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SUPPLEMENTARY INFORMATION

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Graphene quantum dots prevent α-synucleinopathy in Parkinson's disease

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Supplementary Methods

AFM imaging. For AFM imaging, 5 μl of GQDs (10 μg/ml) and α-syn fibrils (10 μg/ml) were prepared on a silicon substrate. The analyses were performed with an XE-100 AFM (Park Systems) via non-contact mode (scan size: 25 μm^2 and scan rate: 0.8 Hz). The images were acquired by equipped XE data acquisition program (XEP 1.8.0).

Complex I activity assay. The commercial microplate assay kit (Cat#: ab109721; abcam) was used for measuring the complex I enzyme activity, following the manufacturer's instruction. Briefly, primary cortical neurons were plated onto poly-*D*-lysine coated 6 cm dishes at a density of 1,000,000 cells/dish. Neurons were then treated with 1 μg/ml of α-syn PFFs with or without 1 μg/ml of GQDs at 10 days *in vitro*. After 7-day treatment, the cytosolic proteins were extracted using 1/10 volume detergent in PBS. The supernatant was isolated by centrifuging at 12,000 × g for 20 mins from 5.5 mg/ml of samples. Isolated samples were loaded and incubated for 3 hours at RT, and rinsed twice with washing buffer. After adding a 200 μl assay solution, the mitochondrial complex 1 enzyme activity was measured at approximately 1-min intervals for 30 mins.

Mitochondrial morphology assessment. Primary cultured cortical neurons were plated onto poly-*D*-lysine coated glass coverslips at a density of 10,000 cells/cm^2. The neurons were treated with 1 μg/ml of α-syn PFFs with or without 1 μg/ml of GQDs at days in vitro (DIV) 10. At DIV 17, the mitochondria in neurons were stained with the MitoTracker® Orange CMTMRos probes (Cat#: M7510; Life technologies). Briefly, mitochondria were stained with the 100 nM of MitoTracker® Orange CMTMRos probes for 30 mins. After washing the stained mitochondria with a live cell imaging solution (Cat#: A1429DJ; Life technologies), the mitochondrial morphological characteristics were captured using a Zeiss confocal microscope (LSM 710). The

length or aspect ratio (AP, the ratio between the major and minor axis) were measured by ImageJ software (http://rsb.info.nih.gov/ij/, NIH).

Determination of oxygen consumption rate. The Seahorse XF cell mito stress test kit (Cat#: 103015; Agilent) was used for measuring the oxygen consumption rate (OCR). Briefly, the primary cultured cortical neurons were plated onto the 24 well culture plate at a density of 500,000 cells/well. Neurons were treated with 1 μg/ml of α-syn PFFs with or without 1 μg/ml of GQDs at DIV 10. At DIV 17, the neurons were incubated in complete Seahorse assay medium at 37 ^{o}C for 1 hour after washing with warmed PBS. The assay plate was then loaded in an XF24 analyser (XF24 EX extracellular flux analyser, Seahorse Bioscience) and the OCR was calculated. The OCR was measured with specific measurement protocol (1-min mix, 1-min wait, and 2-min measurement). To assess the coupling of respiratory, chain, the basal respiration, and mitochondrial respiratory capacity, Oligomycin, carbonyl canide m-chlorophenyllhydrazone (CCCP), and rotenone were sequentially injected to the Seahorse assay plate. The measured OCRs were normalised relative to the protein concentration in each well. Data are presented as the percentage of change as compared with control.

Live imaging. Primary cortical neurons were seeded onto poly-*D*-lysine coated glass bottom dish (Cat#: 150682; Nunc^{TM}) at a density 10,000 cells/ cm^2 and maintained in a 7% CO_2 incubator at 37 ^{o}C. At DIV 7, the neurons were treated with 1 μg/ml FITC labelled α-syn-PFFs, 1 μg/ml GQDs-biotin and streptavidin Qdot complex, and 100 nM LysoTracker™ Blue DND-22 (Cat#: L7525; Life technologies) containing live cell imaging solution for 1 hour. For time-lapse confocal live imaging, the temperature controlled CO_2 incubation system equipped confocal microscope was used. The culture dish was mounted and time-lapse images were taken at the indicated intervals with 488 nm and 561 nm laser excitations.

***In vitro* BBB permeability of GQDs.** For *in vitro* BBB experiments, 10-pups of C57BL/6 mouse were used for preparing primary mouse astrocytes as previously described[1]. Briefly, the cerebral cortex was isolated from 1-d-old C57BL/6 mice and the meninges were removed. The cerebral cortex was mechanically disrupted by 30-mL syringe with 19-gauge needle. The isolated cells were plated onto 75 cm^2 T-flasks with complete DMEM culture medium. After 2 weeks, the 95% pure astrocytes were isolated by an astrocyte isolation kit (Cat#: 130-096-053; Miltenyl Biotec). The primary brain microvascular endothelial cells (BMEC) from C57BL/6 mice were purchased from Cell Biologics as well. The purities of cultured astrocytes (>95%) and BMEC (>95%) were confirmed by fluorescence staining with cell specific marker GFAP (for astrocyte) and CD31 (for BMEC, Cat#: ab28364; 1:500; abcam). For the formation of an *in vitro* BBB, the isolated astrocytes were seeded at a density of 10^6 cells/ml onto the underside of the inserts (collagen-coated 0.4 μm transwell inserts; Cat#: CLS3491; Sigma-Aldrich) and incubated for 48 hours at 37 °C in 5% CO_2 incubator. The inserts were then placed carefully into a 6-well plate where BMEC were plated at a density of 10^6 cells/ml on top of the inserts. The trans-epithelial electrical resistance (TEER) was determined to confirm the structural integrity of the *in vitro* BBB by the epithelial volt/ohm (TEER) meter (Cat#: 300523; EVOM2; world precision instruments) at 0, 2, 4, 6, and 8 days after BMEC seeding. BBB impermeable 3 kDa dextran-fluorescein (Cat#: D3306; Life technologies) were loaded inside (the blood side) of the *in vitro* BBB to confirm the integrity and calculated the ratio using fluorescence spectrophotometer (Ex=494 nm / Em=521 nm for dextran-fluorescein; Ex=555 nm / Em=580 nm for dextran rhodamine). The concentrations of remaining GQDs, GQDs-biotin, nano-GOs, and rGQDs were measured at 520 nm (Ex=310 nm) using the FluoroBrite™ DMEM media (Cat#: A1896701; Life technologies) of inside (the blood side) and outside (the brain side) of the inserts.

Exosome isolation. To measure the concentration of GQDs-biotin from the released exosome, BMEC or astrocytes were plated on 6 cm dishes and 5 μg of GQDs-biotin were treated for 12 hours. After 12 hours, the culture medium was changed, and the exosomes were isolated using an exosome isolation reagent (Cat#: 4478359; ThermoFisher) after 24 and 48 hours.

***In vivo* BBB permeability of GQDs.** For *in vivo* immunostaining of GQDs-biotin, 8-week old C57BL/6 mice were i.p. injected with 2 mg/kg GQDs-biotin. The removed brains were fixed with 4% PFA for 6 hours. The brains were then stored in 30% sucrose for 48 hours for the following immunohistochemical staining. The biotin signals of the olfactory bulb, neocortex, midbrain and cerebellum were visualised using DAB staining kit. The GQDs-biotin positive signals in cells of the aforementioned regions were confirmed by immune-EM staining using GoldEnhance^{TM}EM Plus solution with 20-min incubation, following the manufacturer's instructions. For *in vivo* BBB experiments, 8-week old C57BL/6 mice were i.p. or i.v. injected with GQDs-biotin (2 mg/kg) or vehicle. At 7 days, and 14 days, the brain and blood were harvested, and the brain was homogenated with 1% TX-100 in PBS. The plasma was prepared by clot removed with centrifuging at 2,000 × g for 10 mins after the whole blood storing at RT for 30 mins. The concentrations of GQDs-biotin were measured using QuantTag Biotin Kit (Cat# BDK-2000, Vector Laboratories). The ratio of brain/plasma concentration of GQDs-biotin was calculated against the brain/plasma ratio.

α-syn aggregation formation assay. HEK293T cells were purchased from American Type Culture Collection (ATCC) and authenticated by STR analysis. HEK293T cells were routinely tested and found to be negative of mycoplasma contamination. HEK293T cells were seeded on glass coverslides and transfected the pCMV5 vectors with myc-tagged A53T α-syn mutant (kindly gifted by Dr. Thomas C. Südof), followed by treatment with PBS (pH 7.4) or GQDs (0.1

μg/ml). 48 hours after the treatment, cells were washed three times with PBS and fixed for 20 mins at RT in 4% PFA containing PBS. The fixed cultures were permeabilised for 4 mins in 0.1% Triton X-100 containing PBS. The expression of α-syn was monitored by immunostaining with α-syn antibody (Cat#: 610787; 1:1,000; BD Biosciences). The subcellular localisation of α-syn aggregates were determined by the confocal microscopy with serial excitations at 550 nm and 570 nm. The number of α-syn aggregates per field was measured using ImageJ software (http://rsb.info.nih.gov/ij/, NIH).

Behaviour analysis. Clasping test[2]: For behaviour test of hA53T α-syn mice, the hindlimb clasping function was tested. After clearing all surrounding objects, the test animals were lifted by grasping their tails near the base and their hindlimb positions were observed for 10 secs. The clasping functional scores are rated on the following criteria: 1) Score 0: hindlimbs are spread outwards and away from the abdomen. 2) Score 1: For more than 5 secs, single hindlimb is drawn back towards the abdomen. 3) Score 2: For more than 5 secs, both hindlimbs are partially drawn back towards the abdomen. 4) Score 3: For more than 5 secs, both hindlimbs are completely drawn back towards the abdomen.

Preparation of nano-GOs and rGQDs. To prepared nano-GOs, pristine GOs were synthesised by improved Hummer's method according a previous literature[3]. Obtained GOs powder was subsequently tip-sonicated in DI water (10 mg/ml) for 3 hours to yield nano-GOs. The reduction of GQDs was achieved by the autoclave-based hydrothermal method at 200 ^{o}C for 2 hours.

The viability assay of human neuroblastoma cell line. SH-SY5Y (ATCC® CRL-2266™) was purchased from ATCC and authenticated by STR analysis. Cells were routinely tested for mycoplasma contamination and found to be negative. SH-SY5Y cells were plated at a density 50,000 cells/well in 48-well plate and incubated in 5% CO_2 incubator at 37 ^{o}C. The viability of

SH-SY5Y cells was quantified by alamarBlue cell viability assay kit (Cat#: DAL1025; Molecular Probes™), following the manufacturer's instructions.

Supplementary Table

Supplementary Table 1. Mean ± s.d. values and *P* values of Figure 1b, c, e, and f.

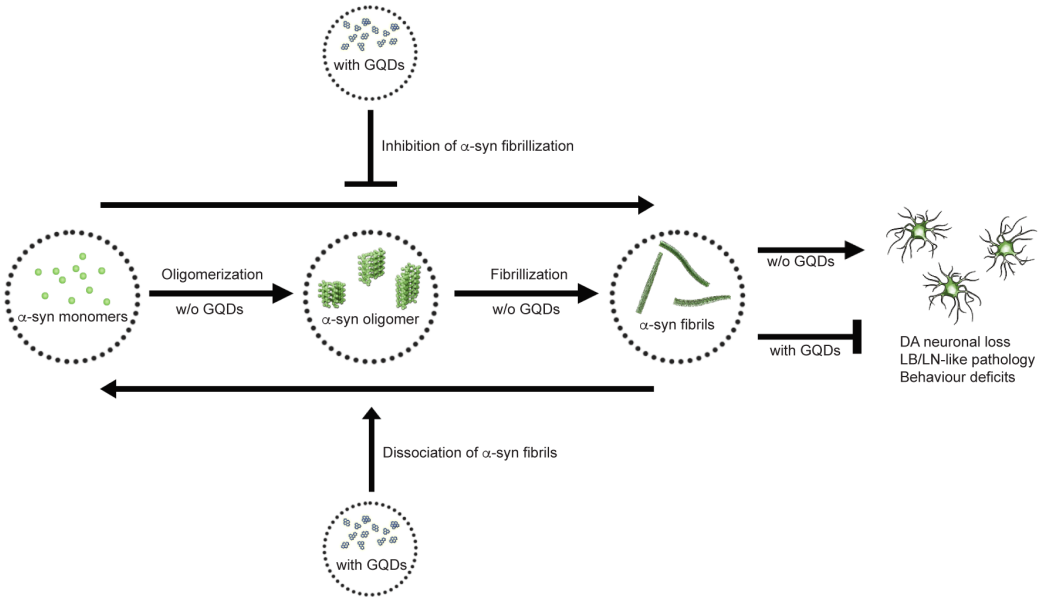
Figures	Sample	0h		2h		4h		6h		8h	
		Mean ± s.d.	*P* value	Mean ± s.d.	*P* value	Mean ± s.d.	*P* value	Mean ± s.d.	*P* value	Mean ± s.d.	*P* value
Figure 1b	α-syn	5.63 ± 5.25	$P > 0.9999$	7.83 ± 5.65	$P > 0.9999$	6.45 ± 3.31	$P = 0.0003$	7.43 ± 5.18	$P < 0.0001$	8.13 ± 2.10	$P < 0.0001$
	α-syn+GQDs	8.75 ± 5.68		16.63 ± 4.67		30.48 ± 11.70		46.10 ± 13.67		70.40 ± 12.83	
Figure 1c	α-syn	9.08 ± 10.69	$P > 0.9999$	9.43 ± 7.20	$P > 0.9999$	9.65 ± 5.21	$P = 0.0143$	9.63 ± 3.64	$P < 0.0001$	8.05 ± 3.79	$P < 0.0001$
	α-syn+GQDs	6.89 ± 5.26		20.02 ± 5.13		34.82 ± 5.13		50.40 ± 14.50		69.29 ± 10.12	

Figures	Sample	10h		12h		24h		72h		168h	
		Mean ± s.d.	*P* value	Mean ± s.d.	*P* value	Mean ± s.d.	*P* value	Mean ± s.d.	*P* value	Mean ± s.d.	*P* value
Figure 1b	α-syn	6.40 ± 4.08	$P < 0.0001$	10.18 ± 5.23	$P < 0.0001$	7.43 ± 3.71	$P < 0.0001$	10.80 ± 2.29	$P < 0.0001$	9.23 ± 5.48	$P < 0.0001$
	α-syn+GQDs	86.88 ± 5.95		90.45 ± 4.28		89.80 ± 5.95		95.63 ± 8.30		100.00 ± 15.77	
Figure 1c	α-syn	7.58 ± 3.97	$P < 0.0001$	9.45 ± 3.55	$P < 0.0001$	8.33 ± 9.26	$P < 0.0001$	13.38 ± 7.02	$P < 0.0001$	13.40 ± 13.89	$P < 0.0001$
	α-syn+GQDs	80.27 ± 6.11		83.78 ± 11.65		89.01 ± 6.72		93.40 ± 13.80		100.00 ± 26.97	

Figures	Sample	0h		1h		3h		6h	
		Mean ± s.d.	*P* value	Mean ± s.d.	*P* value	Mean ± s.d.	*P* value	Mean ± s.d.	*P* value
Figure 1e	α-syn fibrils	100.00 ± 1.88	$P > 0.9999$	94.98 ± 3.51	$P < 0.0001$	95.53 ± 3.21	$P < 0.0001$	90.05 ± 4.88	$P < 0.0001$
	α-syn fibrils+GQDs	99.98 ± 1.88		61.58 ± 13.21		54.38 ± 10.05		39.43 ± 16.53	
Figure 1f	α-syn fibrils	100.00 ± 22.7	$P > 0.9999$	91.48 ± 5.53	$P < 0.0001$	92.73 ± 3.48	$P < 0.0001$	89.45 ± 4.60	$P < 0.0001$
	α-syn fibrils+GQDs	98.43 ± 3.84		49.70 ± 11.09		41.13 ± 10.17		33.58 ± 14.54	

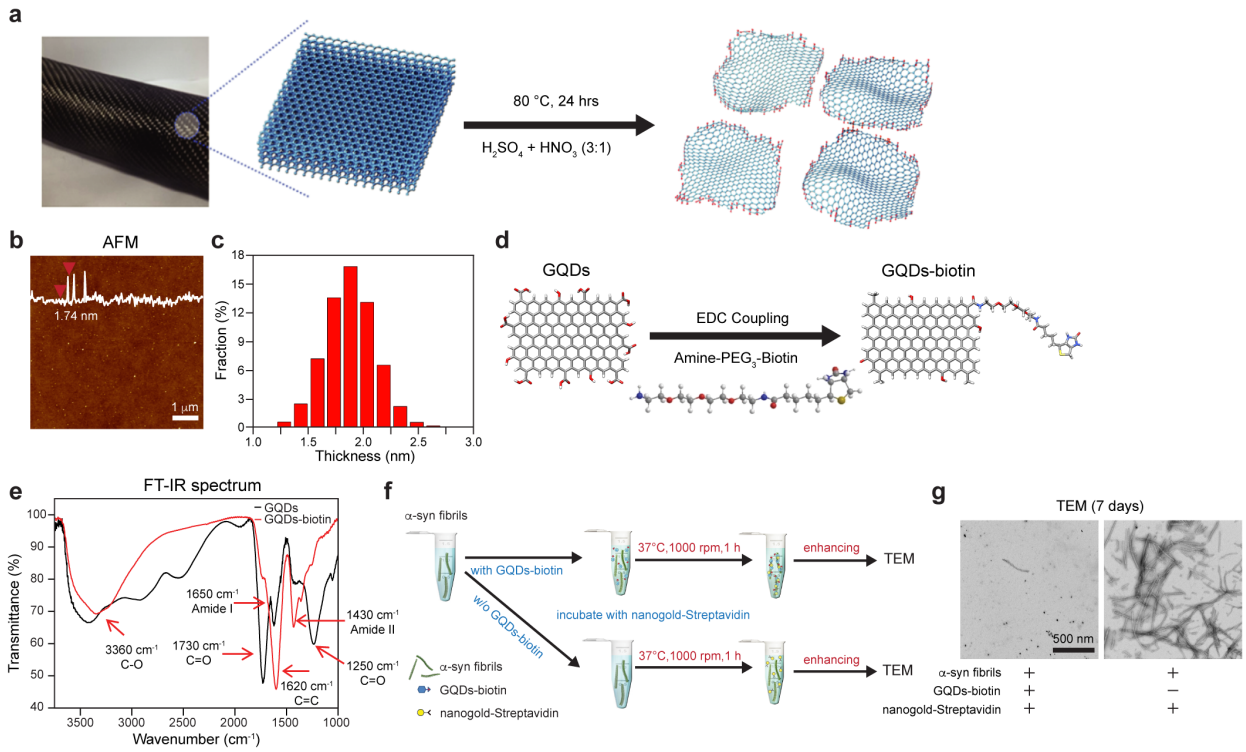
Figures	Sample	12h		24h		72h		168h	
		Mean ± s.d.	*P* value	Mean ± s.d.	*P* value	Mean ± s.d.	*P* value	Mean ± s.d.	*P* value
Figure 1e	α-syn fibrils	88.83 ± 3.78	$P < 0.0001$	90.40 ±2.45	$P < 0.0001$	92.18 ± 6.15	$P < 0.0001$	82.18 ± 11.91	$P < 0.0001$
	α-syn fibrils+GQDs	34.00 ± 10.34		21.03 ± 8.85		22.55 ± 7.70		13.45 ± 5.96	
Figure 1f	α-syn fibrils	86.95 ± 4.39	$P < 0.0001$	85.68 ± 8.86	$P < 0.0001$	85.32 ± 3.18	$P < 0.0001$	84.58 ± 10.53	$P < 0.0001$
	α-syn fibrils+GQDs	26.48 ± 8.09		25.08 ± 7.02		22.50 ± 6.50		13.55 ± 6.13	

Supplementary Fig. 1



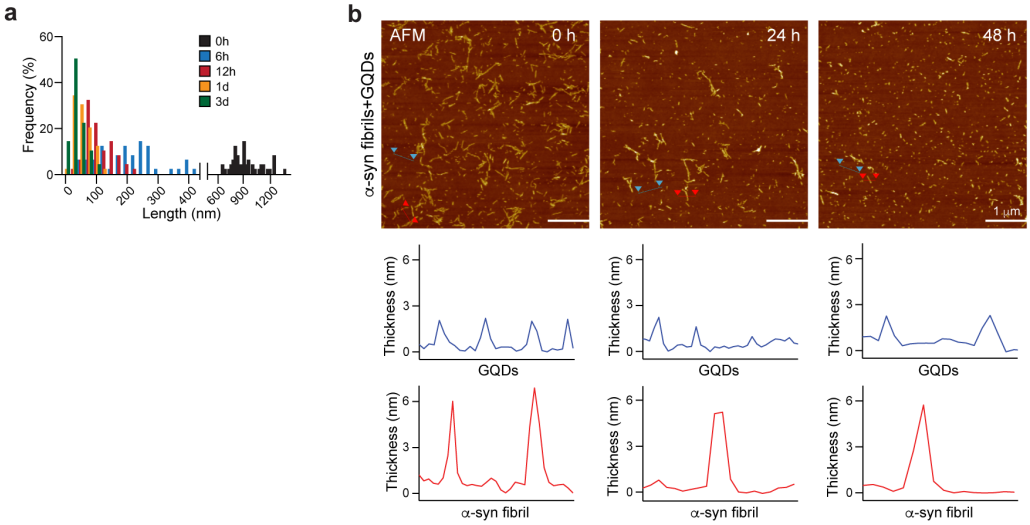
Supplementary Figure 1. Schematic overview of the therapeutic effect of GQDs on the pathogenesis of PD. Without GQDs, pathological α-syn monomers undergo spontaneous fibrillization to form fibrillary aggregates, which ultimately induces loss of dopaminergic neurons, LB/LN-like pathology and behaviour deficits. On the other hand, GQDs treatment inhibits α-syn fibrillization and disaggregates mature fibrils to monomers and thereby prevents dopaminergic neuron loss, LB/LN-like pathology and behaviour deficits provoked by abnormal α-syn fibrillization.

Supplementary Fig. 2



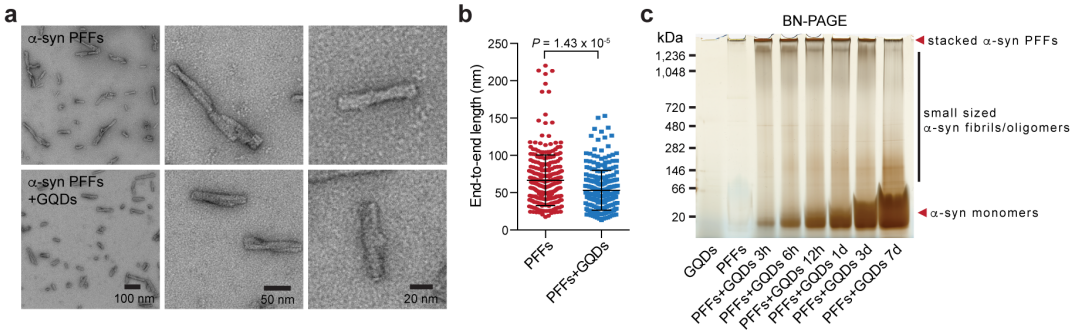
Supplementary Figure 2. Synthesis and biotinylation of GQDs and binding assay between GQDs-biotin and α-syn fibrils. a, Schematic representation of synthetic procedure from carbon fibre. **b**, AFM image of synthesised GQDs. **c**, Fractions of GQDs with different thicknesses. **d,** Schematic representation of synthetic procedure of biotinylation on GQDs. **e,** FT-IR spectrum of GQDs (black line) and biotinylated GQDs (red line) with designated peaks and shifts for the functional groups. **f,** Schematic representation of the preparation steps for the binding assay between biotinylated GQDs and nanogold-streptavidin tagged α-syn fibrils. **g,** TEM images of preformed α-syn fibrils after 7 days in the presence of GQDs-biotin-nanogold-Streptavdin complex (left) and nanogold-Streptavdin only (right).

Supplementary Fig. 3



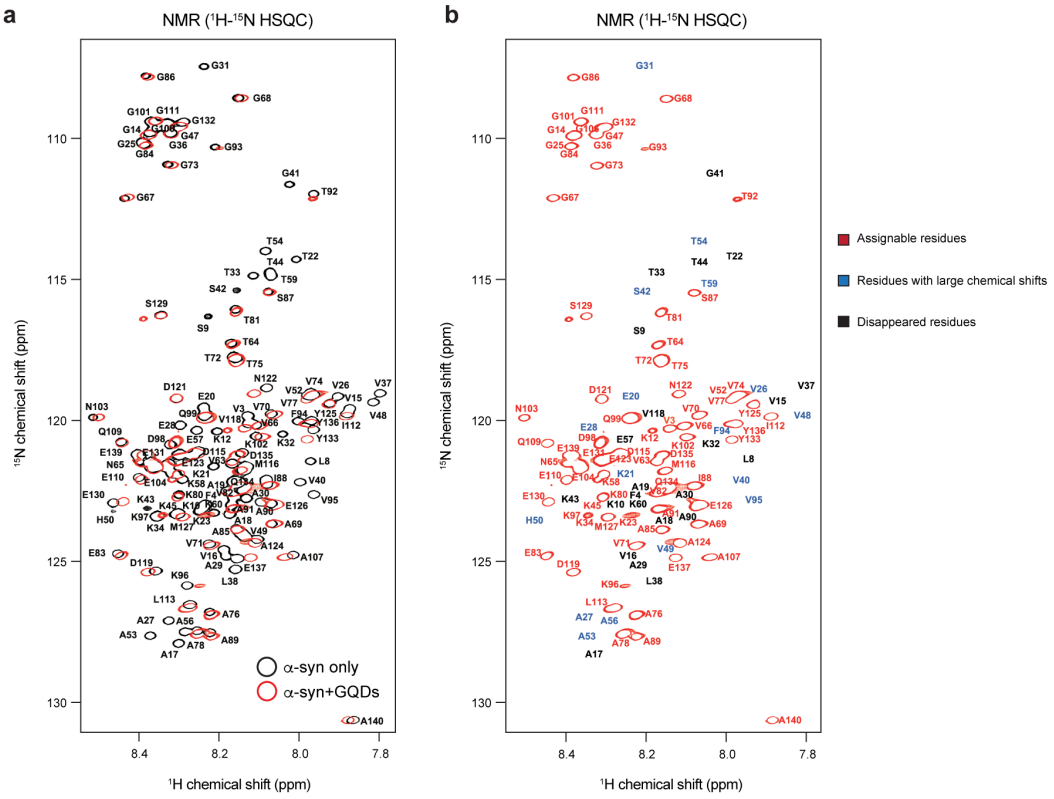
Supplementary Figure 3. The effect of GQDs on disaggregation of α-syn fibrils. a, The distribution of α-syn fibril lengths at various time points (0, 6, 12, 24, and 72 hours, n=50 fibrils at each time point). **b**, AFM images of α-syn fibrils with GQDs after various time points (0, 24, and 48 hours) and the representative line profiles of the designated regions, marked by blue (GQDs) and red (α-syn fibrils) arrows at each time point.

Supplementary Fig. 4



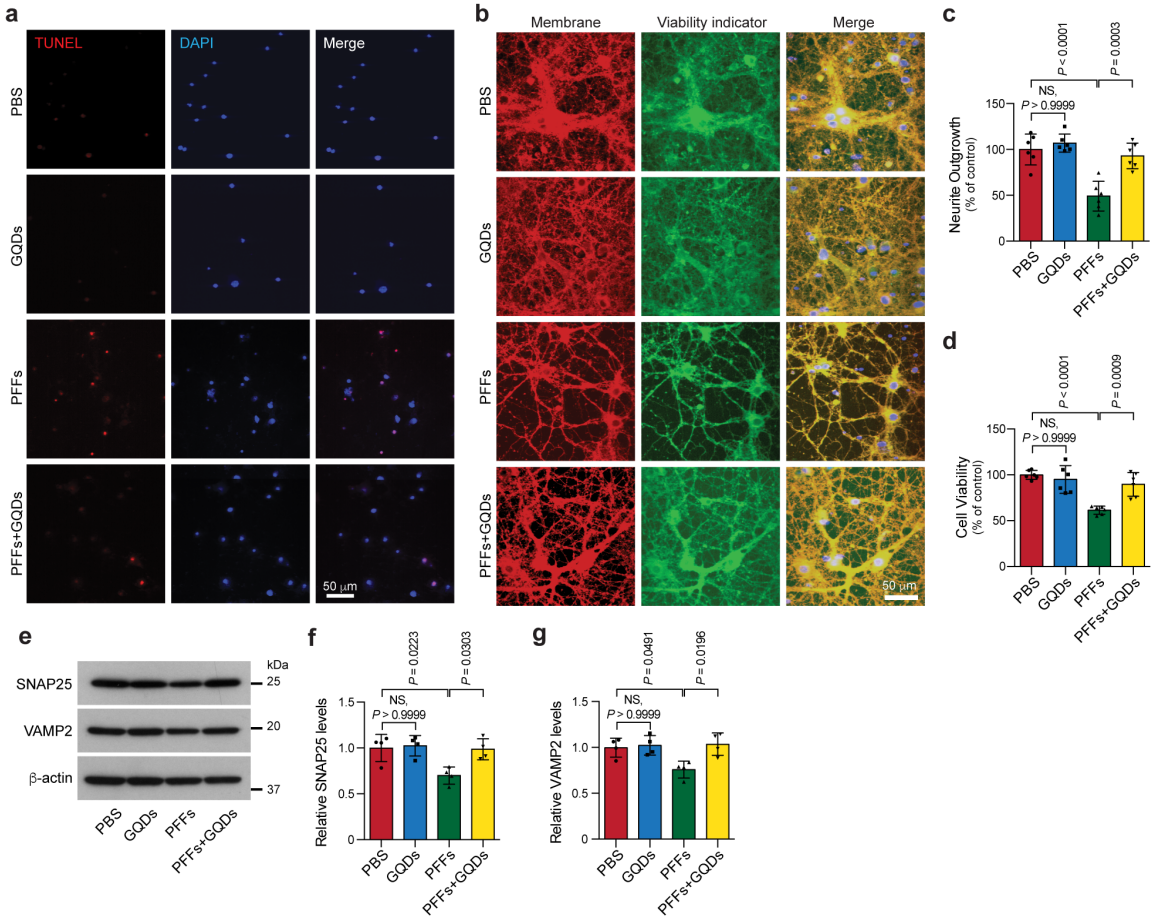
Supplementary Figure 4. The effect of GQDs on disaggregation of α-syn PFFs. a, Representative TEM images of fibrillized α-syn PFFs (5 mg/ml) incubated in the absence of (top) and in the presence of (bottom) GQDs (5 mg/ml) for 1 hour. **b,** The end-to-end length of α-syn PFFs after 1 hour of incubation. Mean ± s.d. values are 66.52 ± 34.53 and 54.49 ± 26.74 for PFFs and PFFs+GQDs (n=286: PFFs, n=249: PFFs + GQDs; two-tailed Student's t-test). **c**, The amount of remaining α-syn PFFs and disaggregated α-syn PFFs assessed by BN-PAGE after various incubation periods. These experiments were independently repeated three times with similar results.

Supplementary Fig. 5



Supplementary Figure 5. ^{1}H-^{15}N HSQC spectral analysis. a, Full, overlapped ^{1}H-^{15}N HSQC spectra of ^{15}N-labelled α-syn monomers only (black) and after incubation with GQDs (red). The assigned residues designate ^{15}N-labelled α-syn monomers only group. **b,** A full HSQC spectrum of ^{15}N-labelled α-syn monomers incubated with GQDs. Based on the peak intensity of ^{15}N-labelled α-syn monomers only group, the residues with no or minimal decrease in peak intensity (red), the residues with significant decrease in peak intensity (blue), and the disappeared residues (black) are distinctively assigned.

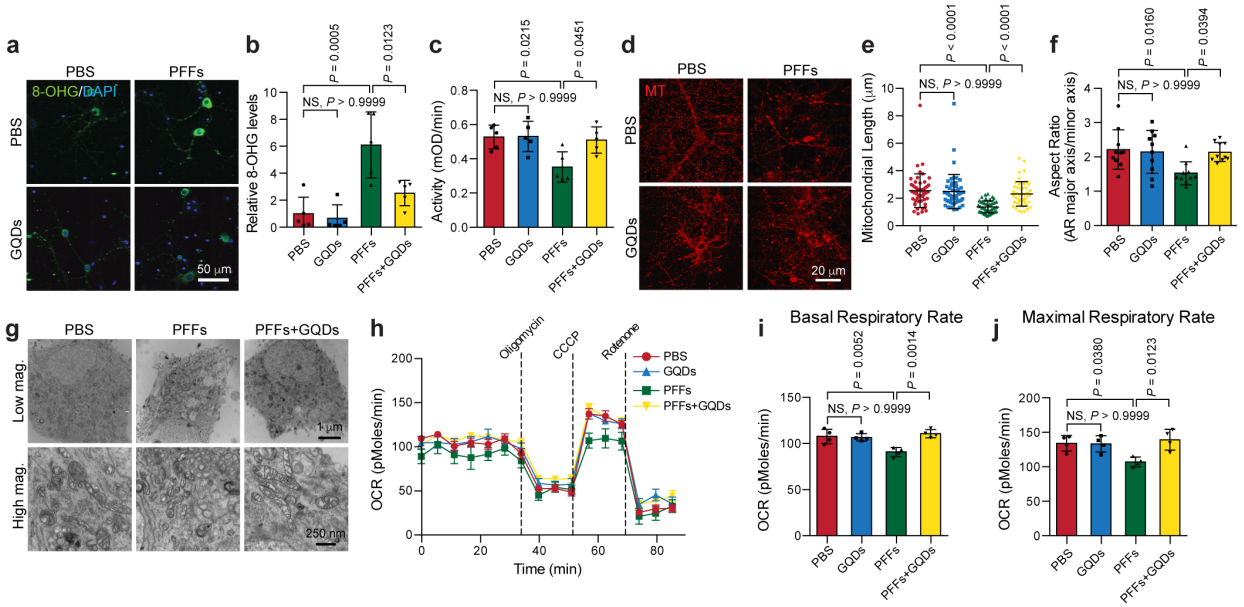
Supplementary Fig. 6



Supplementary Figure 6. The effect of GQDs on α-syn PFFs-induced cell death and restricted neurite outgrowth. a, Representative TUNEL-positive neurons. DIV 10 primary cortical neurons were treated with α-syn PFFs (1 μg/ml) in the absence and presence of GQDs (1 μg/ml). TUNEL assay was performed after 7 days of incubation. **b,** Representative micrographs of neurite outgrowth and cell viability assays stained by outer cell membrane (red) and cell-permeable viability indicator (green). **c-d,** Quantifications of neurite outgrowth and neuron viability from the stained images. Mean values of the neurite outgrowth are 100.00, 106.89, 49.08, and 92.98 for PBS, GQDs, PFFs, and PFFs+GQDs; mean values of the cell viability are 100.00, 95.08, 61.43, and 89.68 for PBS, GQDs, PFFs, and PFFs+GQDs (n=6, biologically independent samples; two-way ANOVA with a post hoc Bonferroni test; NS, not significant; error bars are the standard deviation). **e,** Representative immunoblot analysis of SNAP25 and VAMP2 protein levels. 10 DIV primary cortical neurons were treated with α-syn PFFs (5 μg/ml) in the absence and presence of GQDs (5 μg/ml) and incubated for 7 days. **f,** The expression levels of SNAP25

were normalised to β-actin level and quantified. Mean values are 100.00, 102.6, 69.98, and 98.68 for PBS, GQDs, PFFs, and PFFs+GQDs (n=4, biologically independent sample; two-way ANOVA with a post hoc Bonferroni test; NS, not significant; error bars are the standard deviation). **g,** VAMP2 were normalised to β-actin level and quantified. Mean values are 100.00, 102.33, 75.80, and 103.55 for PBS, GQDs, PFFs, and PFFs+GQDs (n=4, biologically independent samples; two-way ANOVA with a post hoc Bonferroni test; NS, not significant; error bars are the standard deviation).

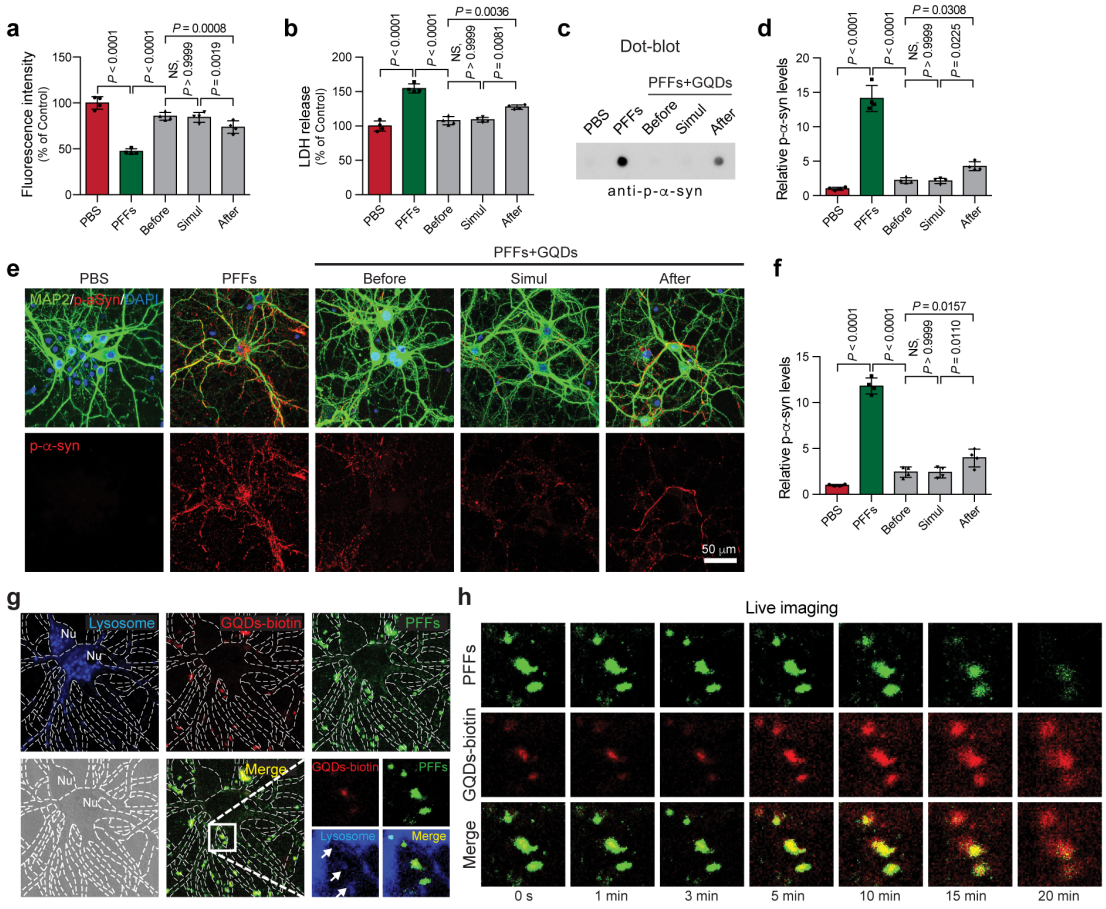
Supplementary Fig. 7



Supplementary Figure 7. The effect of GQDs on α-syn PFFs-induced mitochondrial dysfunction and oxidative stress. a, Representative 8-OHG immunostaining images in primary cortical neurons treated with α-syn PFFs (1 μg/ml) in the absence and presence of GQDs (1 μg/ml) and incubated for 7 days. **b,** Quantifications of 8-OHG content by the immunofluorescence levels. Mean values are 1.00, 0.66, 6.01, and 2.54 for PBS, GQDs, PFFs, and PFFs+GQDs (n=5, biologically independent samples; two-way ANOVA with a post hoc Bonferroni test; NS, not significant; error bars are the standard deviation). **c,** Measurements of the mitochondrial complex I activity after 7 days of α-syn PFFs (1 μg/ml) incubation. Mean values are 0.52, 0.53, 0.35, and 0.51 for PBS, GQDs, PFFs, and PFFs+GQDs (n=5, biologically independent samples; two-way ANOVA with a post hoc Bonferroni test; NS, not significant; error bars are the standard deviation). **d,** Representative MitoTracker-positive micrographs. 10 DIV primary cortical neurons were treated with α-syn PFFs (1 μg/ml) in the absence and presence of GQDs (1 μg/ml). After 7 days of incubation, mitochondria were labelled with MitoTracker® Orange CMTMRos (Red). **e,** Quantifications of the length of the stained mitochondria. Mean values are 2.55, 2.49, 1.38, and 2.33 for PBS, GQDs, PFFs, and PFFs+GQDs (n=50, biologically independent samples; two-way ANOVA with a post hoc Bonferroni test; NS, not significant; error bars are the standard deviation). **f,** Aspect ratio of the stained mitochondria. Mean values are 2.22, 2.15, 1.53, and 2.14 for PBS, GQDs, PFFs, and PFFs+GQDs (n=10, biologically independent samples; two-way ANOVA with a post hoc Bonferroni test; NS, not significant; error bars are the standard deviation). **g,** Representative TEM images with low and high magnifications after the same preparation steps. **h,** Microplate-based respirometry readings for neurons.

The oxygen consumption rate was measured in an XF 24 Seahorse analyser in primary cortical neurons treated with α-syn PFFs (1 μg/ml) in the absence and presence of GQDs (1 μg/ml) for 24 hours. Mean values are 111.84, 116.36, 103.08, 107.07, 105,15, 111,79, 94,42, 54,67, 55,42, 50.81, 139,74, 137.09, 127.98, 28.09, 32.08, and 32.68 for PBS group; 106.87, 107.41, 105.22, 108.21, 113.83, 107.85, 100.25, 61.22, 58.81, 60.01, 140.25, 131.73, 128.53, 37.23, 47.80, and 37.92 for GQDs group; 91.65, 104.99, 93.36, 90.06, 94.11, 100.81, 86.56, 47.58, 56.70, 53.91, 109.52, 112.07, 108.72, 23.60, 27.08, and 35.31 for PFFs group; 104.20, 115.69, 109.04, 115.20, 113.70, 111.38, 106.44, 66.55, 66.09, 65.96, 147.19, 135.62, 129.37, 37.86, 40.70, and 46.95 for PFFs+GQDs (n=4, biologically independent experiments). **i,** Quantifications of the basal respiratory rate. Mean values are 107.84, 106.68, 90.91, and 110.78 for PBS, GQDs, PFFs, and PFFs+GQDs (n=4, biologically independent experiments; two-way ANOVA with a post hoc Bonferroni test; NS, not significant; error bars are the standard deviation). **j,** Quantifications of the maximal respiratory rates from the respirometry results. Mean values are 134.32, 133.26, 107.14, and 139.37 for PBS, GQDs, PFFs, and PFFs+GQDs (n=4, biologically independent experiments; two-way ANOVA with a post hoc Bonferroni test; NS, not significant; error bars are the standard deviation).

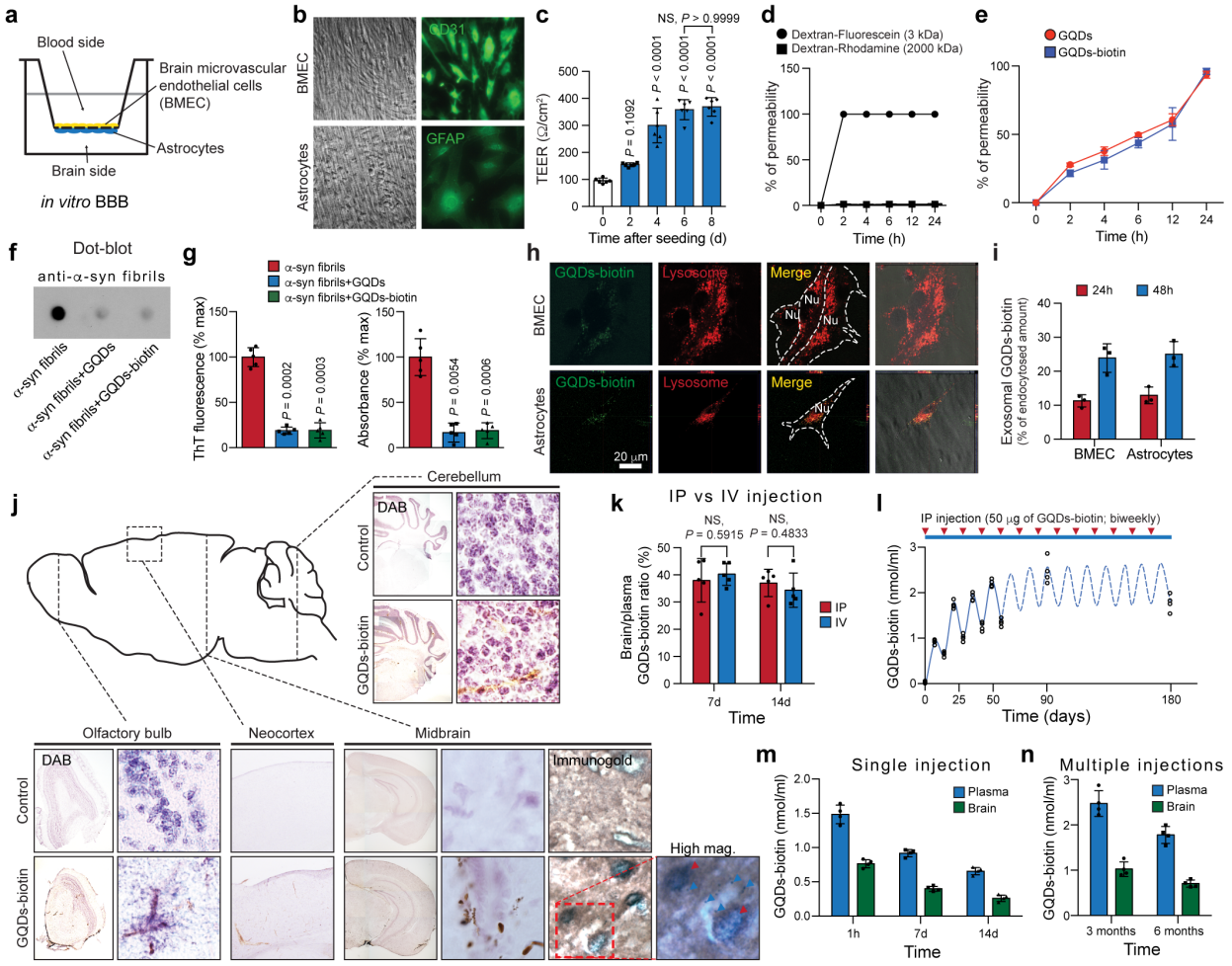
Supplementary Fig. 8



Supplementary Figure 8. The effect of GQDs on α-syn PFFs-induced primary neuronal toxicity and pathology at different treatment points of GQDs and live cell imaging. a, Neuronal toxicities assessed by alamarBlue and **b,** LDH assays. 10 DIV mouse cortical neurons were treated with a-syn PFFs (1 μg/ml) with 3 days pre- (n=4, Before), simultaneous (n=4, Simul), and 3 days post-incubation (n=4, After) of GQDs (1 μg/ml) for 7 days. Mean values are 100.00, 47.14, 85.27, 84.52, and 73.72 for PBS, PFFs, Before, Simul, and After of alamarBlue assay; 100.00, 154.64, 107.72, 109.11, and 127.62 for PBS, PFFs, Before, Simul, and After of LDH assay (n=4, biologically independent samples; two-way ANOVA with a post hoc Bonferroni test; NS, not significant; error bars are the standard deviation). **c,** Representative images of p-α-syn by dot-blot analysis and **d,** quantified intensities normalised to the PBS control. Mean values are 1.00, 14.11, 2.21, 2.14, and 4.26 for PBS, PFFs, Before, Simul, and After (n=4, biologically independent samples; two-way ANOVA with a post hoc Bonferroni test; NS, not significant; error bars are the standard deviation). **e,** Representative p-α-syn immunostaining micrographs with p-α-

syn antibody at 7 days post-incubation. **f,** Quantifications of p-α-syn immunofluorescence intensities normalised to the PBS control. Mean values are 1.00, 11.82, 2.44, 2.39, and 3.98 for PBS, PFFs, Before, Simul, and After (n=4, biologically independent samples; two-way ANOVA with a post hoc Bonferroni test; NS, not significant; error bars are the standard deviation). **g,** Representative images show that GQDs and α-syn PFFs are co-localised in the lysosome of primary cultured neuron with live imaging. The LysoTracker (blue), GQDs-biotin-streptavidin Qdot complex (red), and FITC-labelled α-syn PFFs (green) were used. **h,** α-syn PFFs disaggregation process by GQDs was monitored by time-lapse fluorescence signals during live imaging. Experiment was independently repeated three times with duplication with similar results.

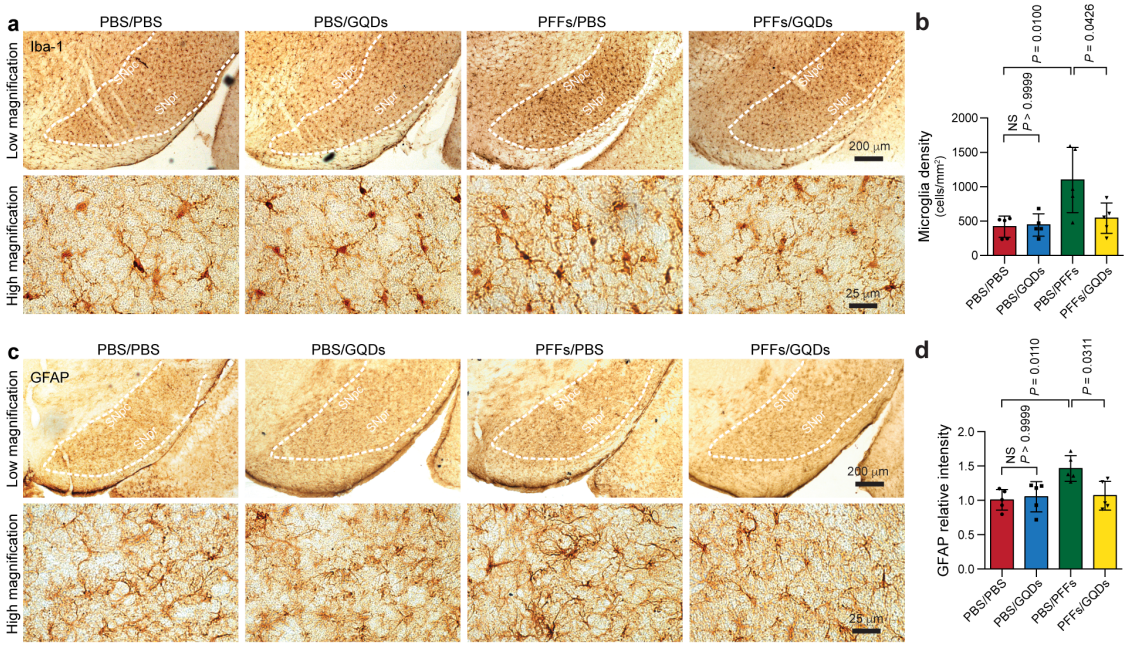
Supplementary Fig. 9



Supplementary Figure 9. The BBB permeability of GQDs. a, Schematic illustration of the *in vitro* BBB model. **b,** The confluent monolayer formations of the brain microvascular endothelial cells (BMEC) and astrocytes were determined by immunofluorescence staining with CD31 (endothelial cell marker) or GFAP (astrocyte marker) antibodies, respectively. **c,** The transepithelial electrical resistance (TEER) was measured at various time points. Mean values are 95.95, 154.67, 299.98, 358.35, and 368.88 at 0, 2, 4, 6, and 8 d (n=6, biologically independent samples; one-way ANOVA with a post hoc Bonferroni test, NS, not significant; error bars are the standard deviation). **d,** The permeabilities of endothelial cell monolayer and astrocyte were monitored by measuring the ratio between the blood side and the brain side using dextran-fluorescein (3 kDa) and dextran-rhodamine (2,000 kDa) (n=4, biologically independent samples). **e,** Quantifications of permeability of GQDs and GQDs-biotin over time. Mean ± s.d. values are 27.86 ± 1.29, 37.71 ± 3.07, 49.65 ± 1.52, 60.78 ± 4.28, and 93.652.55 at 2, 4, 6, 12, and 24 hours of GQDs; 21.65

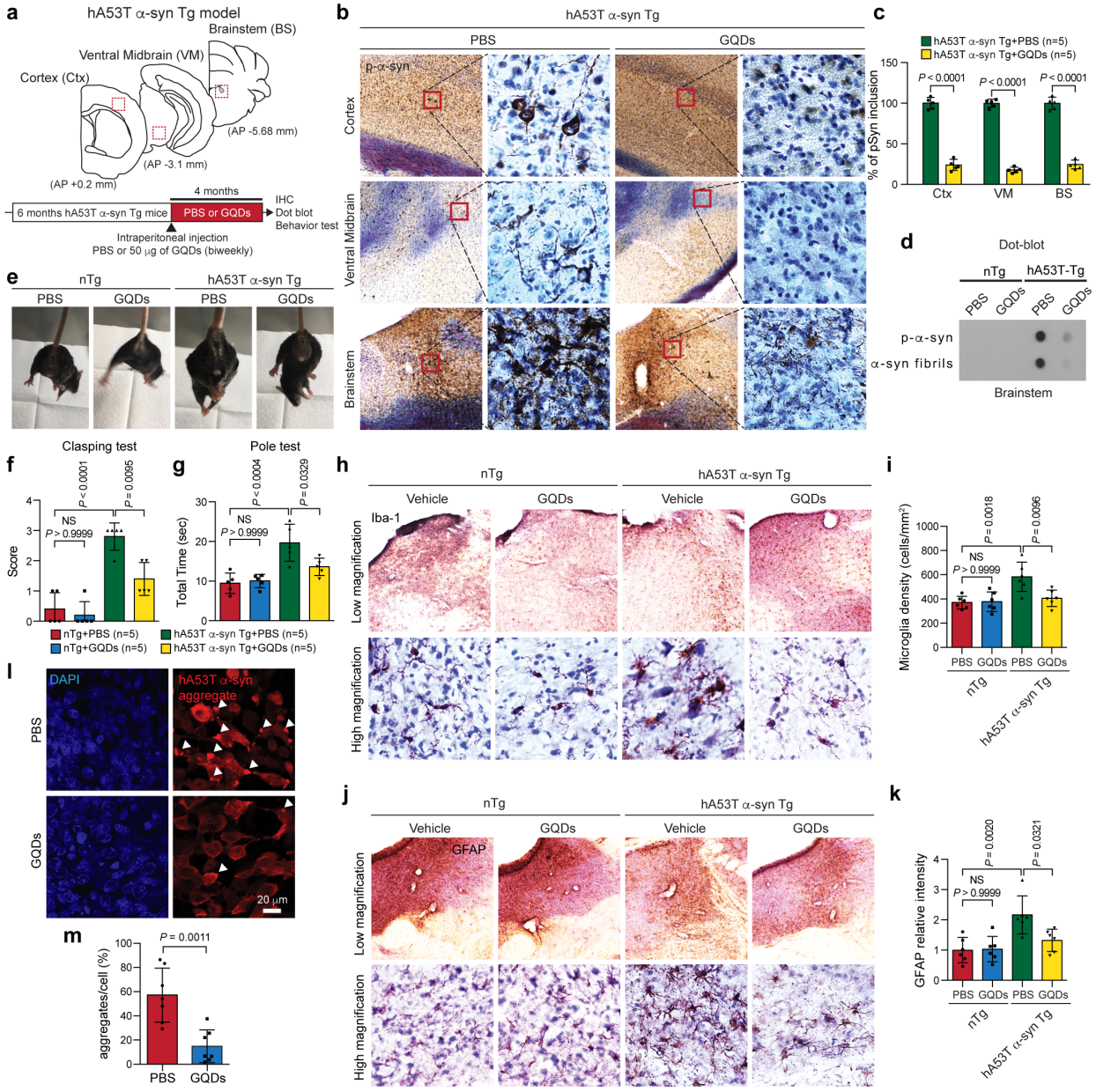
± 2.46, 31.29 ± 6.65, 43.85 ± 3.69, 57.60 ± 11.93, and 96.14 ± 2.27 at 2, 4, 6, 12, and 24 hours of GQDs-biotin (n=4, biologically independent samples). **f,** The α-syn fibril disaggregation abilities of pristine GQDs and GQDs-biotin monitored by dot-blot (n=3, biologically independent samples), **g,** ThT, and turbidity assays. Mean ± s.d. values are 100.00 ± 9.35, 19.04 ± 4.14, and 19.81 ± 8.33 of α-syn fibril, α-syn fibril+GQDs, and α-syn fibril+GQDs-biotin for ThT assay; 100.00 ± 20.35, 18.26 ± 7.60, and 18.79 ± 8.64 of α-syn fibril, α-syn fibril+GQDs, and α-syn fibril+GQDs-biotin for turbidity assay (n=5, biologically independent samples; one-way ANOVA with a post hoc Bonferroni test). **h,** Confocal laser scanning microscope images of primary cultured BMEC and astrocyte after 1 hour incubation with GQDs-biotin. GQDs-biotin are shown with Qdot™ streptavidin, whereas the lysosomes labelled by LysoTracker orange. **i,** Quantifications of the exosomal GQDs-biotin from BMEC or astrocytes. The amount of GQDs-biotin was measured from the isolated exosome at 24 and 48 hours after endocytosis. Mean ± s.d. values are 11.30 ± 1.80 and 23.91 ± 4.22 for 24 h and 48 h of BMEC; 12.91 ± 2.46 and 25.04 ± 3.77 for 24 h and 48 h of astrocytes (n=3, biologically independent samples). **j,** Immunohistochemical analysis of i.p. injected GQDs-biotin (2 mg/kg) by staining the olfactory bulb, neocortex, midbrain, and cerebellum with avidin-biotin complex method or immunogold method at 7 days after injection. The DAB positive stained signals were detected in GQDs-biotin-injected mice compared to vehicle-injected control group. Immunogold positive signals were observed inside (red triangles) or outside of neurons (blue triangles). **k,** The comparison between intraperitoneal (IP; red bar) and intravenous (IV; blue bar) injections of GQDs-biotin. Mean ± s.d. values are 38.02 ± 7.97 and 40.26 ± 4.10 for IP and IV at 7 d; 37.06 ± 5.02 and 34.42 ± 6.27 for IP and IV at 14 d (n=5, biologically independent animals; two-tailed Student's t-test; NS, not significant). **l,** The GQDs-biotin concentrations of the plasma were measured from the non-injected control after 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 3 months, and 6 months (n=4, each group) after biweekly GQDs-biotin injections using biotin quantification kit. **m,** The brain (green bar) and plasma (blue bar) concentrations were measured from 1 hour, 7 days, and 14 days after single i.p. injection (2 mg/kg of GQDs-biotin. Mean values are 1.46 and 0.77 for plasma and brain of 1 hour; 0.92 and 0.40 for plasma and brain of 7 days; 0.66 and 0.26 for plasma and brain of 14 days. (n=4, biologically independent animals; error bars are the standard deviation). **n,** 3 months and 6 months after multiple biweekly injections. NS, not significant; Student's t-test, error bars are the standard deviation. Mean values are 2.47 and 1.03 for plasma and brain of 3 months; 1.78 and 0.71 for plasma and brain of 7 months (n=4, biologically independent animals; error bars are the standard deviation).

Supplementary Fig. 10



Supplementary Figure 10. The effect of GQDs on α-syn PFFs-induced glial cell activation in the SN. **a,** Representative immunohistochemistry images for Iba-1 in the SN with low and high magnifications. Microglia in the SN of α-syn PFFs-injected hemisphere were stained with specific microglial marker Iba-1 (ionised calcium binding protein; specific marker of microglia/macrophage). **b,** Quantifications of Iba-1-positive microglia. Mean values are 420.42, 445.54, 1099.80, and 544.20 for PBS/PBS, PBS/GQDs, PFFs/PBS, and PFFs/GQDs (n=5, biologically independent animals; two-way ANOVA with a post hoc Bonferroni test, error bars are the standard deviation). **c,** Representative immunohistochemistry images for GFAP in the SN with low and high magnifications. Astrocytes in the SN of α-syn PFFs-injected hemisphere were stained with GFAP (glial fibrillary acidic protein; specific marker of astrocytes) antibody. **d,** Quantifications of GFAP intensity. The relative GFAP intensities were normalised against the PBS-injected control group. Mean values are 1.00, 1.05, 1.46, and 1.07 for PBS/PBS, PBS/GQDs, PFFs/PBS, and PFFs/GQDs (n=5, biologically independent animals; two-way ANOVA with a post hoc Bonferroni test, error bars are the standard deviation).

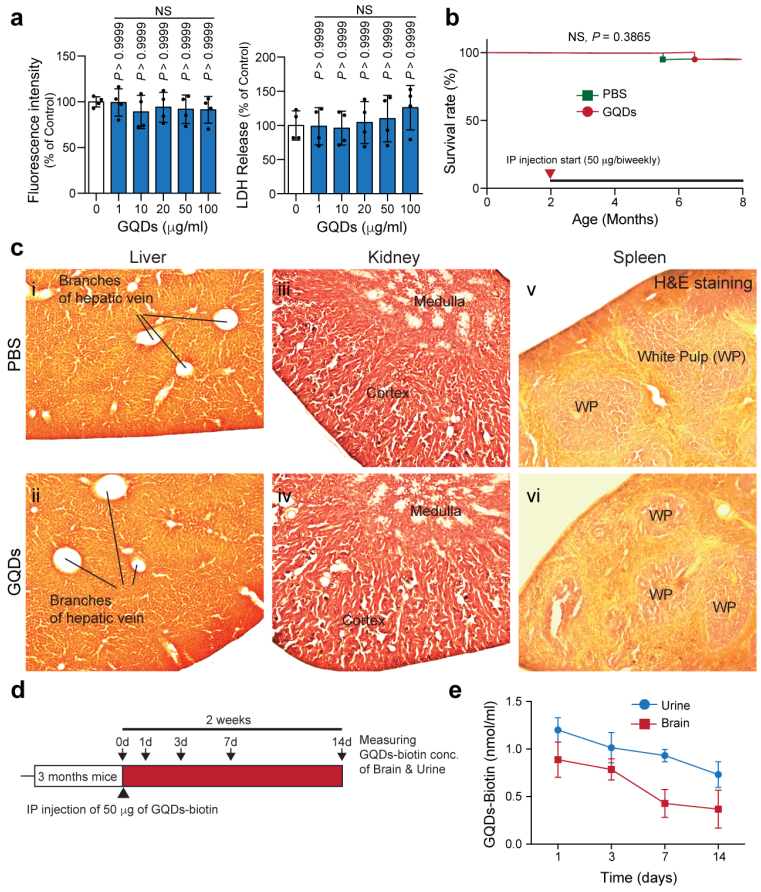
Supplementary Fig. 11



Supplementary Figure 11. The effect of GQDs on pathologies and glial cell activation of hA53T α-syn transgenic mice. a, Schematic illustration of the observing areas of hA53T α-syn Tg model. As a treatment, 50 μg of GQDs or PBS were i.p. injected biweekly for 4 months. **b,** Representative p-α-syn immunostaining images in the cortex, ventral midbrain, and brainstem of hA53T α-syn Tg. **c,** Quantifications of p-α-syn immunoreactive neurons in the cortex (Ctx), ventral midbrain (VM), and brainstem (BS). Mean values are 100.00 and 23.80 for PBS and GQDs of Ctx; 100.00 and 17.80 for PBS

and GQDs of VM; 100.00 and 24.60 for PBS and GQDs of BS (n=5, biologically independent animals; two-tailed Student's t-test; error bars are the standard deviation). **d,** Representative dot-blot assay of p-α-syn and α-syn fibrils. Experiment was independently repeated three times with similar results. **e,** Hindlimb clasping dystonic phenotypes of hA53T α-syn Tg mice were compared with GQDs-injected group. **f,** Assessment of the behavioural deficits measured by the clasping scores. Mean values are 0.4, 0.2, 2.8, and 1.4 for nTg+PBS, nTg+GQDs, hA53T α-syn Tg+PBS, and hA53T α-syn Tg+GQDs (n=5, biologically independent animals; two-way ANOVA with a post hoc Bonferroni test, NS, not significant; error bars are the standard deviation). **g,** Assessment of the behavioural deficits measured by the pole tests. Mean values are 9.49, 10.05, 19.69, and 13.60 for nTg+PBS, nTg+GQDs, hA53T α-syn Tg+PBS, and hA53T α-syn Tg+GQDs (n=5, biologically independent animals; two-way ANOVA with a post hoc Bonferroni test, NS, not significant; error bars are the standard deviation). **h,** Representative immunohistochemistry images of microglia in the LC of the brainstem with low and high magnifications. Microglia in the brainstem of nTg or hA53T α-syn Tg were stained with Iba-1. **i,** Quantifications of Iba-1-positive microglia. Mean values are 371.17, 378.83, 583.67, and 405.67 for nTg/PBS, nTg/GQDs, hA53T α-syn Tg/PBS, and hA53T α-syn Tg/GQDs (n=6, biologically independent animals; two-way ANOVA with a post hoc Bonferroni test, NS, not significant; error bars are the standard deviation). **j,** Representative immunohistochemistry images of GFAP in the brainstem with low and high magnifications. **k,** Quantifications of GFAP intensity. The relative GFAP intensities were normalised against the PBS-injected control group. Mean values are 1.00, 1.03, 2.17, and 1.32 for nTg/PBS, nTg/GQDs, hA53T α-syn Tg/PBS, and hA53T α-syn Tg/GQDs (n=6, biologically independent animals; two-way ANOVA with a post hoc Bonferroni test, NS, not significant; error bars are the standard deviation). **l,** The effect of GQDs on α-syn aggregate formation in HEK293 cells with hA53T α-syn overexpression. HEK293T cells were transfected with pCMV5-myc-A53T α-syn and the test groups were treated with GQDs (0.1 μg/ml). After 48 hours, cells were immunostained with α-syn antibodies. **m,** The number of immuno-positive aggregates per field was quantified and normalised. Mean ± s.d. values are 57.29 ± 8.45 and 14.94 ± 5.15 for PBS and GQDs (n=7, biologically independent samples; two-tailed Student's t-test). Single-headed arrows mark α-syn aggregates.

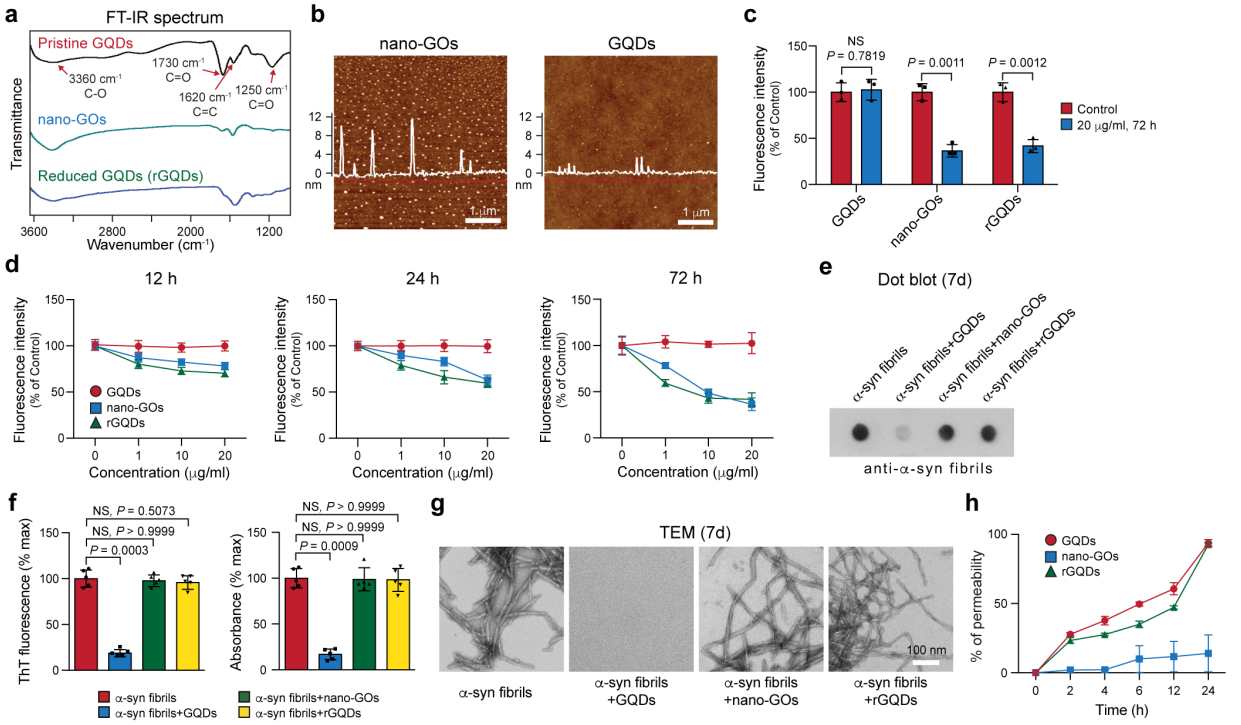
Supplementary Fig. 12



Supplementary Figure 12. Long-term *in vitro* and *in vivo* toxicity of GQDs. a, Cytotoxicity measurements of GQDs on DIV 10 primary cortical neurons after 7 days of incubation with alamarBlue and LDH assays. Mean ± s.d. values are 100.00 ± 5.65, 99.33 ± 15.08, 89.03 ±18.25, 94.10 ± 16.30, 91.95 ± 15.50, and 91.35 ± 7.31 for alamarBlue assay; 100.00 ± 21.21, 99.02 ± 27.10, 96.14 ± 24.87, 104.31 ± 30.70, 110,13 ± 34.19, and 126.05 ± 32.53 for LDH assays. (n=4, biologically independent samples; one-way ANOVA with a post hoc Bonferroni test, NS, not significant). **b,** Survival curves of GQDs injected mice. 50 μg of GQDs or vehicle were i.p. injected in C57BL/6 mice biweekly for 6 months and *in vivo* toxicity of GQDs was monitored (n=20, biologically independent animals; Log-rank test; NS, not significant). Statistically, there is no significant difference between GQDs and vehicle-injected control groups after 8 months. **c,** Representative haematoxylin and eosin (H&E) staining images of the major organs. The nuclei and eosinophilic structure of the liver, kidney, and spleen of GQDs-injected group (n=3, biologically independent animals) were compared to the PBS-injected control group (n=3, biologically independent animals). **d,** Schematic illustration of the injection and sampling methods for *in*

vivo tracking of GQDs-biotin. **e,** 50 μg of GQDs-biotin were i.p. injected in C57BL/6 mice. The concentrations of GQDs-biotin were measured using the brain (red) or urine (blue) of GQDs-biotin-injected mice at various time points (1, 3, 7, and 14 days). Mean ± s.d. values are 1.21 ± 0.13, 1.02 ± 0.16, 0.92 ±0.07, and 0.73 ± 0.14 at 1, 3, 7, and 14 days for Urine; 0.89 ± 0.19, 0.79 ± 0.11, 0.43 ± 0.15, and 0.37 ± 0.20 at 1, 3, 7, and 14 days for Brain (n=4, biologically independent animals).

Supplementary Fig. 13



Supplementary Figure 13. The comparative effects of nano-GOs and rGQDs on α-syn PFFs induced primary neuronal toxicity, disaggregation of fibrils and the BBB permeability. a, FT-IR spectra of pristine GQDs (black), nano-GOs (green), and reduced GQDs (rGQDs, purple) with designated peaks for the functional groups. **b**, AFM images of nano-GOs and GQDs with representative line profiles. **c,** Quantifications of the cell toxicities after 72 hours of incubation with 20 μg/ml of each material. Mean ± s.d. values are 100.00 ± 10.17, 102.59 ± 11.26, 100.00 ± 9.27, 36.52 ± 6.92, 100.00 ± 10.11, and 41.66 ± 7.23 for GQDs-control, GQDs-72 h, nano-GOs-control, nano-GOs-72 h, rGQDs-control, and rGQDs-72 h (n=3, biologically independent samples; two-tailed Student's t-test; NS, not significant). **d,** The cell toxicities monitored in SH-SY5Y cells by alarmarBlue assay at 12, 24, and 72 hours with various concentrations of each material (1, 10, and 20 μg/ml). Mean ± s.d. values are 100.00 ± 5.86, 99.56 ± 5.29, 98.23 ± 4.88, and 99.98 ± 5.43 at 12 hours with PBS, 1, 10, and 20 μg/ml of GQDs; 100.00 ± 4.62, 87.11 ± 4.12, 82.47 ± 3.49, and 78.23 ± 3.86 at 12 hours with PBS, 1, 10, and 20 μg/ml of nano-GOs; 100.00 ± 4.62, 80.30 ± 4.76, 72.83 ± 3.78, and 70.46 ± 3.92 at 12 hours with PBS, 1, 10, and 20 μg/ml of rGQDs; 100.00 ± 5.08, 99.90 ± 5.81, 100.12 ± 6.18, and 99.65 ± 6.88 at 24 hours with PBS, 1, 10, and 20 μg/ml of GQDs; 100.00 ± 5.31, 89.91 ± 5.39, 82.98 ± 4.62, and 63.00 ± 5.48 at 24 hours with PBS, 1, 10, and 20

μg/ml of nano-GOs; 100.00 ± 3.21, 78.96 ± 5.21, 66.12 ± 7.08, and 59.73 ± 4.56 at 24 hours with PBS, 1, 10, and 20 μg/ml of rGQDs; 100 ± 10.17, 104.03 ± 6.78, 101.38 ± 3.27, and 102.59 ± 11.26 at 72 hours with PBS, 1, 10, and 20 μg/ml of GQDs; 100.00 ± 9.27, 78.34 ± 3.30, 48.53 ± 4.28, and 36.52 ± 6.92 at 72 hours with PBS, 1, 10, and 20 μg/ml of nano-GOs; 100.00 ± 10.11, 59.37 ± 3.67, 43.04 ± 5.47, and 41.66 ± 7.23 at 72 hours with PBS, 1, 10, and 20 μg/ml of rGQDs. (n=3, biologically independent samples). **e,** The comparative dissociation effects of GQDs, nano-GOs, and rGQDs assessed by dot blot, **f,** ThT and turbidity assays. Mean ± s.d. values are 100.00 ± 9.35, 18.86 ± 4.11, 97.86 ± 6.57, and 95.42 ± 8.77 for α-syn fibrils, α-syn fibrils+GQDs, α-syn fibrils+nano-GOs, and α-syn fibrils+GQDs of ThT assay and 100.00 ± 10.46, 17.06 ± 5.99, 98.84 ± 12.86, and 98.14 ± 12.35 for α-syn fibrils, α-syn fibrils+GQDs, α-syn fibrils+nano-GOs, and α-syn fibrils+GQDs of turbidity assay (n=5, biologically independent animals; one-way ANOVA with a post hoc Bonferroni test, NS, not significant). **g,** TEM images after 7 days of incubation (each with 5 mg/ml concentration; n=3, independent experiments). **h,** *In vitro* BBB permeabilities of GQDs, nano-GOs, and rGQDs using the *in vitro* BBB model in Supplementary Fig. 9. Mean ± s.d. values are 27.9 ± 1.29, 37.71 ± 3.07, 49.65 ± 1.52, 60.78 ± 4.28, and 93.65 ± 2.55 at 2, 4, 6, 12, 24 hours of GQDs; 1.92 ± 0.90, 2.22 ± 0.20, 9.97 ± 9.55, 11.73 ± 11.06, and 14.06 ± 13.29 at 2, 4, 6, 12, 24 hours of nano-GOs; 23.33 ± 4.27, 27.70 ± 1.97, 35.10 ± 4.73, 47.29 ± 2.24, and 92.74 ± 2.28 at 2, 4, 6, 12, 24 hours of rGQDs (n=4, biologically independent samples).

Supplementary References

1 Park, C. *et al.* TLR3-mediated signal induces proinflammatory cytokine and chemokine gene expression in astrocytes: differential signaling mechanisms of TLR3-induced IP-10 and IL-8 gene expression. *Glia* **53**, 248-256, doi:10.1002/glia.20278 (2006).

2 Guyenet, S. J. *et al.* A simple composite phenotype scoring system for evaluating mouse models of cerebellar ataxia. *J Vis Exp*, doi:10.3791/1787 (2010).

3 Marcano, D. C. *et al.* Improved synthesis of graphene oxide. *ACS Nano* **4**, 4806-4814, doi:10.1021/nn1006368 (2010).