Supporting Information

Wash-Free 3D Imaging and Detection of Glioma with a Novel Neuropotential Targeted AIE Probe

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Contents

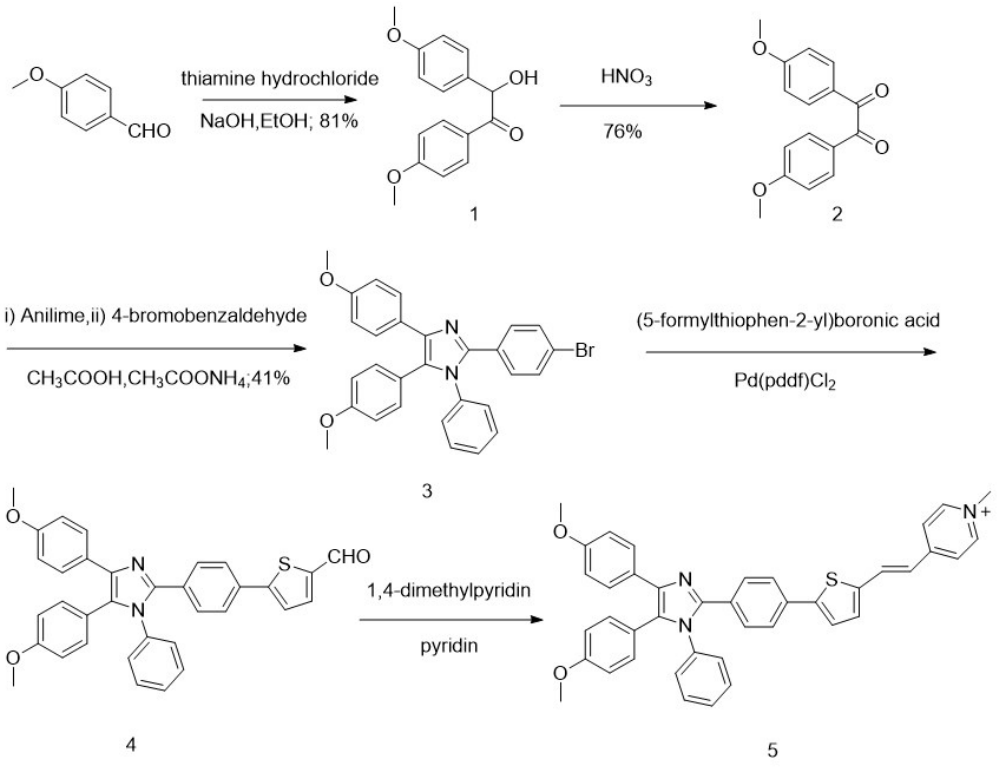
S1 Synthesis and characterization

S1.1 Materials and methods

All chemicals and reagents were used as received useless otherwise specified. Vitamin B1, benzaldehyde, and p-chloroaniline (99.5%) were purchased from Energy Chemical Co., Ltd (China). 4-Pyridine carboxaldehyde (98%) and pyridine were purchased from Sinopharm Chemical Reagent Co., Ltd (China). 4',6-diamidino-2-phenylindole (DAPI) and calf thymus DNA were purchased from Sigma-Aldrich. Roswell Park Memorial Institute (RPMI)-1640 medium, phosphate buffered saline (PBS), fetal bovine serum (FBS), penicillin and streptomycin were purchased from Invitrogen. The cell cytotoxicity assay kit (MTT) was a commercial product of Beyotime Biotechnology (China). Milli-Q water was supplied by Milli-Q Plus System (Millipore Corporation, United States).

^{1}H and ^{13}C NMR spectra were measured on a Bruker ARX 400 NMR spectrometer using $CDCl^{3}$ or DMSO-d6 as the solvent and tetramethylsilane (TMS; $\delta = 0$) as the internal reference. UV-vis Absorption spectra were performed on a UV-2450 scanning spectrophotometer (Shimadzu, Japan). Fluorescent spectra were recorded on a Shimadzu RF-5301 equipped with a 1 cm quartz cell. Dynamic light scattering measurements were performed at 25 ^{o}C on Zestier Nano ZS (Malvern Instruments Ltd, UK). Fluorescent images were collected on Leica DMI 4000B fluorescence microscope. Flow cytometry measurement was performed by BD FACSCanto II, Size of TPIG-NP was measured by Tecnai G2 Spirit TWIN (FEI). The glioma in mouse brain was scanned by LEICA CM1950.

Cell lines: derived Endothelial cells.3 (bEnd.3) and human glioma cells (U87-MG, HS683, U251and T98 cells) were continuously cultured in our laboratory.



Scheme S1 Synthesis of Probe **TPIG**

Synthesis of compound **2**

The mixture of compound 1 (2.72 g, 10.0 mmol) and 50 mL conc. HNO_3 was taken in a 100 mL round bottom flask and heated with occasional shaking until the evolution of oxides of nitrogen seized. The reaction mixture was then poured onto crushed ice, and stirred well till the green solid separated out. It was then filtered and recrystallized in ethanol to yield compound **2** (1.944 g, 76% yields) ^{1}HNMR (500 MHz, $CDCl_3$, δ): 7.91-7.93 (d, 4H), 6.93-6.95 (d, 4H), 3.87 (s, 6H). ^{13}CNMR (125 MHz, $CDCl_3$, δ): 193.53, 164.87, 132.34, 126.24, 114.30, 55.64.

Synthesis of compound **3**

4-bromobenzaldehyde (1.85 g, 10.0 mmol) and aniline (930 mg, 10.0 mmol) were dissolved in acetic acid (100 mL) and stirred for 1 h at room temperature. Compound 2 (2.70 g, 10.0 mmol) and ammonium acetate (5.40 g, 70.0 mmol) were added subsequently. The mixture was heated at 120 °C overnight. After termination of the reaction, the solution was poured into copious amounts of water. After neutralization, the mixture was filtered and washed with water. The organic compounds were

reprecipitated in methanol from dichloromethane solution, which was dried over anhydrous $MgSO_4$ and then concentrated in vacuum. Silica gel column purification with EtOAc: $CHCl_3$: n-hexane (1:10:45, v/v/v) gave a green powder (1.89 g, yield = 41%). ^{1}HNMR (500 MHz, $CDCl_3$, δ): 7.73-7.75 (d, 2H), 7.59-7.61 (d, 2H), 7.53-7.55 (d, 2H), 7.30-7.32 (m, 3H), 7.03-7.08 (m, 4H), 6.81-6.83 (d, 2H), 6.76-6.78 (d, 2H), 3.79(s, 3H), 3.78 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 159.36, 158.61, 144.85, 138.65, 136.93, 136.05, 135.34, 132.32, 131.06, 129.46, 129.37, 129.01, 128.68, 128.50, 128.35, 126.72, 122.31, 113.94, 113.70, 55.21, 55.15.

Synthesis of compound **4**

A flame-dried three-necked, round-bottomed flask equipped with a condenser was charged with a ((5-formylthiophen-2-yl)boronic acid (156 mg, 1.0 mmol), compound **3** (510.5 mg, 1.0 mmol), Pd(pddf)Cl_2 (58.0 mg, 0.05 mmol) and K_2CO_3 (280.0 mg, 2.0 mmol) following with the degassed solvents toluene (10.0 mL), methanol (3.0 mL) under N_2. The reaction system was then evacuated and backfilled with N_2 for twice. After stirring at 90 ^{o}C for 12 h, solvents were removed under reduced pressure and the residue was dissolved in CH_2Cl_2 After being washed with water and brine, the organic phase was dried over anhydrous Na_2SO_4, concentrated and purified by column chromatography on silica gel (EtOAc/petroleum ether = 1/4 to 1/1, v/v) afforded **4** as a yellow solid (376.9 mg, 59% yield). ^{1}HNMR (500 MHz, $CDCl_3$, δ): 9.84 (s, H), 7.90-7.85 (d, 2H), 7.73-7.75 (d, 2H), 7.59-7.61 (d, 2H), 7.53-7.55 (d, 2H), 7.30-7.32 (m, 3H), 7.03-7.08 (m, 4H), 6.81-6.83 (d, 2H), 6.76-6.78 (d, 2H), 3.79(s, 3H), 3.78 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ =182.4, 159.36, 158.61, 144.85, 138.65, 138.20, 136.93, 136.05, 135.34, 132.32, 131.06, 129.46, 129.37, 129.01, 128.68, 128.50, 128.35, 126.72, 122.31, 113.94, 113.70, 55.21, 55.15.

Synthesis of Probe **TPIG** (**Compound 5**)

To a solution of compound **4** (0.260 g, 0.50 mmol) in 5 mL MeCN and 2-mercaptoethanol (108.10 mL, 1.00 mmol) was added. After addition of 10 μL pyridine, the mixture was stirred under reflux for 6 h under a nitrogen atmosphere. After cooling to room temperature, the solvent was removed under vacuum. The residue was purified by silica gel column chromatography with MeOH/CH_2Cl_2 (20/1,

v/v) to give a white solid powder (385.3 mg, yield 62%). ^{1}HNMR (500 MHz, DMSO-d6, δ): 8.20-8.15 (d, 2H), 7.90-7.85 (d, 2H), 7.85-7.83 (s, H), 7.65-7.60 (d, 2H), 7.63-7.61 (s, H), 7.55-7.50 (s, H), 7.50-7.45 (s, H), 7.45-7.43 (d, 2H),7.43-7.40 (s, H), 7.40-7.38 (d, 2H), 7.38-7.35 (d, H), 7.35-7.30 (d，2H), 7.20-7.05 (m, 5H), 6.98 (t, 1H), 6.90 (d, H), 6.85-6.80 (d, 2H), 4.23 (s, 3H), 3.73 (3, 6H). ^{13}CNMR (125 MHz, DMSO-d6): δ = 175.08, 165.19, 159.33, 158.36, 153.64, 152.31, 147.65, 147.63, 146.66, 146.65, 145.34, 145.33, 140.63, 140.62, 132.84, 132.83, 130.01, 130.30, 129.91, 129.90, 129.89, 129.88, 129.85, 129.78, 129.77, 129.30, 129.29, 129.29, 129.28, 125.87, 125.86, 125.85, 125.85, 123.47, 123.45, 115.14, 115.13, 114.37, 114.00, 56.17, 55.36.

S3. Calculate the fluorescence quantum yield.

Fluorescence quantum yield (Φf) was determined by using quinine sulfate (Φf = 0.58, in 0.1 M H_2SO_4 aqueous solution) as the fluorescence standard. The quantum yield was calculated using the following equation. $\Phi_F(X) = \Phi_F(S)$ (ASFX/AXFS) $(nX/nS)^2$ Where Φ_F is the fluorescence quantum yield, A is the absorbance at the excitation wavelength, F is the area under the corrected emission curve, and n is the refractive index of the solvent used. Subscripts S and X refer to the standard and to the unknown, respectively. The quantum yield of **TPIG** was calculated 0.27.

S4. Absorption and Fluorescence emission spectra of TPIG

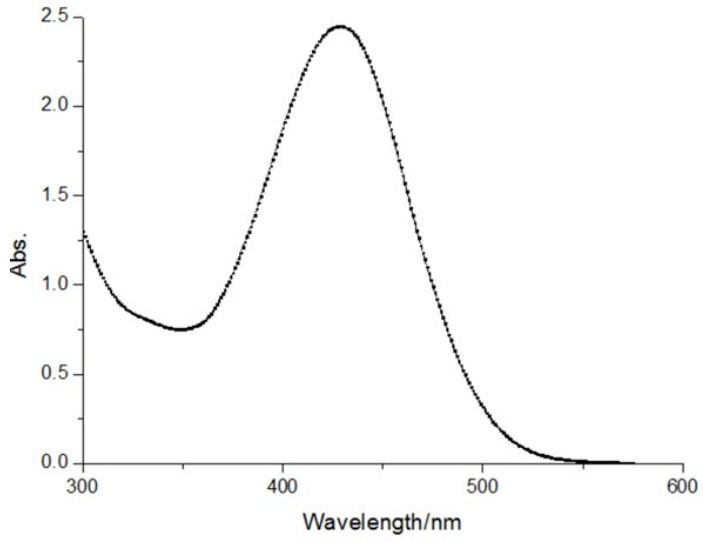


Figure S1. UV-Vis spectrum of **TPIG** in DMSO.

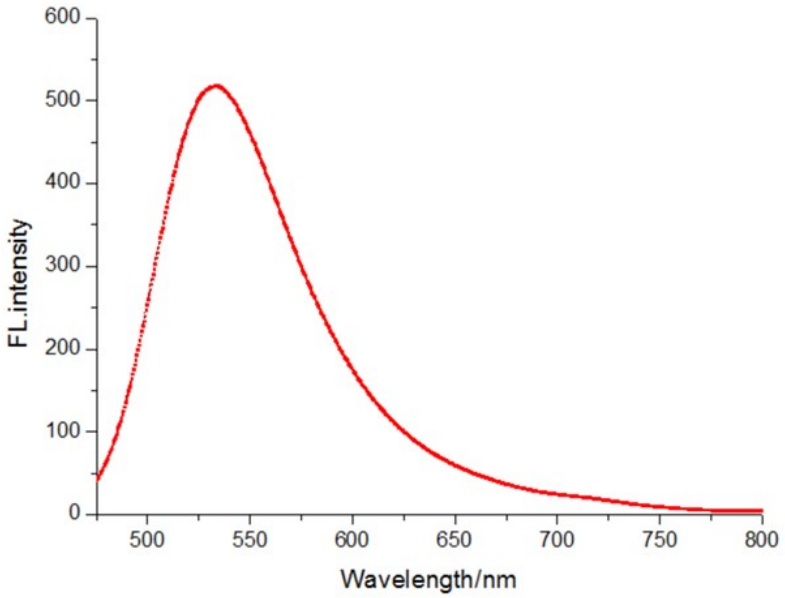


Figure S2. Fluorescent spectrum of **TPIG** in different solution.

S5.The AIE characteristics of TPIG

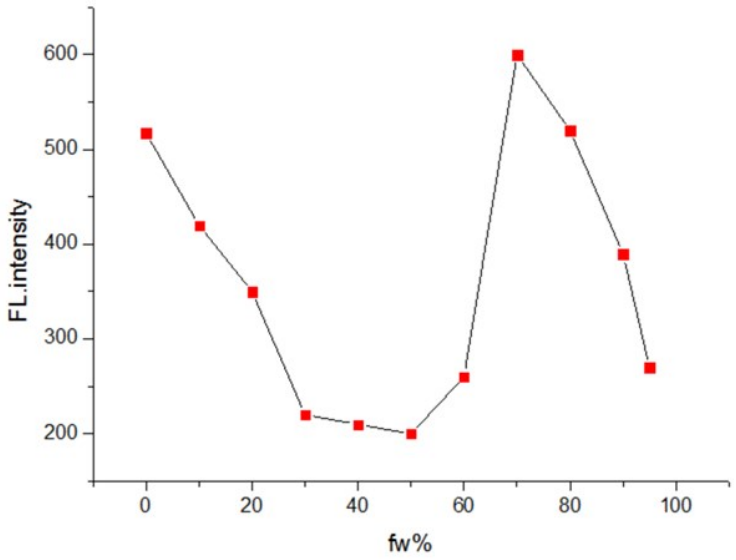


Figure S3. Fluorescence intensity of **TPIG** (10 μM) at 535 nm versus the different water fractions. The excitation wavelength was 430 nm.

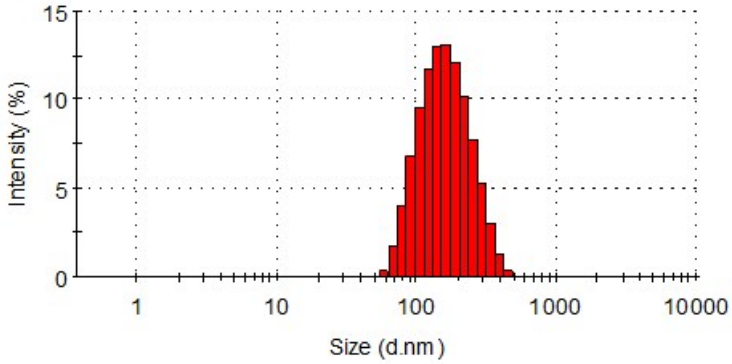


Figure S4. Particle size of **TPIG-NP** by dynamic light scattering.

Average size=145 nm.

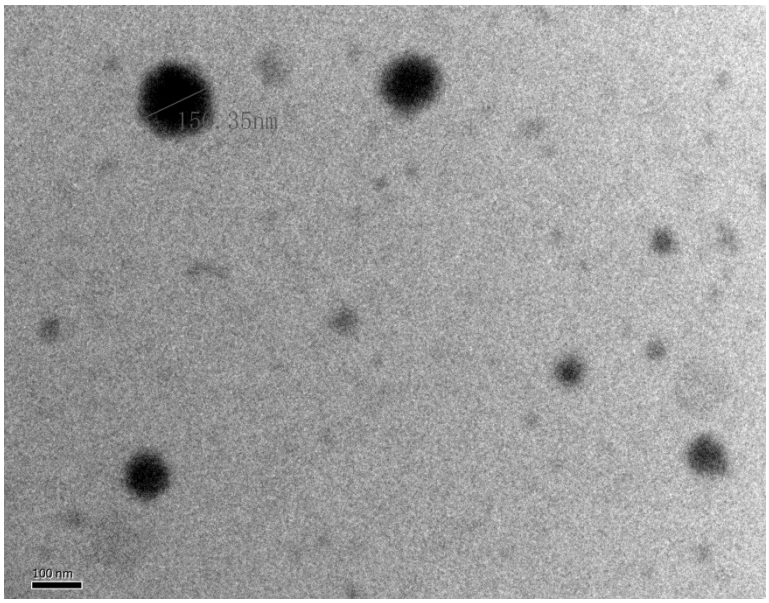


Fig. S5 TEM image of **TPIG** at pH=7.4.

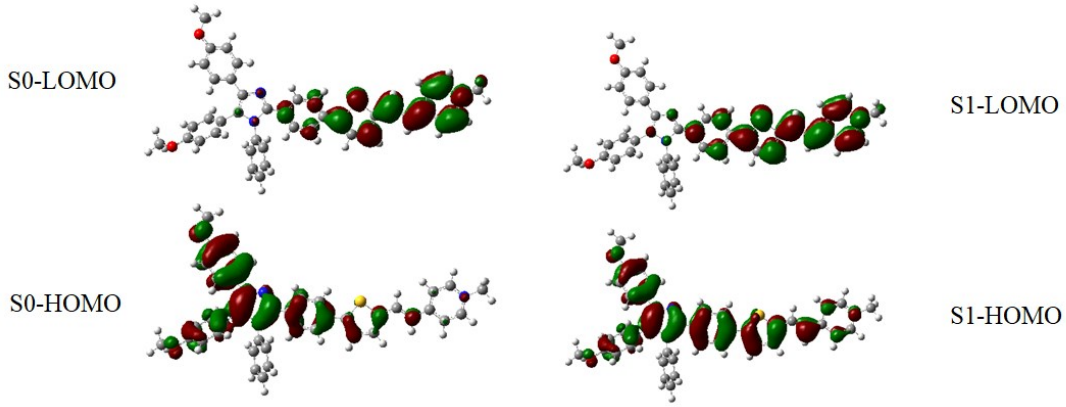
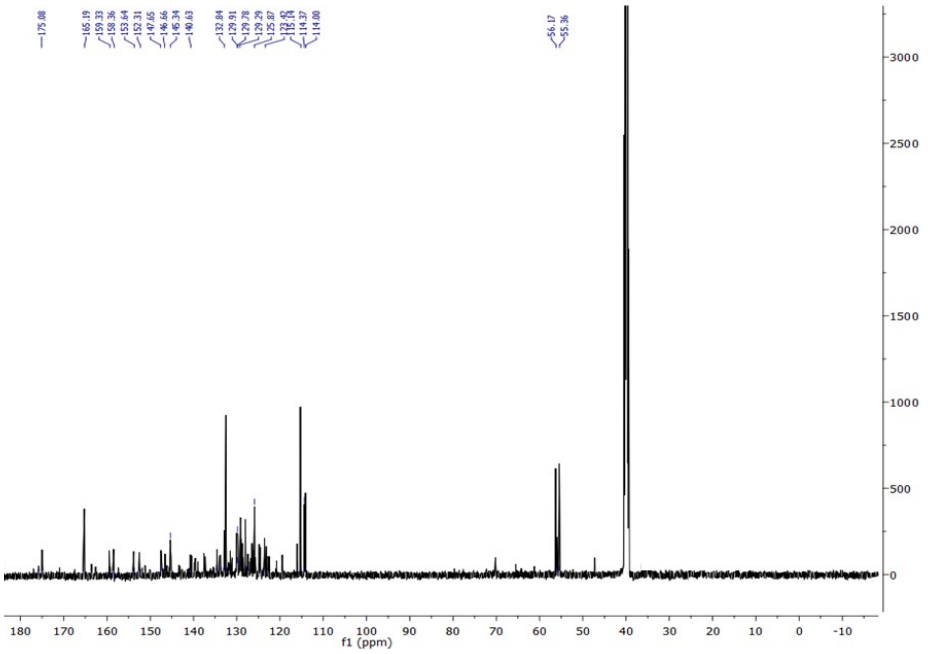


Fig. S6 calculated HOMO and LUMO electron cloud distribution of **TPIG.**



^{13}CNMR (125 MHz, DMSO-d6) of **TPIG**

Cell Viability Assay

The cytotoxicity of **TPIG** was studied by MTT assay. Briefly, 100 μL of cell suspension was placed in a 96-well plate. The plates were preincubated in an incubator for 24 h (37 °C, 5% CO_2). A volume of 10 μL of different concentrations of **TPIG** was added to the plates. The plates were incubated for 48 h in an incubator. A volume of 10 μL of MTT solution was added to each well. The plates were incubated for 1 h in an incubator. The absorbance at 450 nm was measured with a microplate reader to reflect the cell viability of each well.

Cell line: U87-MG，bEnd.3, HS683 T98 U251 cells were maintained in DMEM (Thermo Fisher Scientific, USA), supplemented with 10% FBS (fetal bovine serum, Thermo Fisher Scientific, USA) and 1% penicillin−streptomycin (Thermo Fisher Scientific, USA). Cells were incubated at 37 °C with 5% CO_2.

Flow cytometry measurement

Flow cytometry measurement of cell uptake fluorescence: inoculate 5×10^5 cells per well into a 6-well plate, culture in an incubator for 48 hours, add dye to each well (10 um) incubator and incubate for 2 hours, discard the dye, Digest and collect the cells, wash 3 times with PBS, and measure the average fluorescence intensity of each cell

by flow cytometry.

Measuring cell membrane potential

Use the Cellular Membrane Potential Assay Kit (ab176765) to measure the membrane potential of each cell. In short: Plate cells overnight in growth medium at 40,000 to 80,000 cells/well/100 μL for 96-well, then added with 100 μL/well MP Sensor dye-loading solution into the cell plate, Incubate the dye-loading plate in a 5% CO_2 incubator for 30 minutes. Run the membrane potential assay by monitoring the fluorescence at Ex/Em = 620/650 nm.

3D multicellular spheroids

Take 100 μL/well of Matrigel (10 mg/mL) and add it to a 96-well plate, place it in a cell incubator for 30 minutes to make it solidify into gel, and inoculate 1×10^5 corresponding cells per well (U87-MG or T98, U251), incubate in an incubator for 6 days, and change the medium every two days to form tumor balls on the surface of the gel.

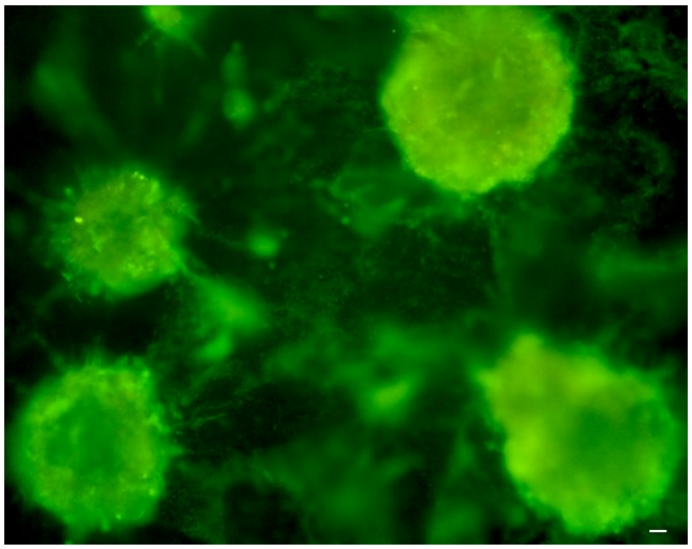


Fig. S6 Imaging of **TPIG-NP** in 3D glioma cell multicellular spheroids.

Scale bar: 50 μm.

Animal Model

The animal experiment was approved by the Animal Ethics and welfare Committee, at the second Xiangya Hospital, Central South University (No. 2020460). All animal studies were carried out using the Institutional Animal Care and Use Committee

(IACUC) approved procedures.

Establish an in situ U87-MG glioma model: Six- to eight-week-old nude mice (Department of Pharmacy the second Xiangya Hospital Animal Laboratory, China) were suspend 1×10^7 U87-MG cells in 40 μL of PBS, anesthetize the nude mice, and fix the mice with a mouse stereotactic fixation device. Position the left striatum of nude mice, drill a hole with a miniature hand-held cranial drill, and use a micro syringe pump to inject 10 ul cell suspension into the left striatum (Left side of anterior skull 2 mm, 3 mm deep), suture with surgical suture the wound, after the tumor has grown to ten days, was anesthetized with nuclear magnetic imaging.

Fig. S7 Establish an *in situ* U87-MG glioma model.

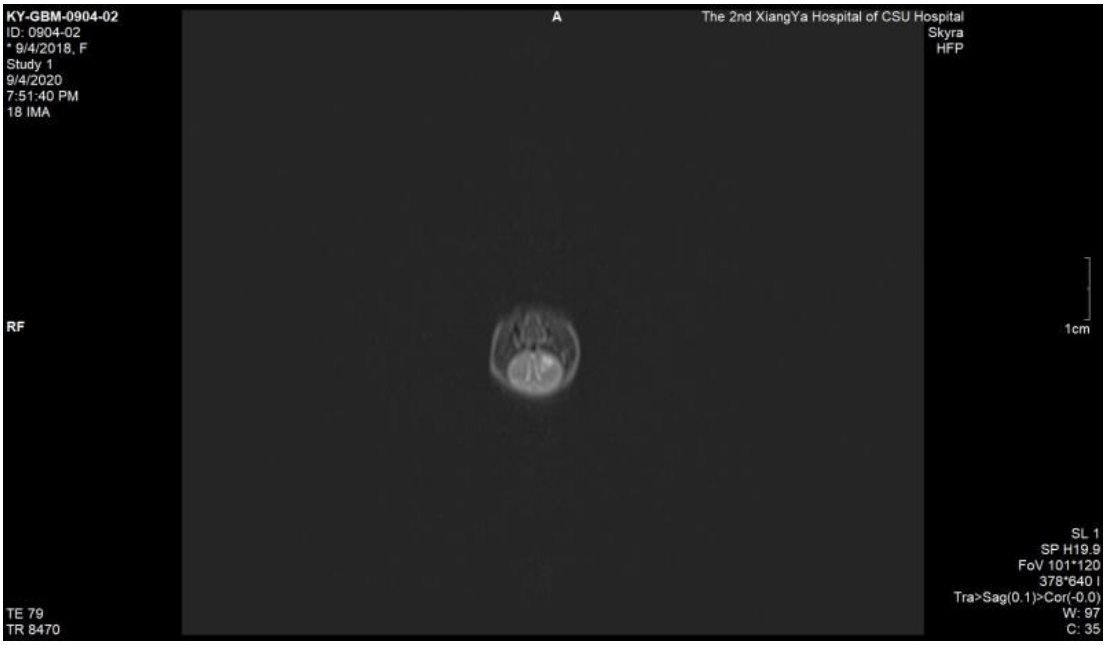


Fig. S8 MRI (1 T) of in situ U87-MG glioma model.