Supplementary

“Cell-addictive” dual-target traceable nanodrug for Parkinson’s disease treatment via flotillins pathway

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Results

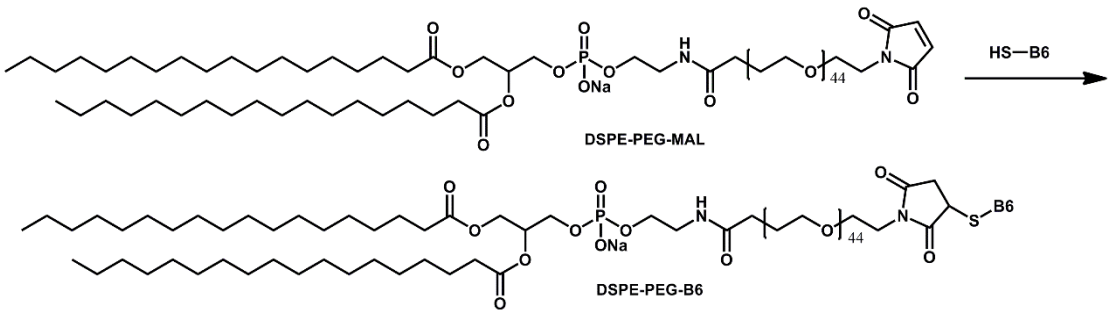


Figure S1. The synthetic routes of DSPE-PEG2000-B6

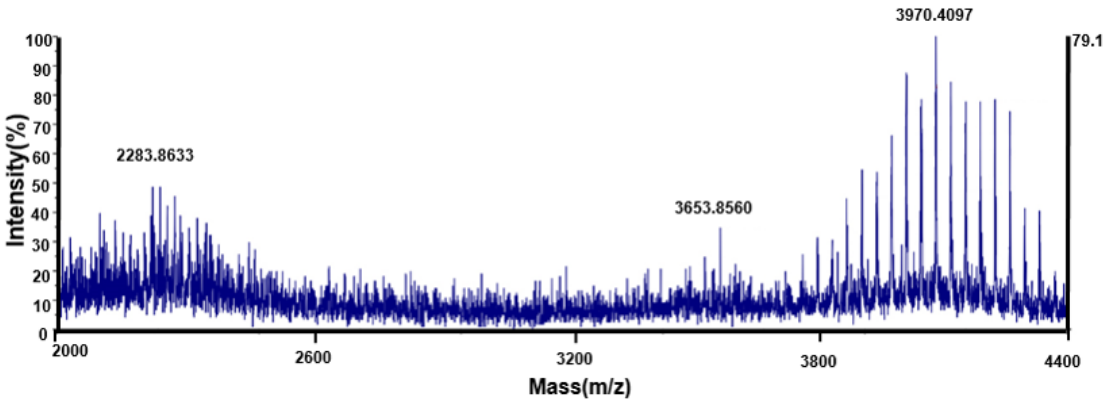


Figure S2. MALDI-TOFMS image of DSPE-PEG2000-B6.

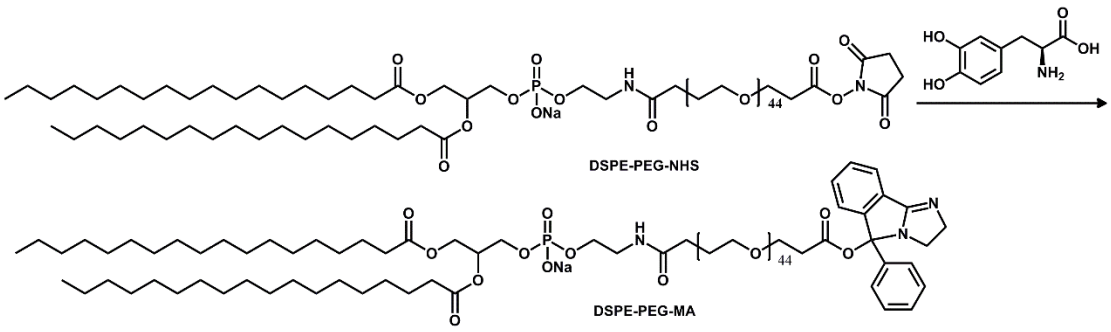
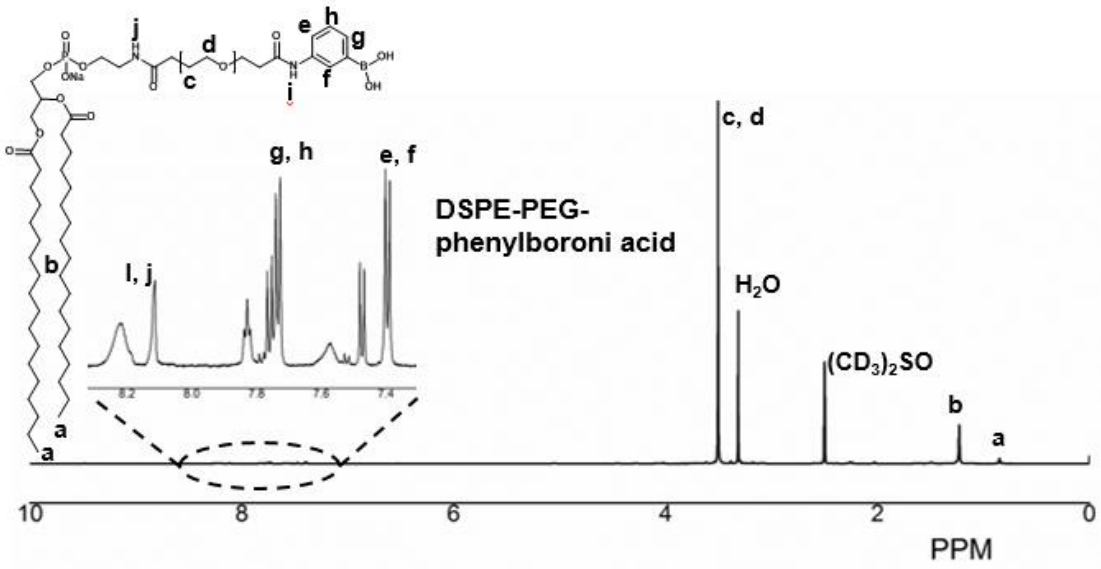


Figure S3. The synthetic routes of DSPE-PEG2000-MA



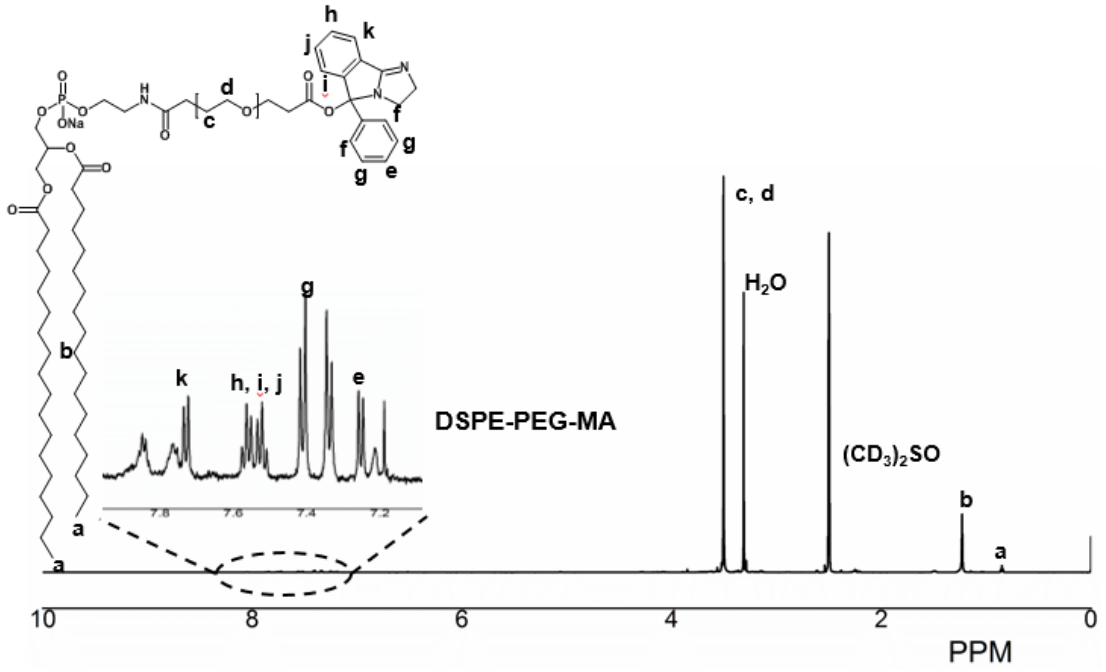


Figure S4. ^{1}H NMR spectra of purified intermediates including DSPE-PEG2000-phenylboronic acid and DSPE-PEG2000-MA

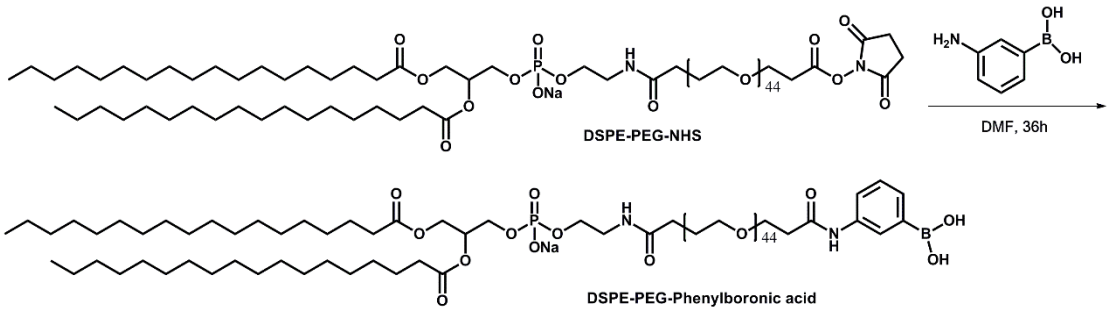


Figure S5. The synthetic routes of DSPE-PEG2000-Phenylboronic acid.

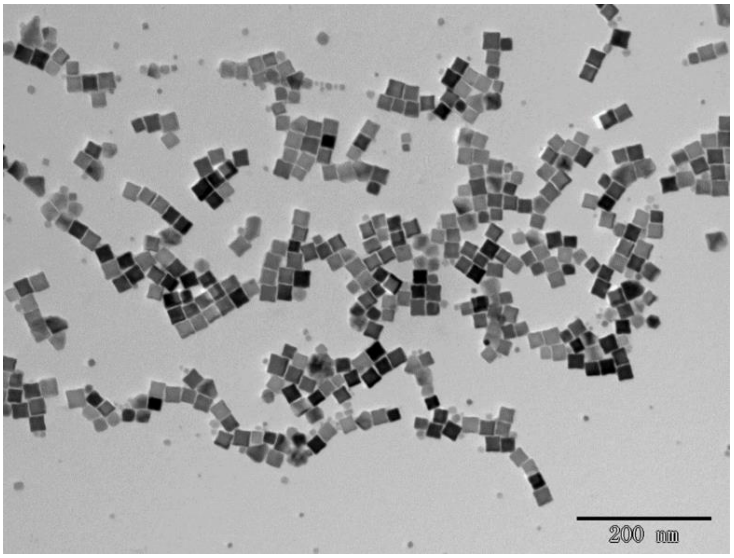


Figure S6. The TEM image of SPIONs, and the scale bar corresponds to 200 nm.

Figure S7. The water dispersity of B6ME-NPs and SPIONs.

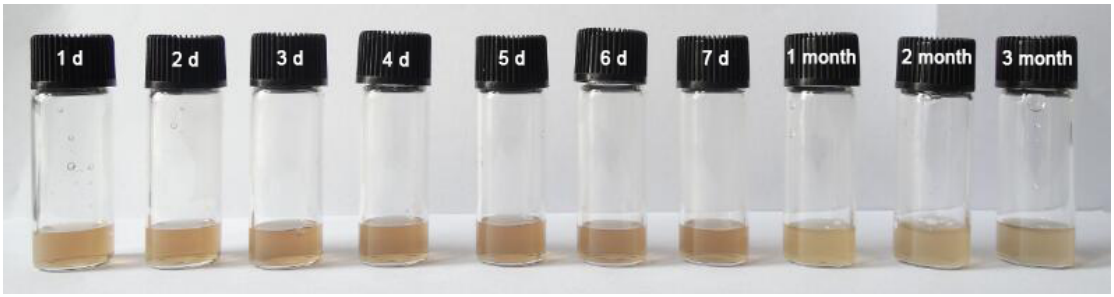


Figure S8. The photos of the serum stability of B6ME-NPs after incubation in culture medium supplemented with 10% FBS for different times.

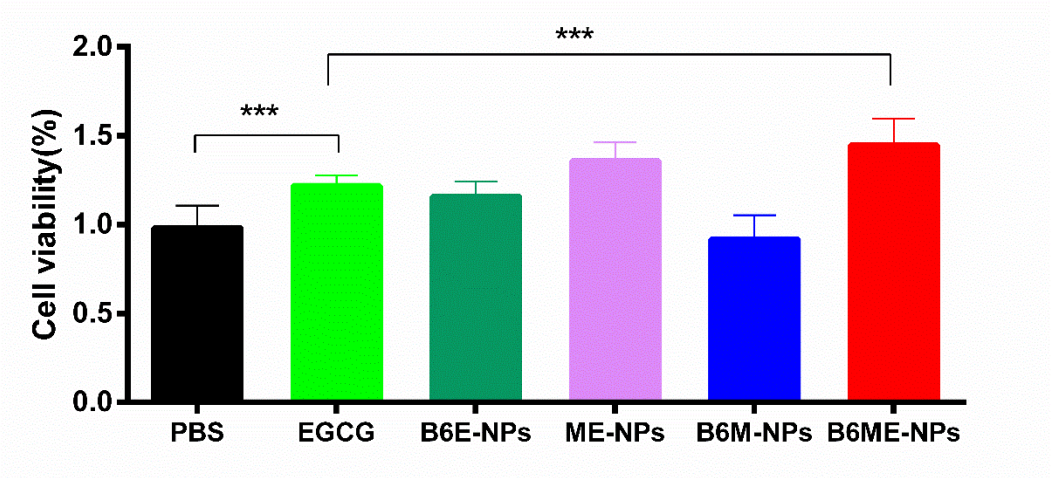


Figure S9. The NPs toxicity. The cell viability of every group were detected by MTT after treating with different samples (bulk EGCG, B6E-NPs, ME-NPs, B6M-NPs, B6ME-NPs), PBS as the control. *: differences between groups below the black line. *P < 0.05, **P < 0.01, ***P < 0.005. The mean ± SD is shown (n= 3).

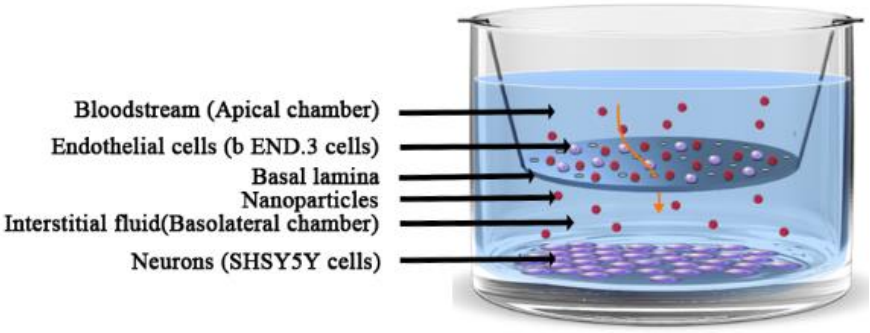


Figure S10. Schematic of a TranswellTM chamber for assaying transport across an endothelial monolayer. Particles were counted in the upper chamber (supernatant), endothelial layer (intracellular) and lower chamber (filtered). b END.3 was seeded on the apical as in vitro BBB cell models, and the SHSY5Y cell was seeded on the basolateral sides as dopaminergic neuron model, respectively.

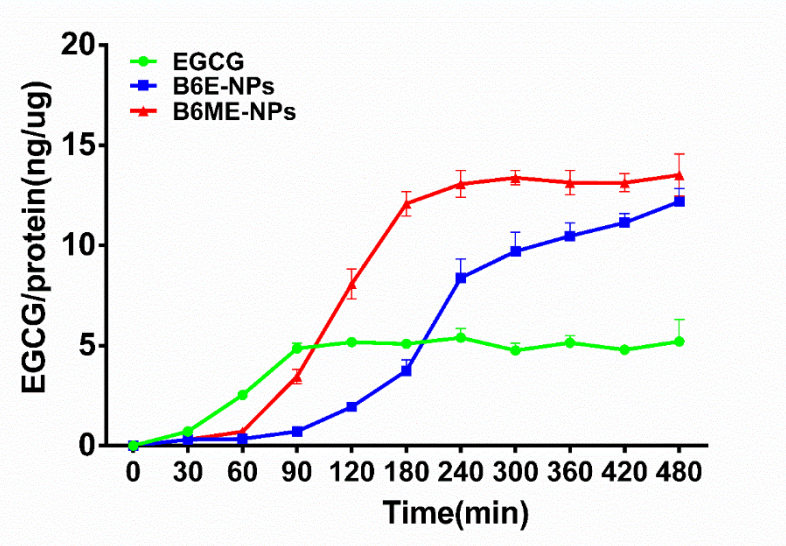


Figure S11. The intracellular concentration of EGCG in SH-SY5Y cells were detected via LC-MS. Samples were from SH-SY5Y cells treated by different samples (bulk EGCG, B6E-NPs, B6ME-NPs). The mean ± SD is shown (n= 3).

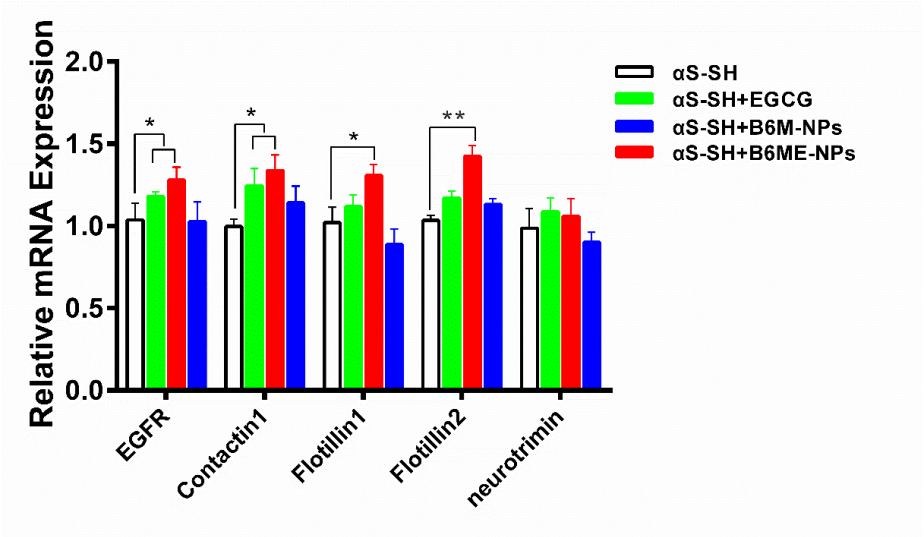


Figure S12. The lipid raft related (EGFR, Contactin1, flotillin1, flotillin2 and neurotrinmin) mRNA were detected by RT-PCR. The mean ± SD is shown (n = 3). *: differences between groups below the black line. *P < 0.05, **P < 0.01, ***P < 0.005

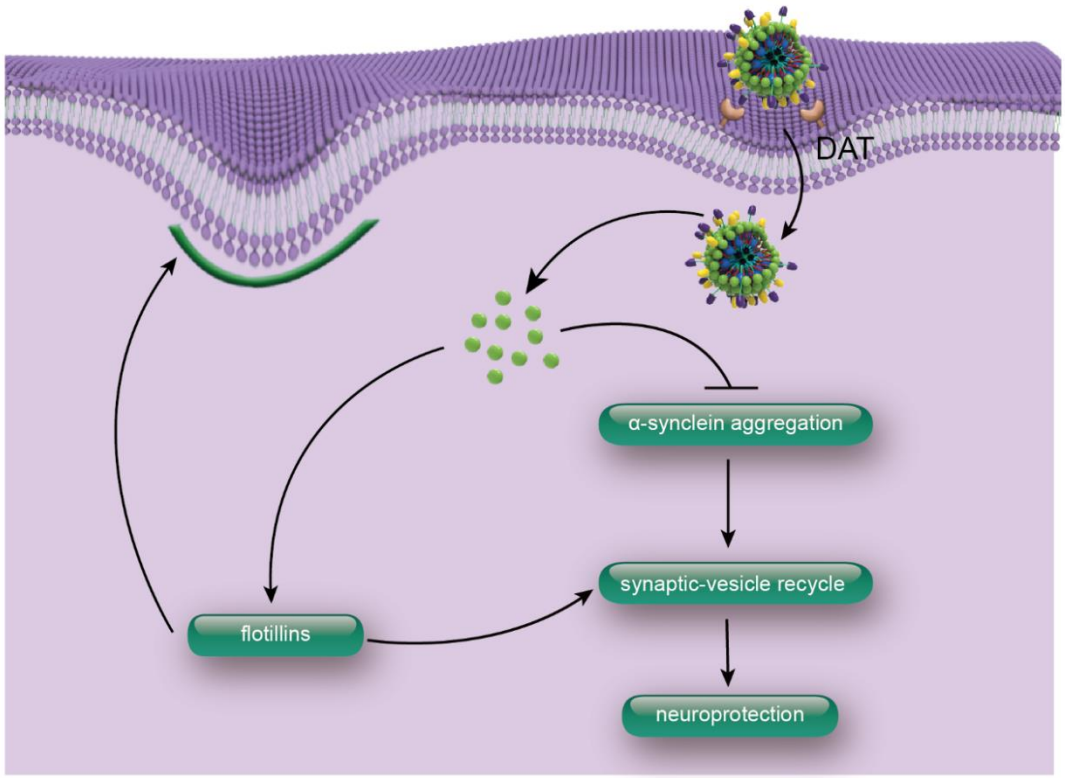


Figure S13. Proposed schematic model for the role of EGCG in neuronprotection through activation of the flotillins signaling pathway: On the basis of experimental studies we found that B6ME-NPs prevents αS aggregation. In addition, B6ME-NPs induced flotillins express of mRNA and protein, obviously. This may affect formation membrane curvature and vesicle budding. It may concern with synaptic-vesicle recycling. Herein, B6ME-NPs activate the flotillins pathway for PD therapy.

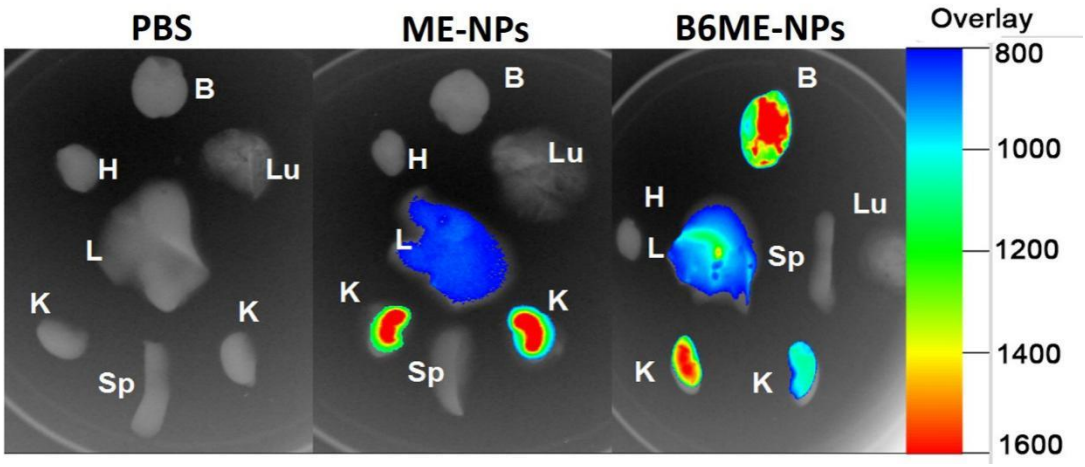


Figure S14. The accumulation of Cy7 in organs (Heart (H), Lung (Lu), Liver (L), Kidney (K), Spleen (Sp) and Brain (B)) at 24 h after intravenous injection of different formulations. The mean ± SD is shown (n= 3).

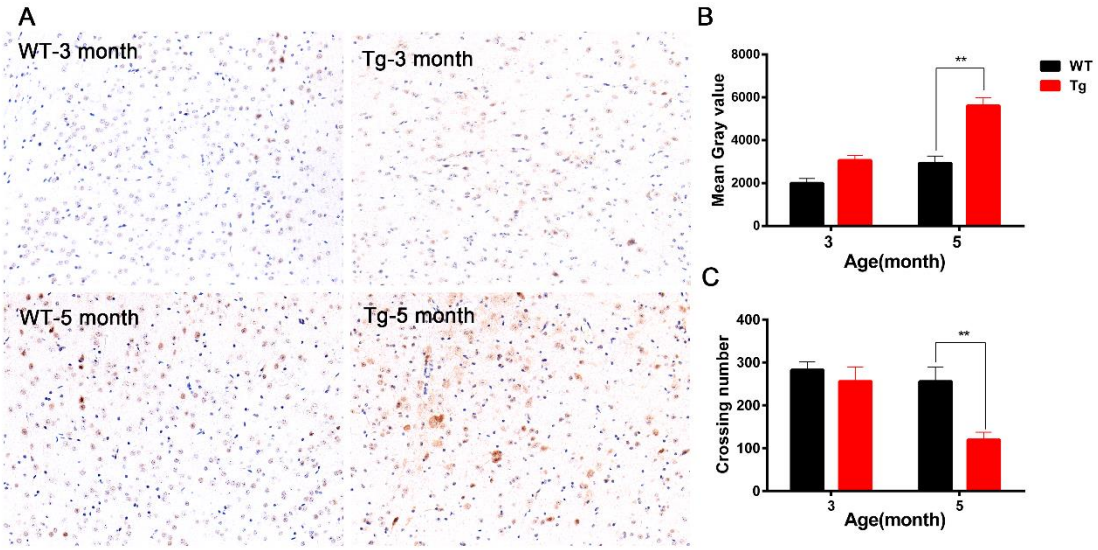


Figure S15. The behavioral test and the biochemical analysis of un-treatment WT and aS overexpression transgenic mice (3 month and 5 month). (A) αS immunohistochemical slices of un-treated mice brain SN region. (B) was the quantization of (A). (C) Un-treated mice open field test scores. The mean ± SD is shown (n = 6). *: differences between groups below the black line. *P < 0.05, **P < 0.01, ***P < 0.005.

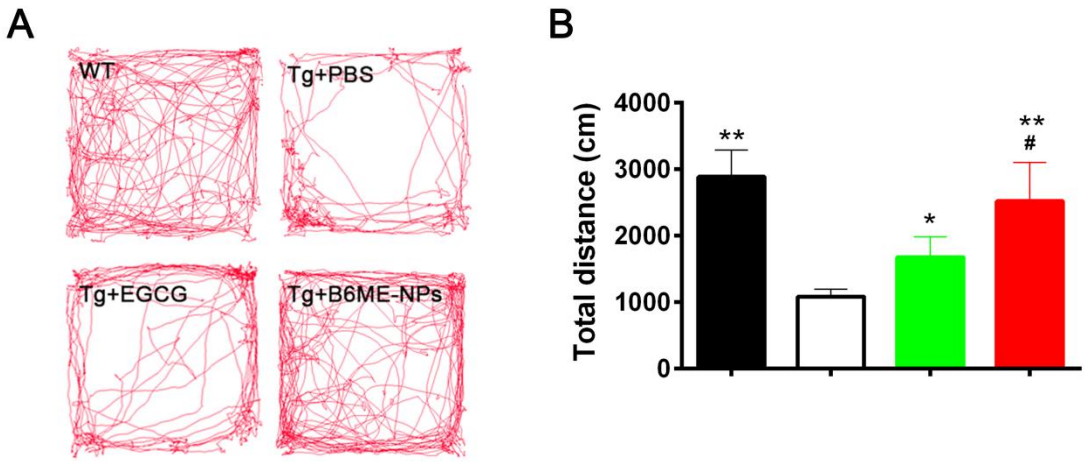


Figure S16. Behavior *Test with Different Groups Treatment*. (A) αS-overexpressing transgene mice as PD model. Representative walking trails of open field test after treatment with different

samples (PBS, bulk EGCG and B6ME-NPs) for 1.5 months. WT mice were the control. Both EGCG and the NPs were administered i.v. **(B)** Quantitation of (A). The mean ± SD is shown (n = 6). *: differences between groups below the black line. *P < 0.05, **P < 0.01, ***P < 0.001; (Tg + PBS), (Tg + B6ME-NPs) vs. WT. #P < 0.05, ##P < 0.01, ###P < 0.001; (Tg + EGCG) vs. (Tg + B6ME-NPs).

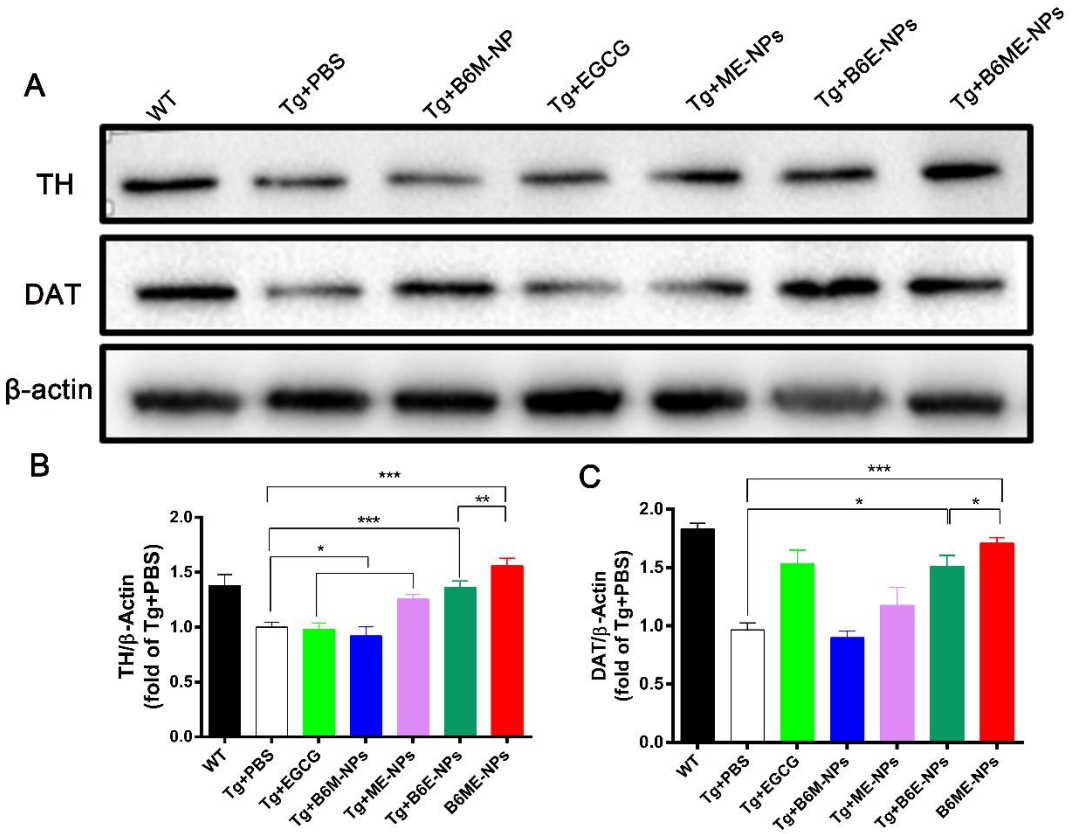


Figure S17. The protein expression of DAT and TH in treated mice of SN. (A) was detected by Western Blot. The samples of Western Blot were from PD model of SN tissue. PD model mice treated via intravenous injection of different formulation (EGCG, B6E-NPs, ME-NPs, B6M-NPs, B6ME-NPs). WT and PBS groups as the negative and positive controls. (B) is quantization of TH. (C) is the quantization of DAT. The mean ± SD is shown (n = 3). *: differences between groups below the black line. *P < 0.05, **P < 0.01, ***P < 0.005.

Table S1. The abbreviation of NPs prepared in the experiments.

NPs	ingredients	Abbreviation
Traceable NPs	$^{(B6+MA+EGCG+SPIONs)}$NPs	B6ME-NPs
Traceable NPs without B6	$^{(MA+EGCG+SPIONs)}$NPs	ME-NPs
Traceable NPs without MA	$^{(B6+EGCG+SPIONs)}$NPs	B6E-NPs
Traceable NPs without EGCG	$^{(B6+MA+EGCG+SPIONs)}$NPs	B6M-NPs

Table S2. The sequences for primers are listed.

Primer name	**The sequence (5'to3')**	**Bases**	**The length of the product**
GAPDH	F：TGAAAGTCGGAGTCAACGGAT	**21**	**230bp**
	R：ACGCTCCTGGAAGATAGTGAT	**21**	
neurotrimin	F：GAAAGAAGGGAGTCAAAGTGGAA	**23**	**132bp**
	R：TGATGCTGGCGTTGGTGT	**18**	
Contactin1	F：GTCAGGAGCACGGAGGCAACT	**21**	**163bp**
	R：ATTCATTCAGAAGCCAGCGGTAGC	**24**	
EGFR	F：	**21**	**105bp**

	CGTTCCCTCAAGGAGATCAGT		
	R：ATTGGGTGTCCCGAAGAGTT	**20**	
Flotillin1	F：CCGAGTGTTTGTCCTACCC	**19**	**209bp**
	R：CAATCTCCGCCTCTGTCTT	**19**	
Flotillin2	F：AATCTCCGAGTGCTGAACG	**19**	**178bp**
	R：CCTTGCTTCTGTGCCTCC	**18**	

Table S3. Parameters of EGCG and B6ME-NPs after intravenous injection to rat.

(mean ± SD, n = 6)

Parameters	**EGCG**	**B6ME-NPs**	**P value**
C_{max} (μg/L)	3.817 ± 0.623	4.367 ± 0.127	0.208198
T_{max} (min)	5 ± 0.001	160 ± 34.641**	0.00149353
$t_{1/2}$ (min)	144.849 ± 32.242	374.889 ± 21.874**	0.00751311
$AUC_{(0-t)}$ (μg/L*min)	322.08 ± 48.514	2650.475 ± 113.355***	< 0.0001
CL (L/min/kg)	0.027 ± 0.021	0.007 ± 0.001**	0.00487786

*C_{max}: peak plasma EGCG concentration. T_{max}: the time when peak plasma concentration was reached. t1/2: the time taken for blood concentration to fall to half its peak value after a single dose. AUC_{0-t}: area under the concentration-time curve. CL: clearance. * p＜0.05, **p＜0.01，***p＜0.001.*