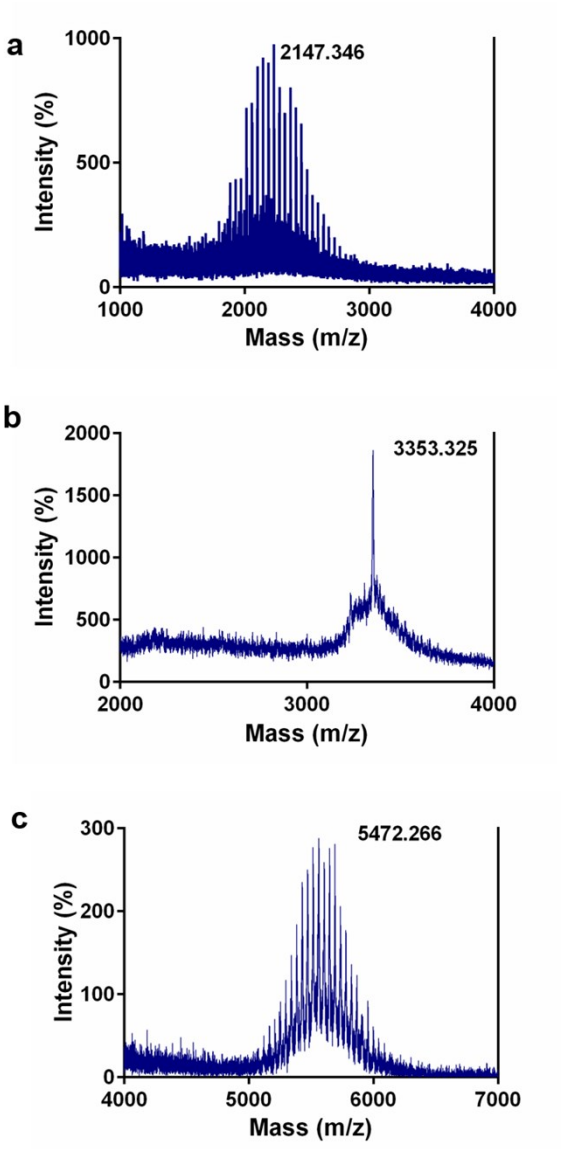

Fig. S14 Matrix-assisted laser desorption ionization time-of-flight mass spectrometry image of (a) DSPE-PEG2000, (b) RVG29-Cys and (c) DSPE-PEG-RVG29.

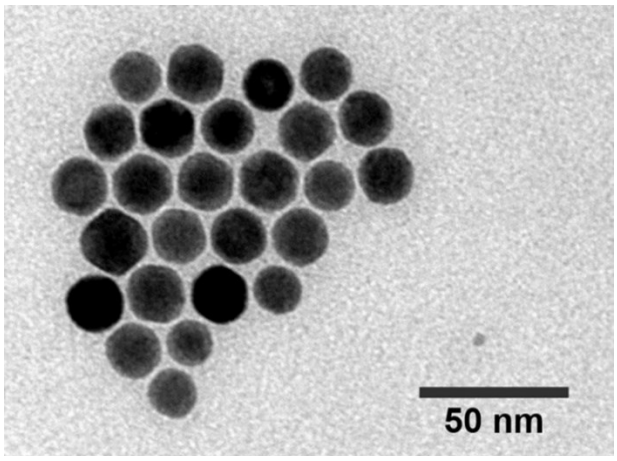

Fig. S15 TEM image of SPIONs. Scale bar: 50 nm.

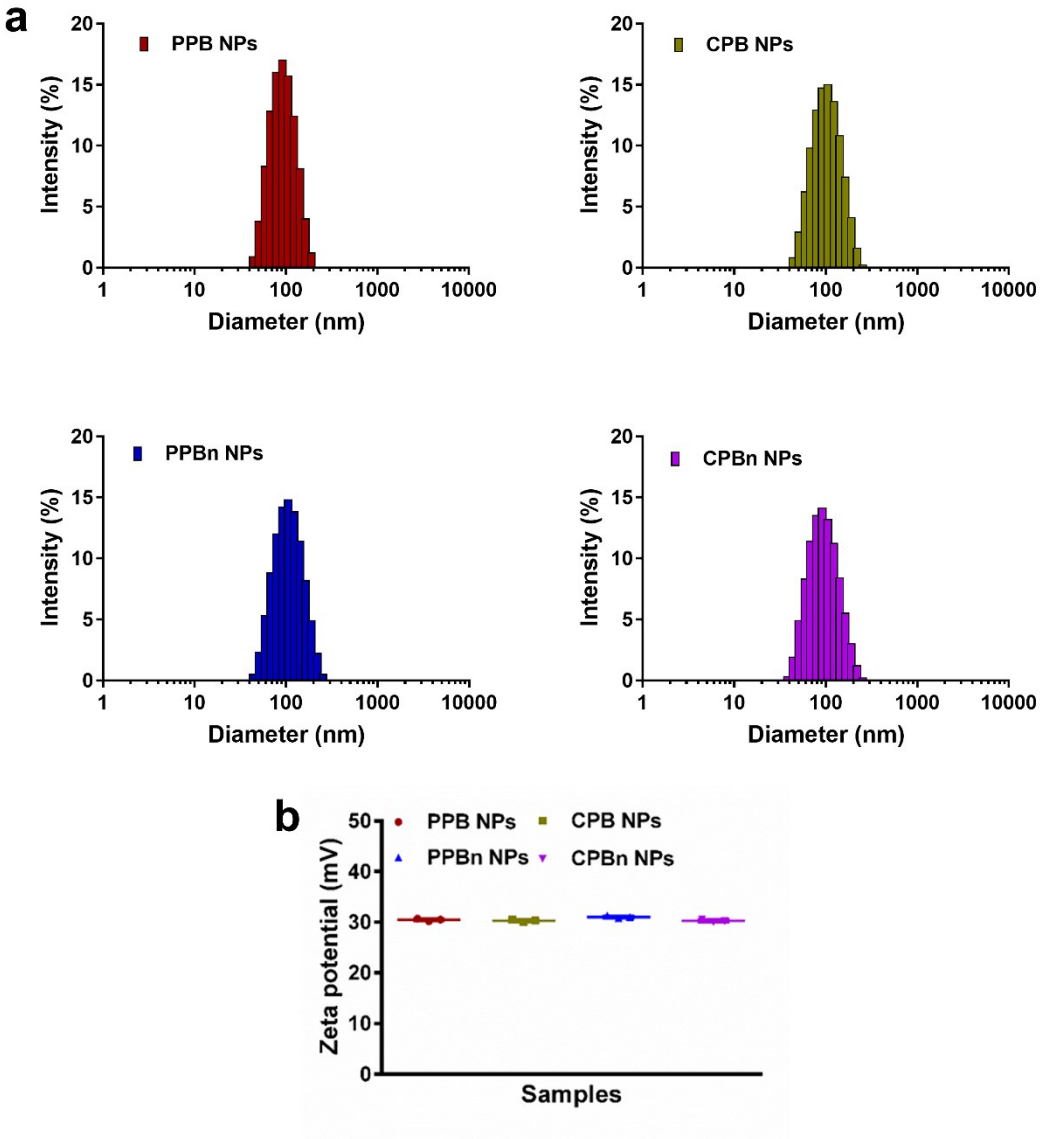

Fig. S16 (a) The diameters and (b) zeta potentials of four core NPs without siRNA (n=3).

Table S2 PDI values of four core NPs without siRNA (n=3).

Nanoparticles	PPB NPs	CPB NPs	PPBn NPs	CPBn NPs
PDI	0.175±0.027	0.138±0.035	0.217±0.012	0.206±0.016

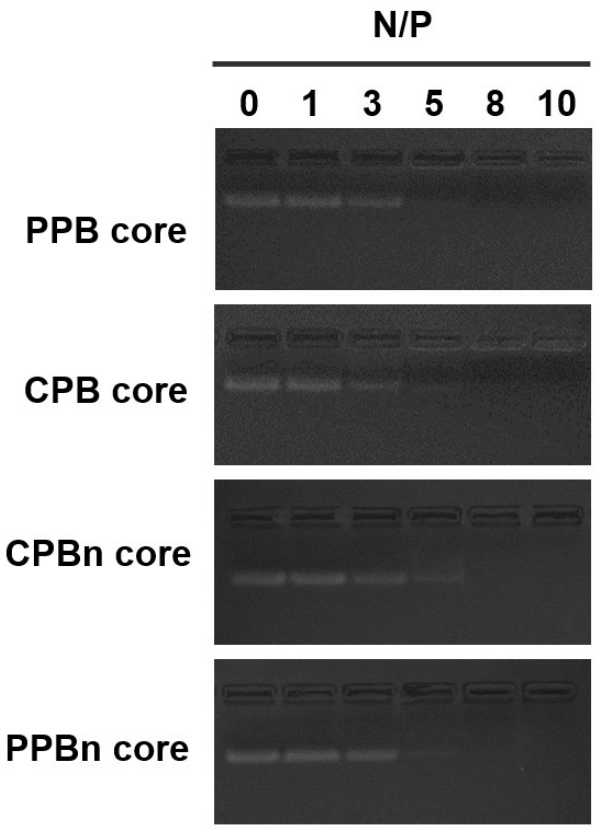

Fig. S17 The gel retardation assay of siRNA at various N/P ratios after complexing with four core NPs.

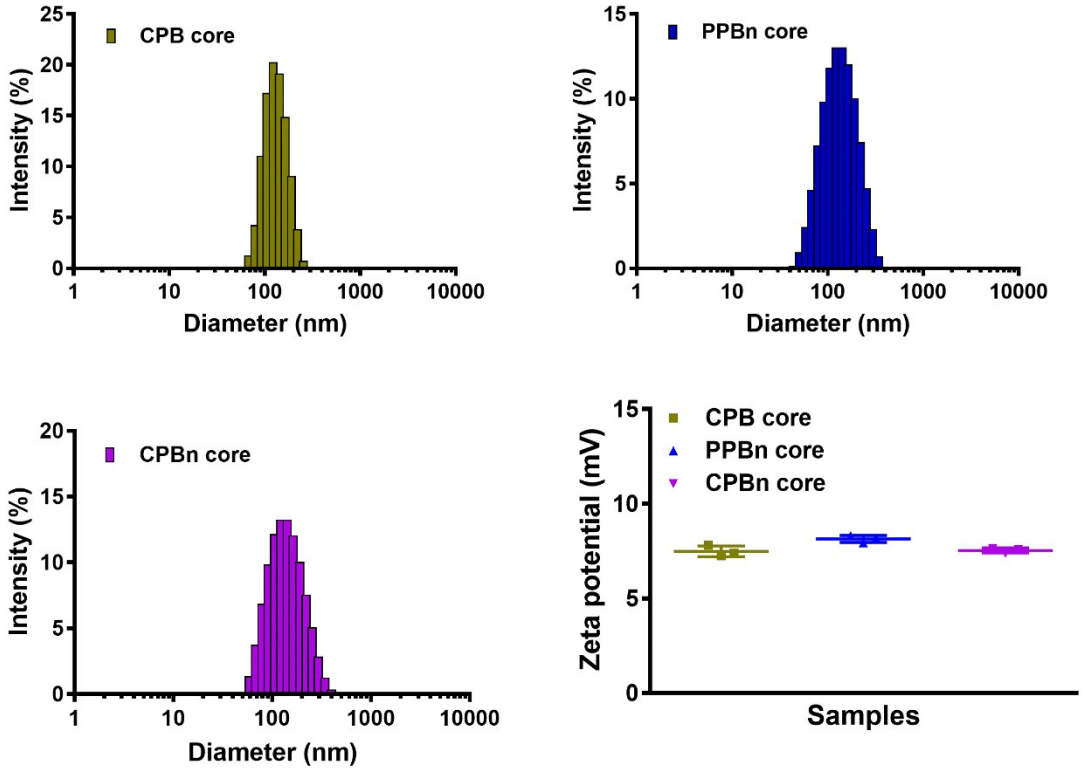

Fig. S18 The diameters and zeta potentials of other three core NPs, CPB core, PPBn core, and CPBn core at the N/P ratio of 8 (n=3).

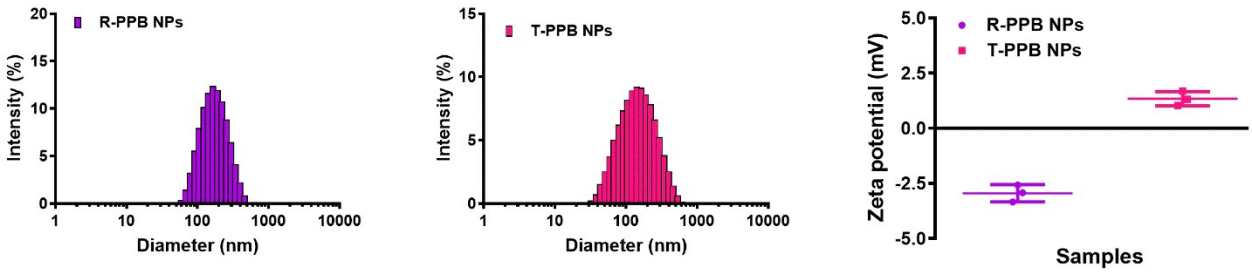

Fig. S19 The diameters and zeta potentials of RVG-Liposome(PPB/RAPA/SPIONs@sinSMase2) nanoparticles (R-PPB NPs) and TMS-Liposome(PPB/RAPA/SPIONs@sinSMase2) nanoparticles (T-PPB NPs) (n=3).

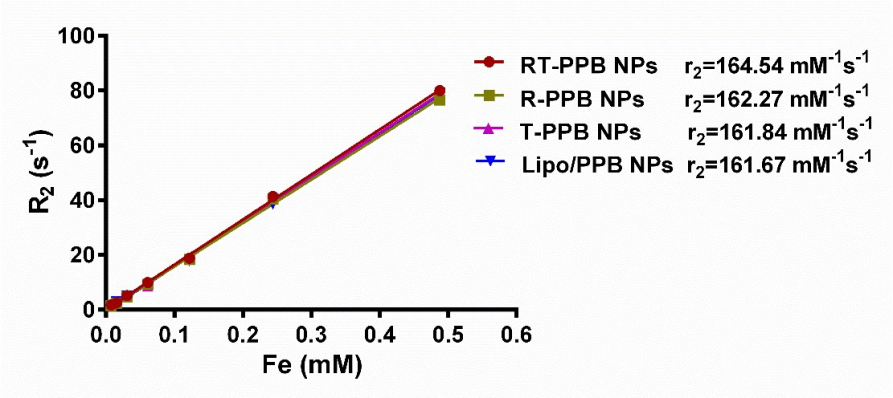

Fig. S20 The r_2 value of RT-PPB NPs, R-PPB NPs, T-PPB NPs, and Lipo/PPB NPs.

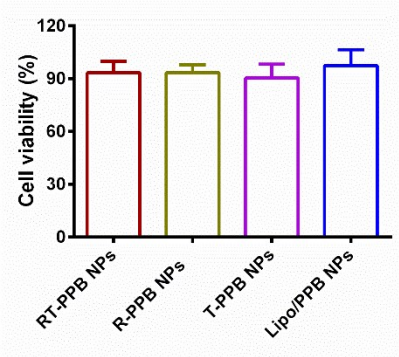

Fig. S21 MTT assay of cell viability after 24 h treatment with RT-PPB NPs, R-PPB NPs, T-PPB NPs, and Lipo/PPB NPs (1 μg mL^{-1} sinSMase2 and 100 nM RAPA) (n=5). Data are presented as the mean ± SD. Student's *t*-test.

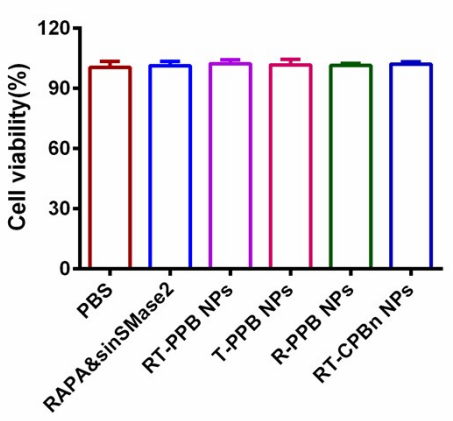

Fig. S22 Alamar blue assay of cell viability after 24 h treatment with different treatment groups (1 μg mL^{-1} sinSMase2 and 100 nM RAPA) (n=5). Data are presented as the mean ± SD. Student's *t*-test.

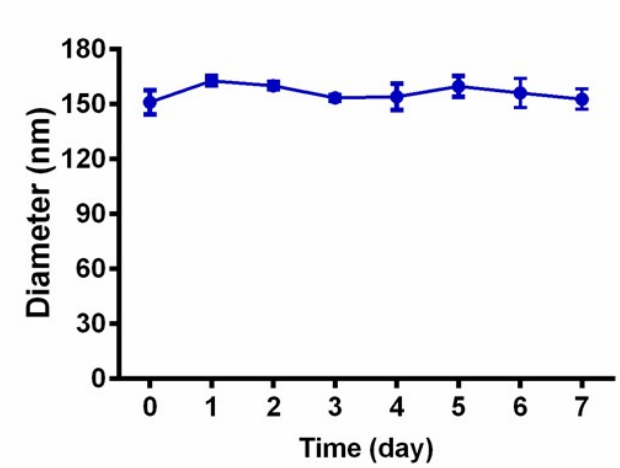

Fig. S23 The serum stability test conducted by measuring the average diameters of RT-PPB NPs at 37 °C (n=3).

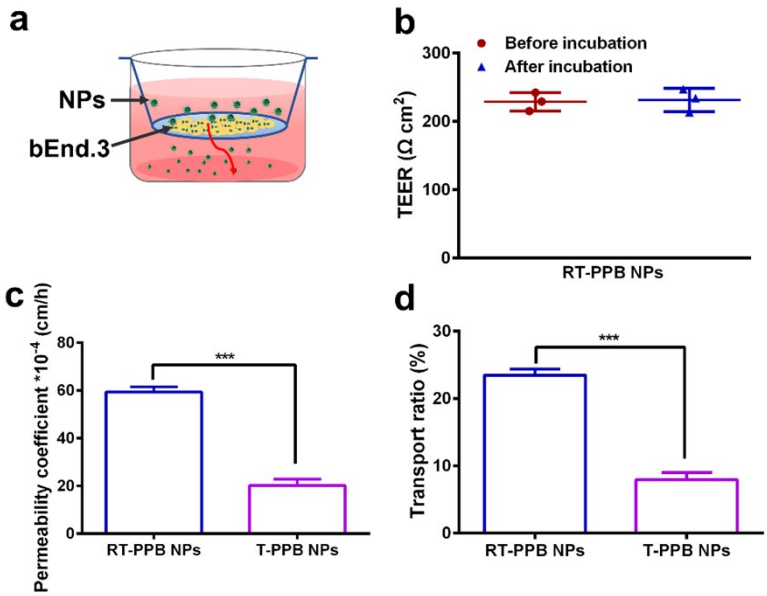

Fig. S24 Blood brain barrier permeability of RT-PPB NPs *in vitro*. (a) The schematic illustration of the transwell model. (b) The transepithellal electric resistance (TEER) of bEnd.3 cells in tranwell inserts before and after RT-PPB NPs incubation for 12 h. (c) The permeability coefficient of RT-PPB NPs and T-PPB NPs (n=3). (d) The transport ratio of RT-PPB NPs and T-PPB NPs in transwell model (n=3). Data are presented as the mean ± SD. ***$P < 0.001$, Student's *t*-test.

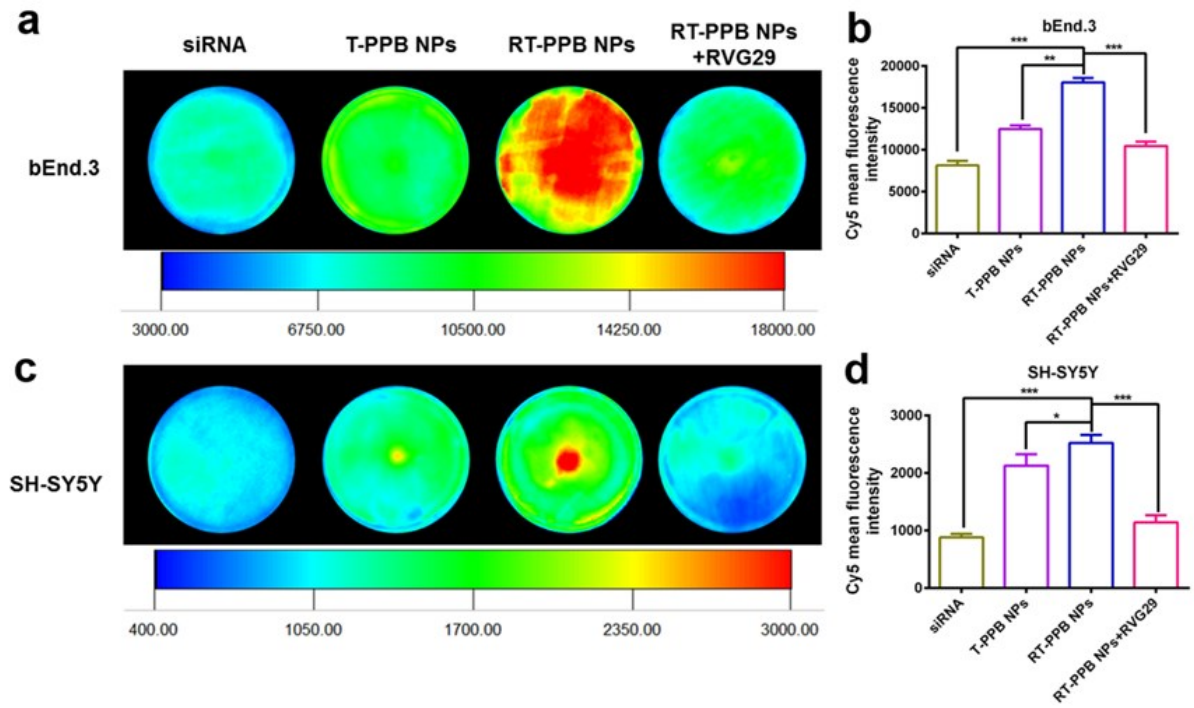

Fig. S25 The cellular uptake of NPs in bEnd.3 cells (a, b) and SH-SY5Y cells (c, d) in transwell model (n=3). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, Student's *t*-test.

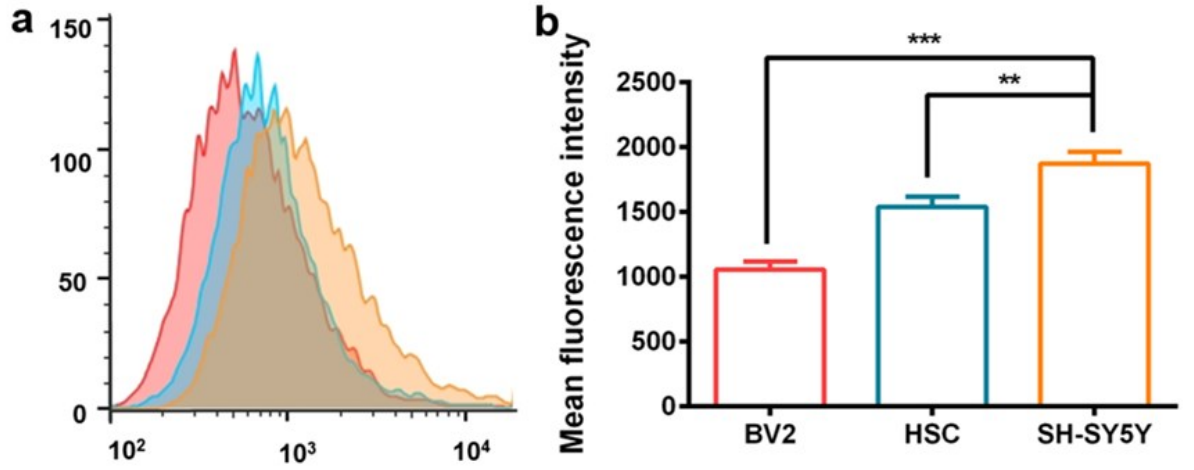

Fig. S26 The cellular uptake of NPs with 1 μg Cy5-siRNA (a) in BV2, HSC and SH-SY5Y cells detected by flow cytometry for 3 h and (b) quantified results (n=3). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, Student's *t*-test.

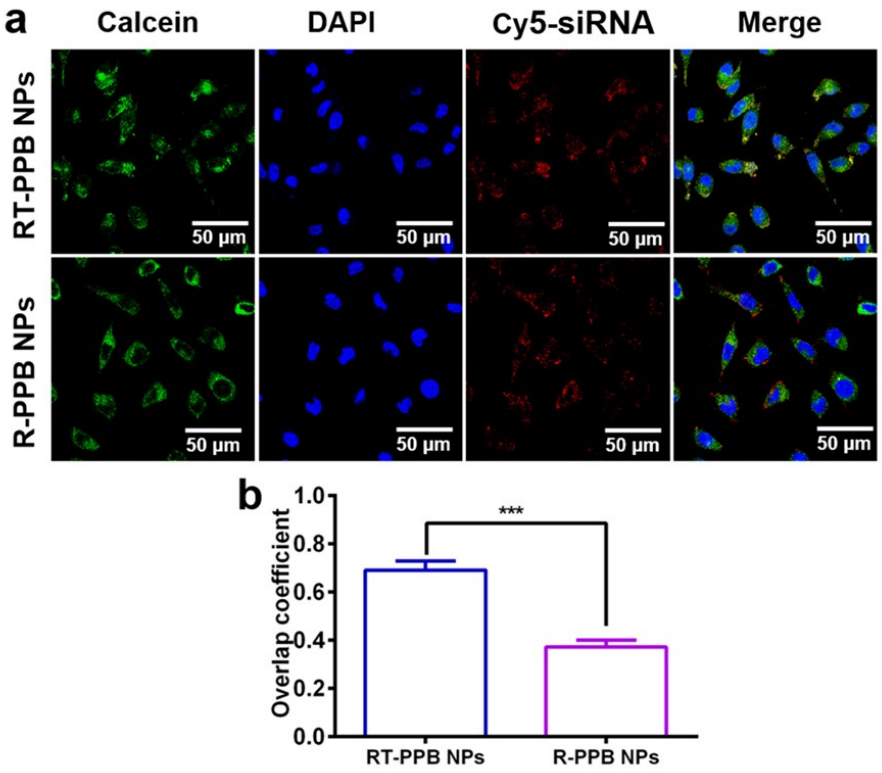

Fig. S27 The cellular internalization (a) and the overlap coefficient (b) of Cy5 and cytoplasm quantified results (n=3). More than 100 cells were analyzed for the quantification. ***P < 0.001, Student's *t*-test.

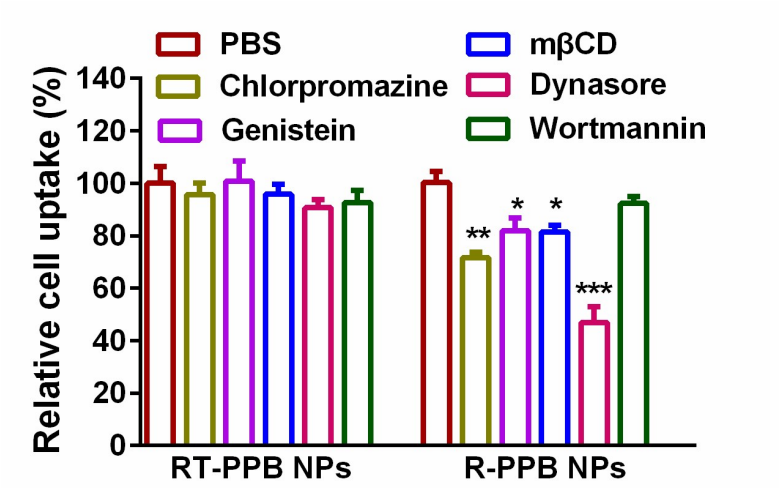

Fig. S28 Cellular uptake of RT-PPB NPs and R-PPB NPs after treatment with endocytosis inhibitors (n=3). Data are presented as the mean ± SD. *P < 0.05, **P < 0.01, ***P < 0.001, Student's *t*-test.

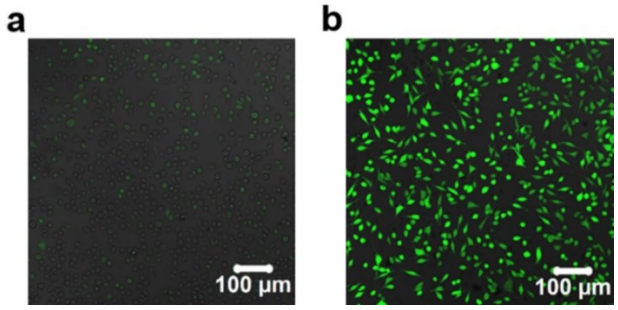

Fig. S29 The ROS level in (a) SH-SY5Y cells and (b) α-syn-mCherry over-expression SH-SY5Y ($^{\alpha\text{-syn-mCherry}}$SH-SY5Y) cells. Cells were cultured for 24 h, and labeled with 10 μM DCFH-DA dye in culture medium at 37 °C for 20 min. After being washed three times with PBS, the fluorescence images were taken by CLSM.

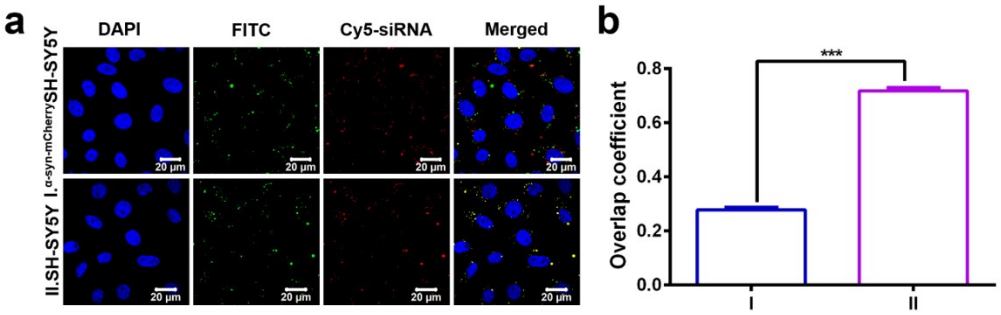

Fig. S30 The ROS-responsive drug release of RT-PPB NPs with 1 μg Cy5-siRNA in (a) $^{\alpha\text{-syn-mCherry}}$SH-SY5Y cells and SH-SY5Y cells and (b) the overlap coefficient quantified results (n=3). More than 100 cells were analyzed for the quantification. Data are presented as the mean ± SD. ****P* < 0.001, Student's *t*-test.

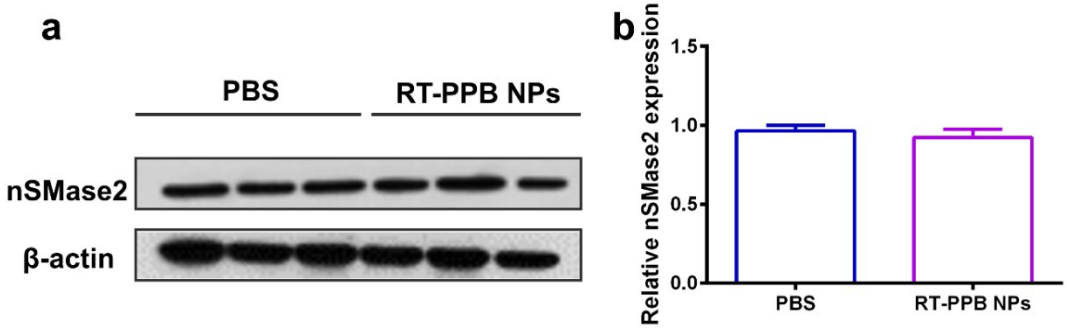

Fig. S31 Western blot of (a) nSMase2 protein in SH-SY5Y cells after treatment with RT-PPB NPs and (b) the quantitative results (n=3). Data are presented as the mean ± SD. Student's *t*-test.

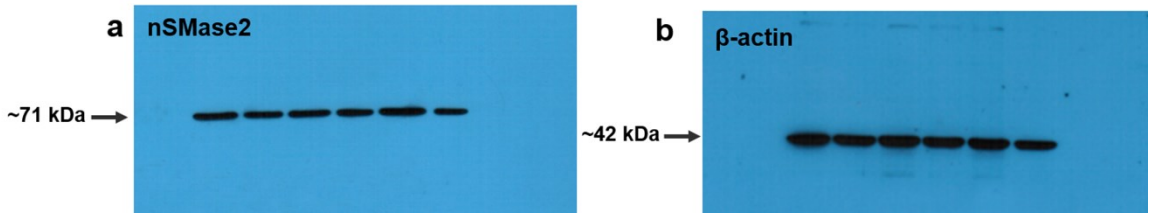

Fig. S32 The original western blot images corresponding to Fig. S31a. (a) Western blot image of nSMase2 (71 kDa). (b) Western blot image of β-actin (42 kDa).

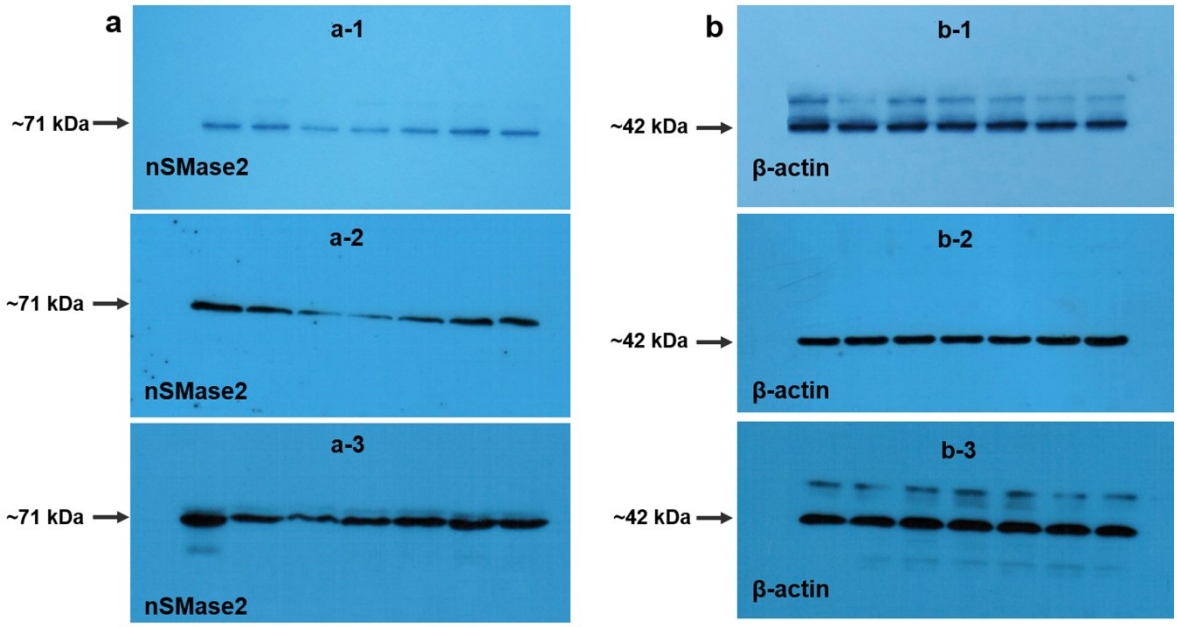

Fig. S33 The original western blot images corresponding to Fig. 3a. (a) Western blot images of nSMase2 (71 kDa). (b) Western blot images of β-actin (42 kDa).

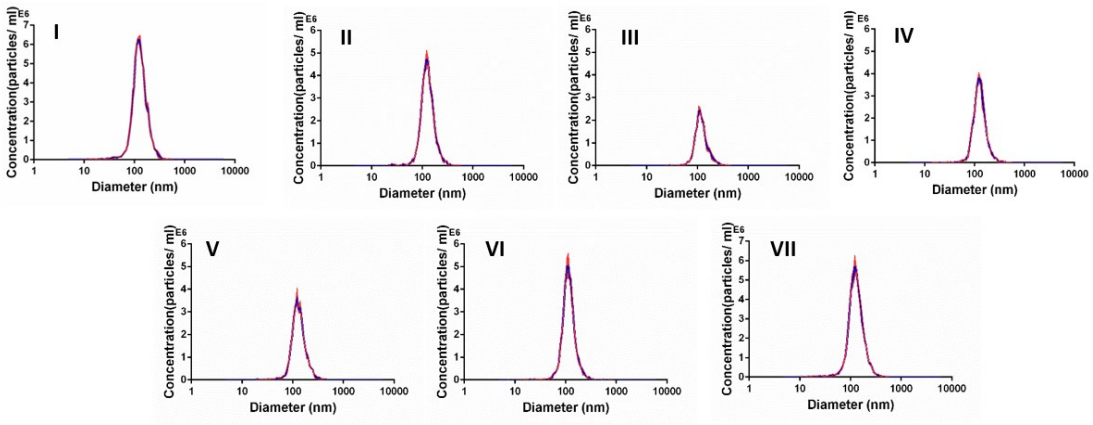

Fig. S34 Concentrations of exosomes secreted by the cells with different treatment (n=3). (I) PBS, (II) sinSMase2, (III) RT-PPB NPs, (IV) T-PPB NPs, (V) R-PPB NPs, (VI) RT-CPBn

NPs, (VII) RT-PPB(siNC) NPs.

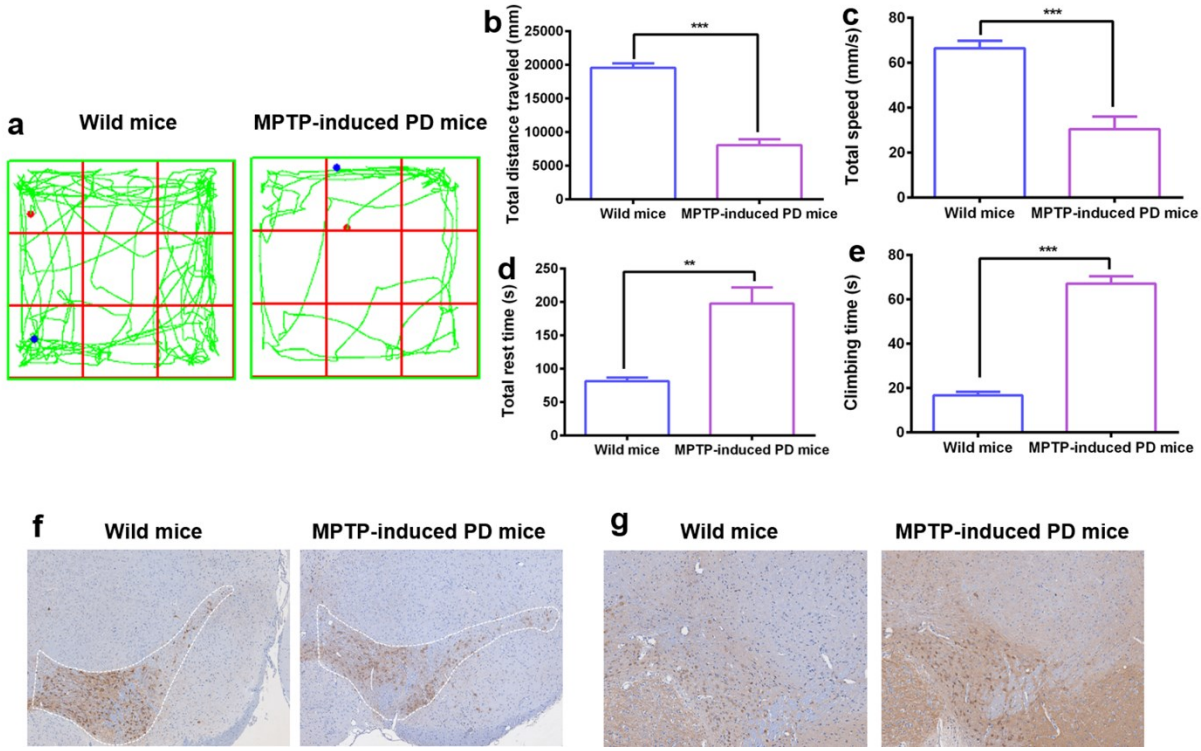

Fig. S35 PD model mice evaluation. (a) Open-field experiment of wild and MPTP-induced PD mice. (b) Total distance traveled (c) total speed, (d) total rest time and (e) climbing time in pole test of wild and MPTP-induced PD mice (n=5). (f) Tyrosine hydroxylase and (g) α-syn immunohistochemistry of brain slides in wild and MPTP-induced PD mice. Data are presented as the mean ± SD. $^{**}P < 0.01$, $^{***}P < 0.001$, Student's *t*-test.

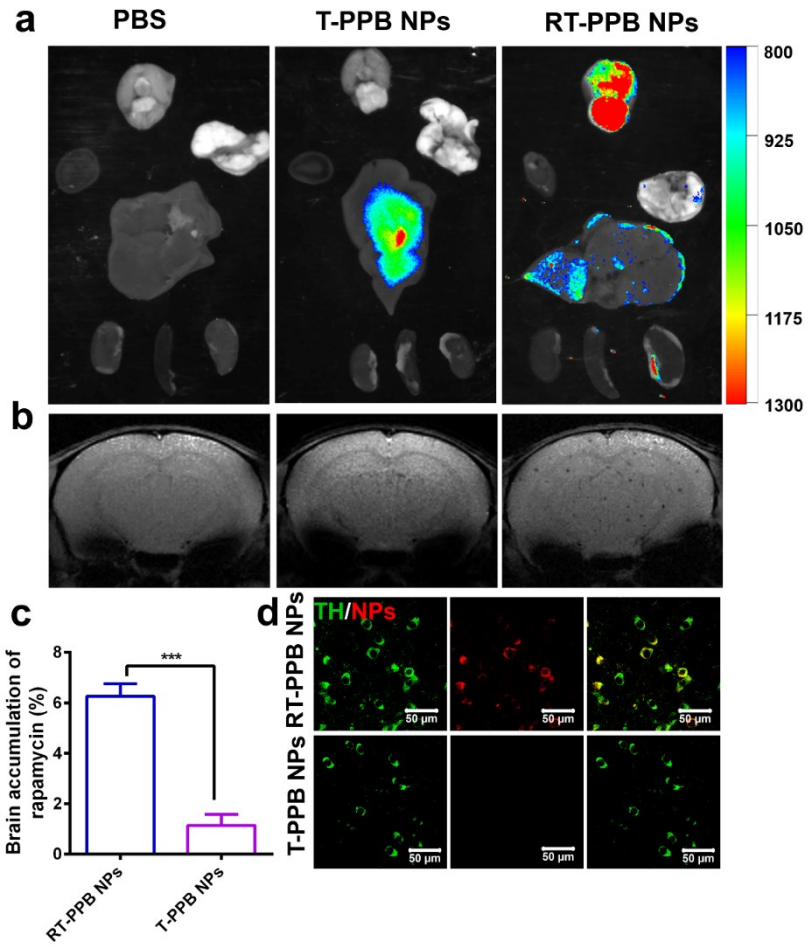

Fig. S36 *In vivo* brain targeting of RT-PPB NPs. (a) Ex-vivo biodistribution of NPs in PBS, T-PPB NPs and RT-PPB NPs treated PD mice after 12 h intravenous injection (n=3). (b) Representative in vivo T_2* MRI images of brain in PBS, T-PPB NPs and RT-PPB NPs treated PD mice after 12 h intravenous injection (n=3). (c) The RAPA concentration of brain homogenate of PD mice (n=3). (d) Immunofluorescence staining of NPs treated PD mice brain: dopaminergic neurons (green) and Cy5-labeled NPs (red). Data are presented as the mean ± SD. ***$P < 0.001$, Student's *t*-test.

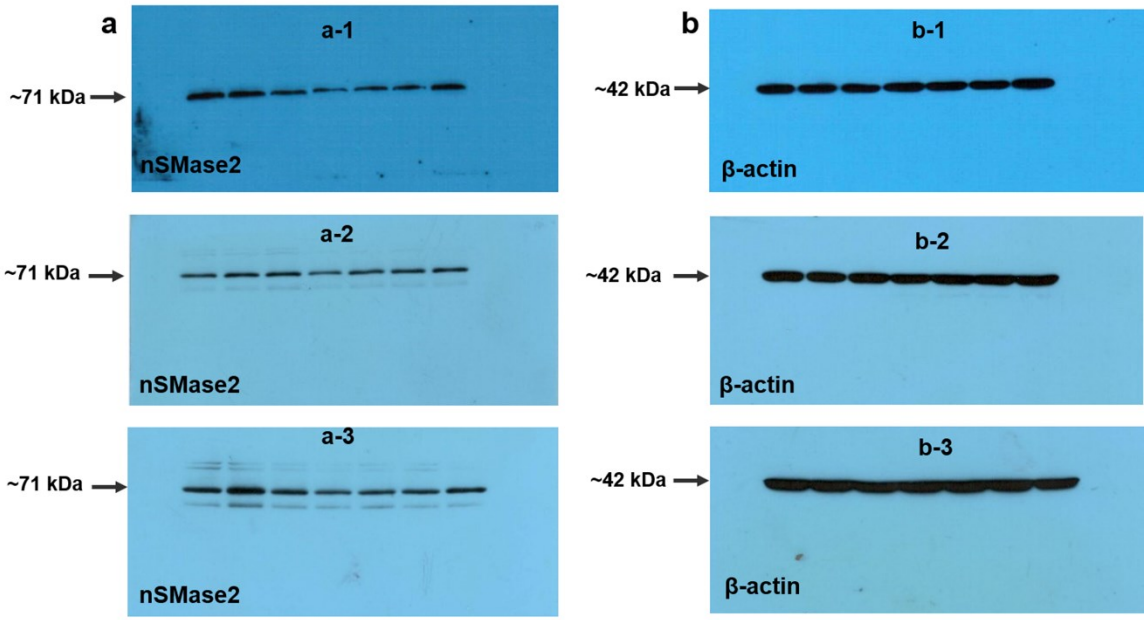

Fig. S37 The original western blot images corresponding to Fig. 6a. (a) Western blot images of nSMase2 (71 kDa). (b) Western blot images of β-actin (42 kDa).

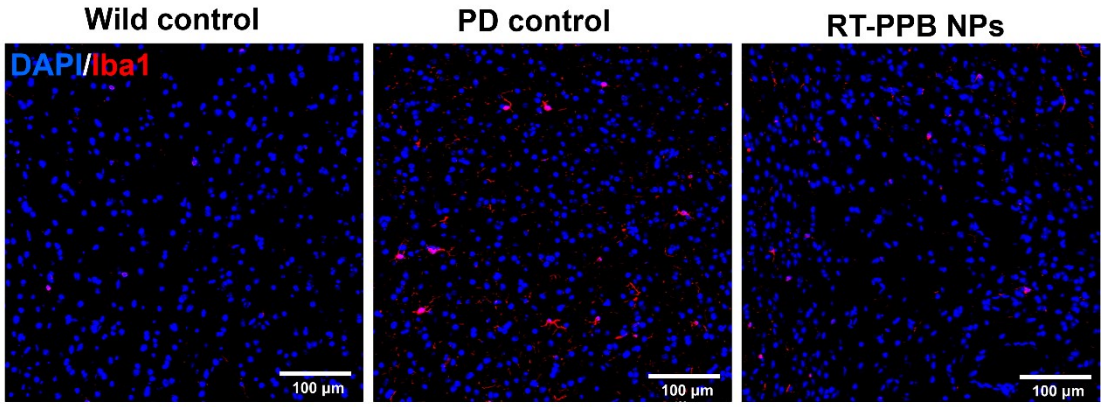

Fig. S38 Immunofluorescence staining for Iba1 in the mice striatum.

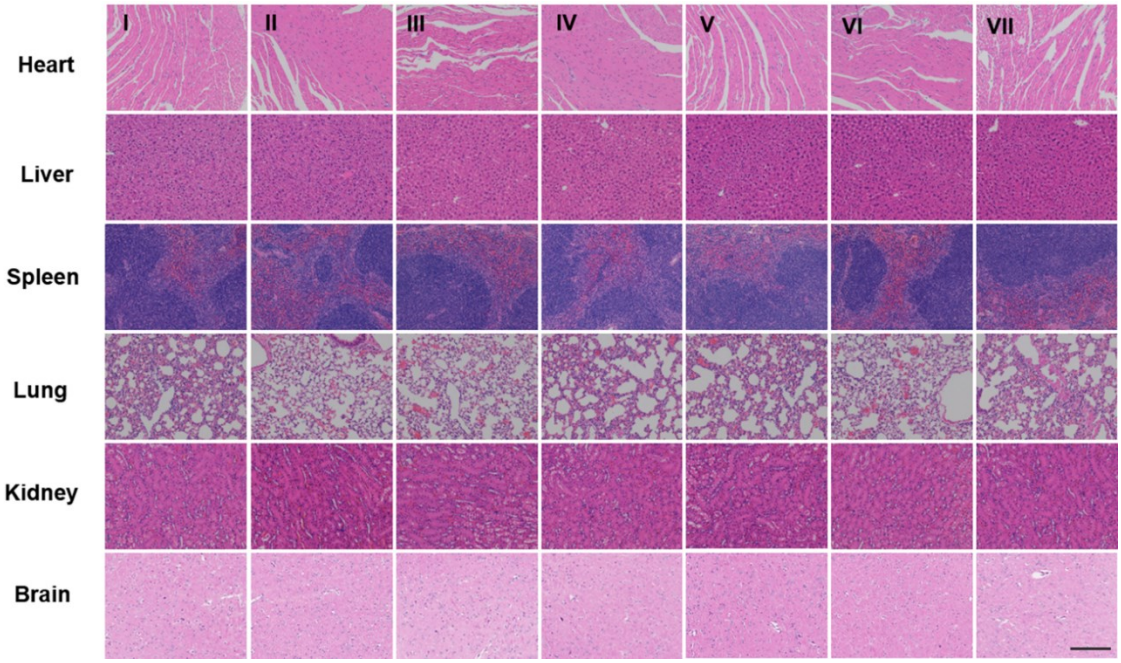

Fig. S39 Hematoxylin and eosin (H&E) staining of treated mice organs' slices. (I) Wild control, (II) PD control. (III) RAPA&sinSMase2, (IV) RT-PPB NPs, (V) T-PPB NPs, (VI) R-PPB NPs, (VII) RT-CPBn NPs. Scale bar: 200 μm.

References

1. Y. Li, Q. Cheng, Q. Jiang, Y. Y. Huang, H. M. Liu, Y. L. Zhao, W. P. Cao, G. H. Ma, F. Y. Dai, X. J. Liang, Z. C. Liang and X. Zhang, *J. Controlled Release*., 2014, **176**, 104-114.
2. J. R. Lakowicz and B. R. Masters, *J. Biomed. Opt*., 2008, **13**, 9901.