Supporting Information

Wenlong Zhang[1,6], Xiangyu Chen[2,6], Dong Ding[1,3,6], Guoxin Zhang[1], Ziwei Zhu[1], XingJiu Yang[1],

Mengyuan Li[1], Lijun Liang[4], Xudong Shi[1,*], Tie Wang[5,*], Ran Gao[1,*]

1. NHC Key Laboratory of Human Disease Comparative Medicine, Beijing Engineering Research Center for Experimental Animal Models of Human Critical Diseases, Institute of Laboratory Animal Sciences, Chinese Academy of Medical Sciences (CAMS) and Comparative Medicine Center, Peking Union Medical College (PUMC), Beijing, 100021, China.

2. State Key Laboratory of Inorganic Synthesis and Preparative Chemistry, College of Chemistry, Jilin University, Changchun,130015, China.

3. Department of Otolaryngology-Head and Neck Surgery, Beijing Tongren Hospital, Capital Medical University, Beijing, 100730, China.

4. State Key Laboratory of Molecular Oncology, National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital, Chinese Academy of Medical Sciences (CAMS) and Peking Union Medical College (PUMC), Beijing, 100021,China.

5. Life and Health Research Institute, Tianjin University of Technology, Tianjin, 300072, China.

6. These authors contributed equally: Wenlong Zhang, Xiangyu Chen, Dong Ding.

*Corresponding author

Email to Dr. Ran Gao: gaoran@cnilas.org

Materials and Methods

1. Nanoparticles preparation

1.1 Synthesis of SNPs

Silica nanoparticles were prepared via the classic Stöber method. Briefly, ethanol (50 mL), water (1.0 ml), and ammonia solution (2 ml) were mixed in a three-neck flask. After magnetic stirring (500 rpm) for 2 min, 1 ml tetraethyl orthosilicate was added. These mixtures were then kept for 3 h. The products were collected by centrifugation and washed three times with ethanol.

1.2 Synthesis of SNPs-FITC-NH_2

(3-Aminopropyl)triethoxysilane (0.025 ml) and (3-mercaptopropyl)triethoxysilane (0.025 ml) were added into the SNPs suspension (50 mg SNPs in 25 ml ethanol) and refluxed for 6 hours. After washing with ethanol, the SNPs-NH_2-SH was obtained. Fluorescein-5-maleimide ethanol solution (1 mg/ml, 1 ml) was mixed with the SNPs-NH_2-SH suspension. After stirring at room temperature for 2 h, the SNPs-FITC-NH_2 was collected by centrifugation. The products were washed three times with ethanol and resuspended in water.

1.3 Synthesis of SNPs-PEG-FITC and SNPs-PEG-RGD-FITC

To synthesize the SNPs-PEG-FITC, PEG-NHS (MW 5000 Da) was added into the suspension of SNPs-FITC-NH_2 at a final concentration of 8 mM, and thenthe suspension wasstirred for 8 hours at room temperature. Afterwards, SNPs-PEG-FITC was washed twice by centrifugation and resuspened in PBS. To make RGD-PEG-modified SNPs, a mixture of 5 mM PEG-NHS (MW 5000 Da), 3 mM MAL-PEG-NHS (MW 5000 Da) and SNPs-FITC-NH_2 was stirred for 8 hours, and then 10 mg of RGD was added and stirred for another 8 hours. The SNPs-PEG-RGD-FITC were washed twice by centrifugation and resuspended in PBS.

2 Dye leaking

Dye leaking experiments were conducted for investigating any leakage of FITC molecules from SNPs on dissolution in water. Aqueous solutions of SNPs-PEG-FITC and SNPs-PEG-RGD-FITC were prepared at 1 mg/ml and the supernatant of both solutions was collected by centrifugation. The supernatants were placed on a 365 nm UV lamp to detect fluorescence leakge. The material was

left to stand for 7 days and then subjected to the same fluorescence test.

3 Cell culture and transfection

GL-261 cells were cultured in Dulbecco's Modified Eagle' Medium (DMEM, Gibico, catalog No. 41965-039) with 10% Fetal Bovine Serum (FBS, Gibico,catalog No. 10099141). To obtain red fluorescent tumor cells for *in vivo* TMLSM imaging, lentivirus vector Ubi-MCS-SV40-Neomycin (Genechem Co.,Ltd, catalog No. CV084) containing Tdtomato sequence was transfected into GL-261 cells, thus GL-261-Tdtamtocell line was established. Transfection procedures were followed according to the manufacturer's protocols. Briefly, $2x10^5$ cellswere planted into a 6-well plate and cultured overnight. And then, 10 μl lentivirus (virus tilter $4x10^8$ Tu/ml) (MOI 20), were added in each well. 24 to 72 hours later red fluorescent tumor cells could be observed under the microscope, and be sub-cultured. All the cells mentioned above should be cultured in a condition of 5% CO_2 and 37℃ in the incubator.

4 Cranial Window Preparation and Orthotopic Tumor Implantation

Cranial windows were prepared as previously described[25,15]. Briefly, mouse was anesthetized with intraperitoneal (i.p.) injection of ketamine, xylazine, and benpiate hydrochloride (0.0375/0.0375/0.000125 mg/g body weight).Penicillin (40 mg/kg) was subcutaneously injected to reduce infection. Then, animals' heads were shaved, with additional use of depilatory cream. After completion of the preliminary treatment, the mice could be fixed on the stereotaxic apparatus. And then, eye ointment was applied to avoid eye dehydration and irritation. Afterwards, the cranial bone and dura mater was removed. And then the brain was covered with a 6-mm-diameter transparent cranial window and sealed with a dental cement ethylcyanoacrylate liquid (Cyano veneer, Hager Werken, Duisbrug, Germany). 2-3 weeks later, cranial window should be removed, and 1 μl cell suspensions containing $5×10^4$ GL-261-Tdtomato cells will be stereotactically injected into mouse brain using a 10 μl Hamilton microsyringe and a 2 pt style needle. The injection point was about 1 mm lateral to the sagital sinus and 2 mm posterior to the bregma at an intraparenchymal depth of 1 mm.

5 *In-vivo* Multiphoton Laser Scanning Microscopy (MPLSM)

Images were acquired using Leica TCS SP8 DIVE Upright Microscope, equipped with a Chameleon Ultra Laser System (680-1300 nm) and a 25× water immersion objective (numerical aperture 0.95, Leica). Single images were acquired from 0-300 μm depth, with z-intervals of 3 μm. 920 nm was

used as an excitation wavelength, with 1024 ×1024 pixels. While 500-540 nm and 570-620 nm were used as the emission wavelengths for FITC-Nanoparticles and GL-261-Tdtomato tumor cells, respectively. For cerebral vessel visualization, we injected 0.1 ml 10 mg/ml FITC-dextran (2M molecular weight, green, Sigma-Aldrich) through mouse tail vein. Mosaic images were obtained for big tumor mass. Mice were anesthetized by isoflurane and maintained with a constant flow from 0.8% to 2.0% (as low as possible according to the physical condition of the mouse). Laser power should also be limited to the lowest percentage to avoid photo-toxicity.

6 Small animal magnetic resonance imaging (MRI)

In vivo MRI wasperformed on a 7.0Tsmall-animalMRIsystem (Varian, Palo Alto, CA, USA). Mouse was administeredwith 0.1 mmol/kg Magnevist(gadolinium-DTPA) from tail vein 30 minutes before MRIimaging. During imaging process, mouse was anesthetized with medical oxygen (1 L/min) containing 2% isoflurane. T1-weighted MRI images of mouse brain in coronalplane were acquired with aspinecho (sems) sequence with following parameters: FOV (field of view) = 20 × 20 mm; No slices = 12; slice thickness = 1 mm (zero slice gap); TR (repetition time) = 400 ms; TE (echo time) = 18.0 ms; image matrix = 256×256; number of averages = 10. ROIs (region of interests, tumor of mousemodel at 7, 14 and 21 days) were then manually drawn on the T1images to determine the change oftumor volume.

7 Imaging data analysis

Bitplane Imaris Software was used for further analysis. To obtain high quality images, brightness, contrast, or color balance were regulated manually for the whole images. Surface were created for vasculature and tumor (TPLSM images), thus parameters including area and volume could be calculated. For MRI data, we also used Imaris for tumor volume/area quantification. However, surface were created manually slice per slice for MRI data, while surface were created automatically for TPLSM images.For quantification of conjugated nanoparticles volume in tumor core, corresponding regions of interest (ROIs) were chosen with the size of 500 μm×500 μm×300 μm (X,Y,Z) over time. Therefore, surface could be created and volume were obtained. Image J/Fiji was used for analysis of confocal images. Statistical analysis was performed using Graphpad Prism 8.4.2. Statistical differences between groups were assessed by unpaired T test, with P values of ≥0.05, <0.05, <0.01, <0.001 and <0.0001 denoted as ns, *, **, ***, ****, respectively.

Supplementary Figure legend

Supplementary Figure 1 *In vivo*TPLSM imaging showed solid glioma growth over time. Left column shows 3D images of glioma in the corresponding region, while right column created surface for these solid tumors. Thus, tumor area and volume could be calculated.

Supplementary Figure 2 Gliomavasculature changes over time. Left column indicates *in vivo* TPLSMof vasculature phenotype, and right column shows surface reconstruction of these vessels thus vessel area and volume could be calculated.

Supplementary Figure 3 Quantification results of both volume and area of vessels and tumor. These data are obtained from images in supplementary Figure 1 and supplementary Figure 2.

Supplementary Figure 4 A. Schematic illustrates the synthesis and modification of SNPs-PEG--RGD-FITC. B. Flurescent images of FITC leakage experiment.

Supplementary Figure 5 Merged *in vivo* TPLSM images illustrate GL-261-Tdtomato tumor growth and green auto-fluorescence in control mouse without nanoparticle injection.

Supplementary Figure6 Green channel *in vivo*TPLSM images indicate auto-fluorescence in control mouse of corresponding regions in supplementary Figure 5.

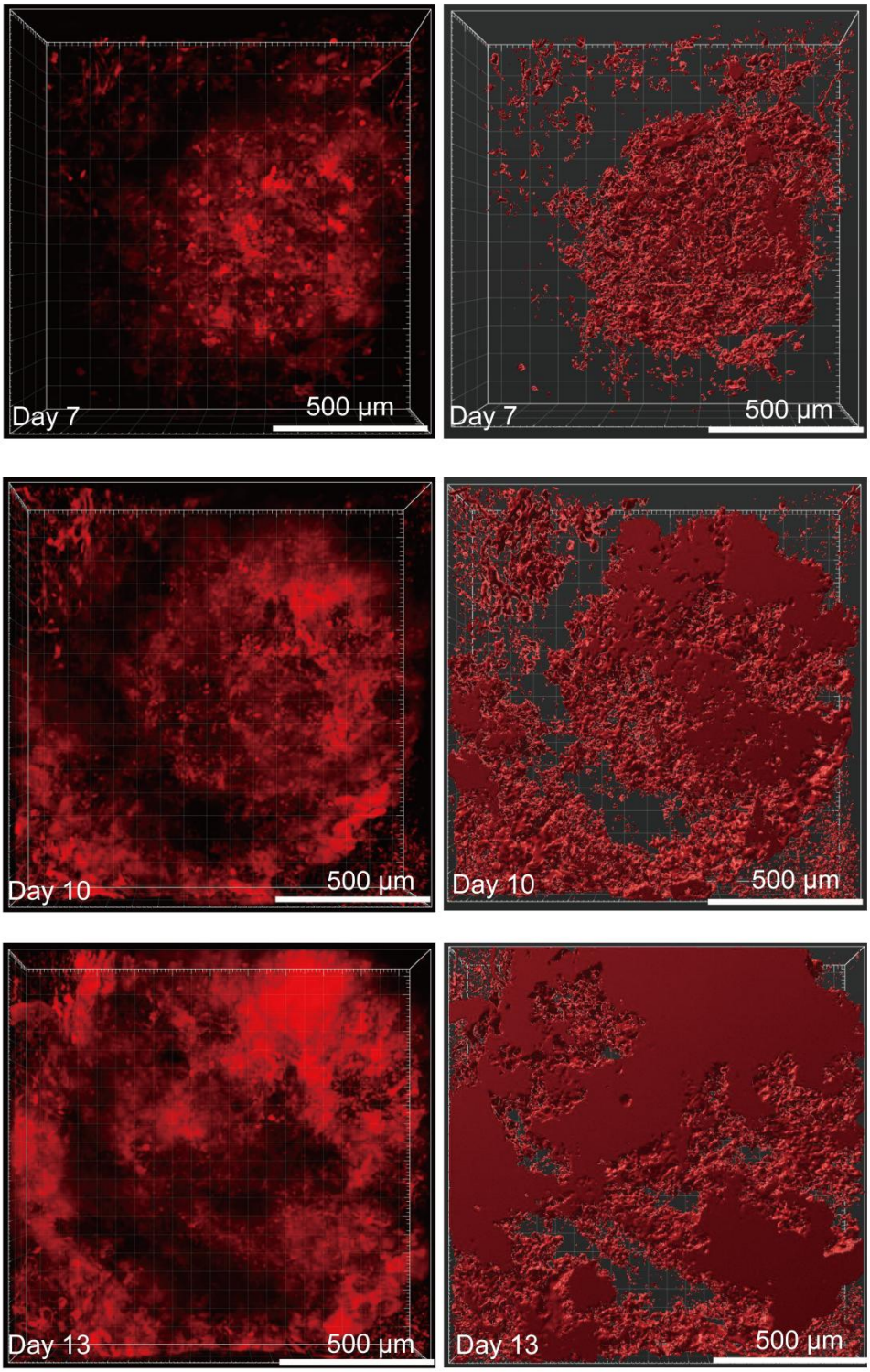
Supplementary Figure 7 Quantification results reveal SNPs-PEG-FITC, SNPs-PEG-RGD-FITC, and green auto-fluorescent volume in glioma from Figure 4, Figure 5, and supplementary Figure 5. RGD conjugated nanoparticles have high penetration in glioma, while penetration of PEG conjugated nanoparticles remains in a low level.

Supplementary Figure 8 Up two rows are merged images of SNPs-PEG-RGD-FITC and GL-261-Tdtomato cells. While lower two rows only shows the green channel of SNPs-PEG-RGD-FITC infiltration. Rectangular box illustrates the region of interest (ROI) with the size of 500 μm×500 μm×300 μm (X,Y,Z), and thus volume of conjugated nanoparticles could be calculated accordingly.

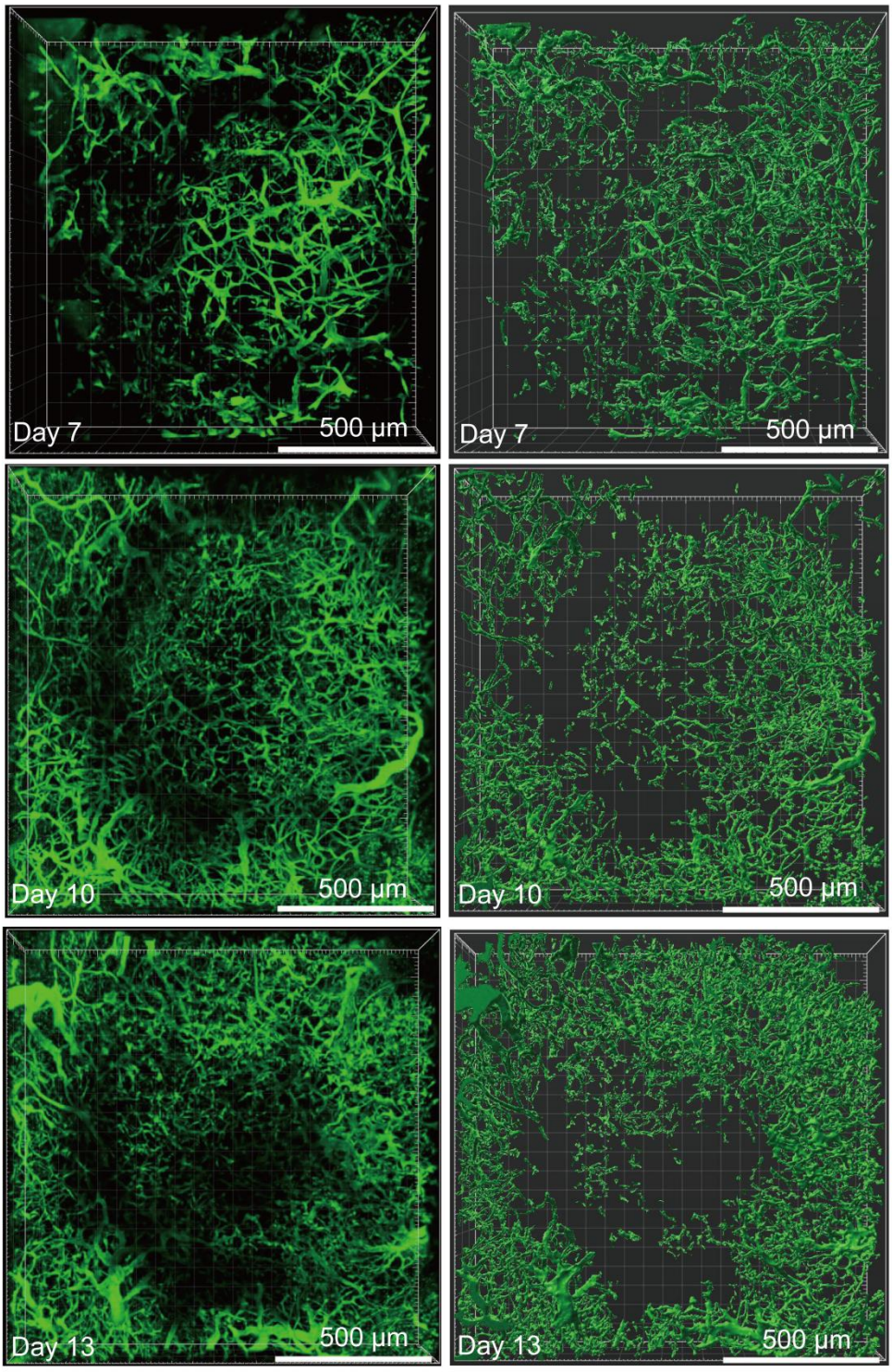
Supplementary Figure 9 Quantification results reveal SNPs-PEG-FITC, SNPs-PEG-RGD-FITC, and green auto-fluorescent volume in regions of interest in (ROIs, with the size of 500 μm×500 μm×300 μm/X,Y,Z) in glioma. RGD nanoparticles have high penetration in glioma, while penetration of PEG nanoparticle remains in a low level.

Supplementary Figure 10 Coronal MRI images reveal glioma growth over time. Solid GL-261-Tdtomato grows underneath the cranial window and has clear boundaries with brain parenchyma.

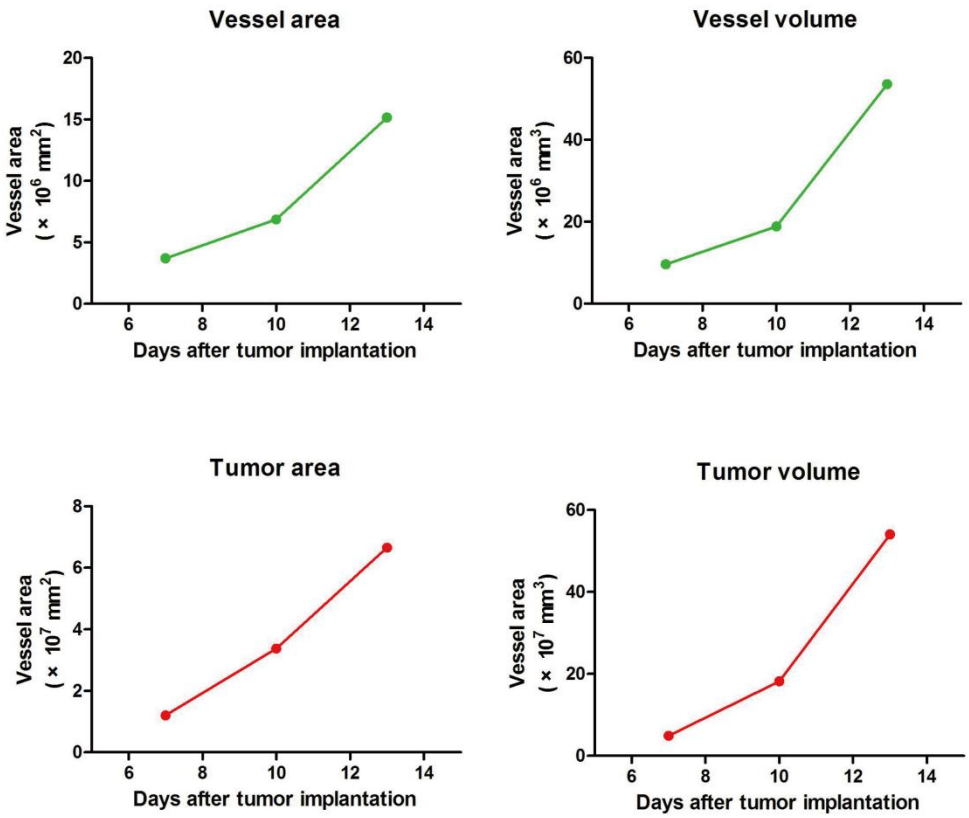
Supplementary Figure



Supplementary Figure 1

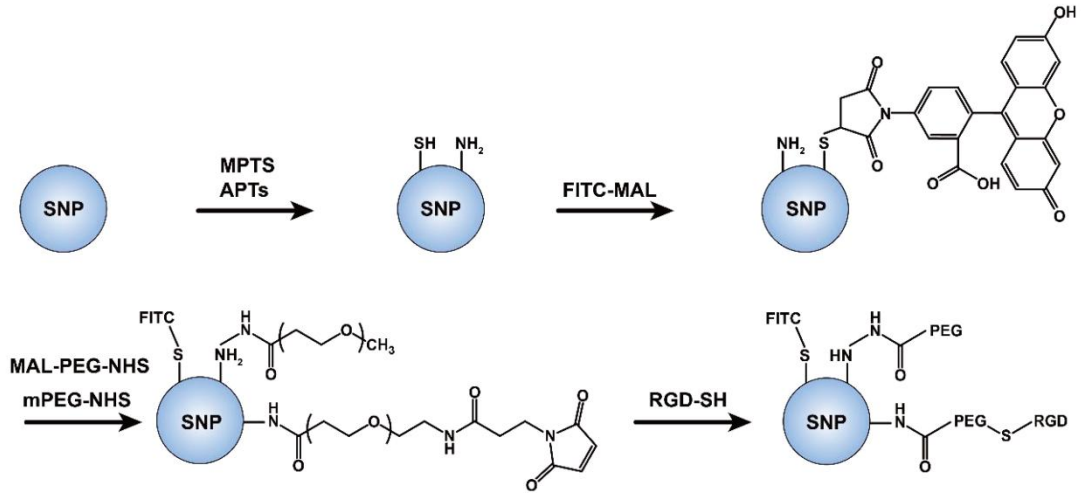


Supplementary Figure 2

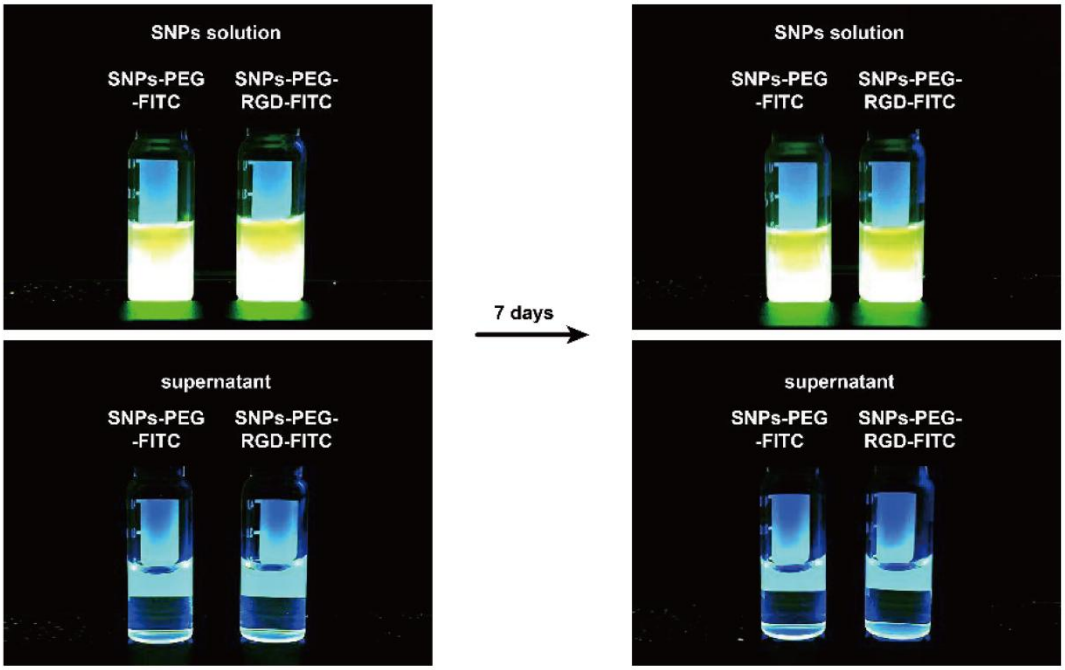


Supplementary Figure 3

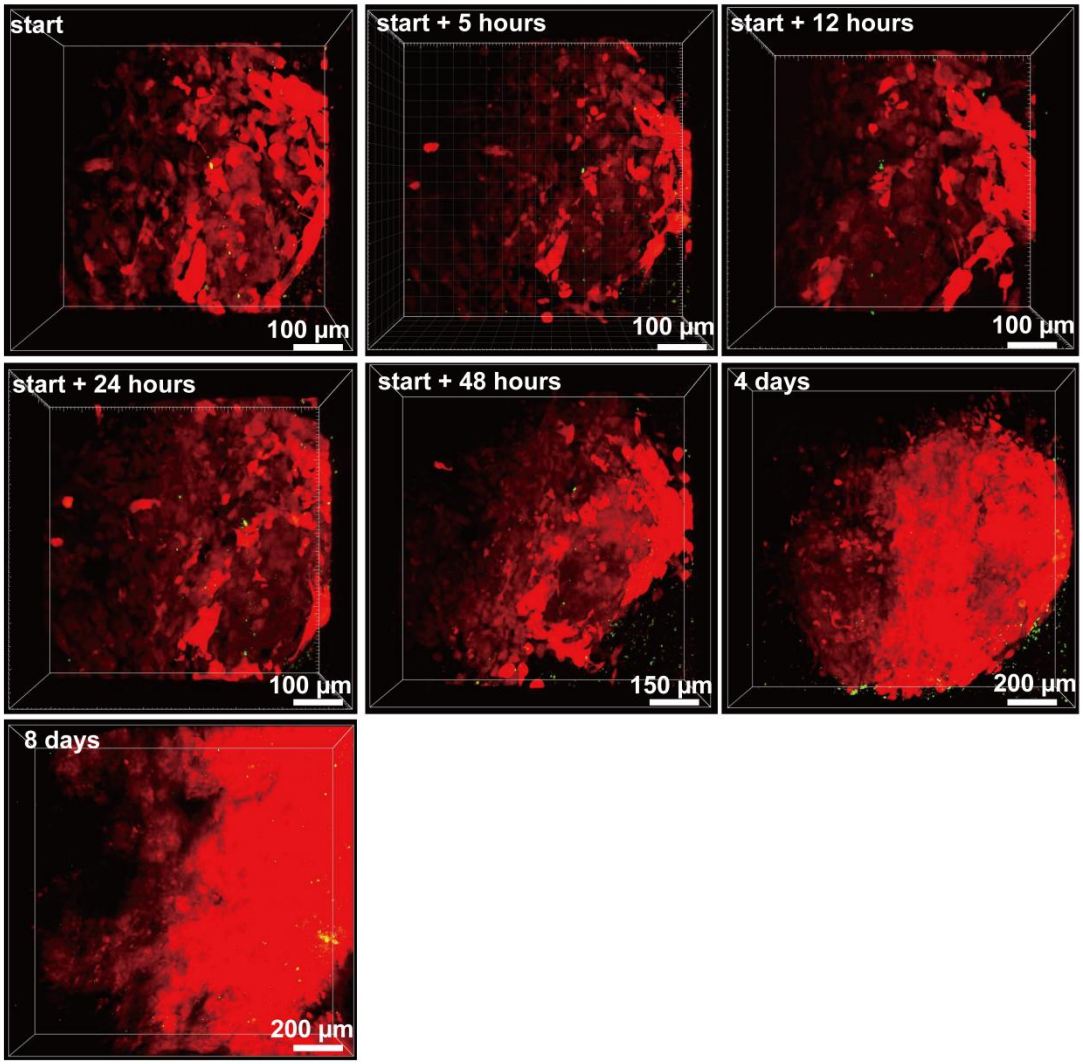
A



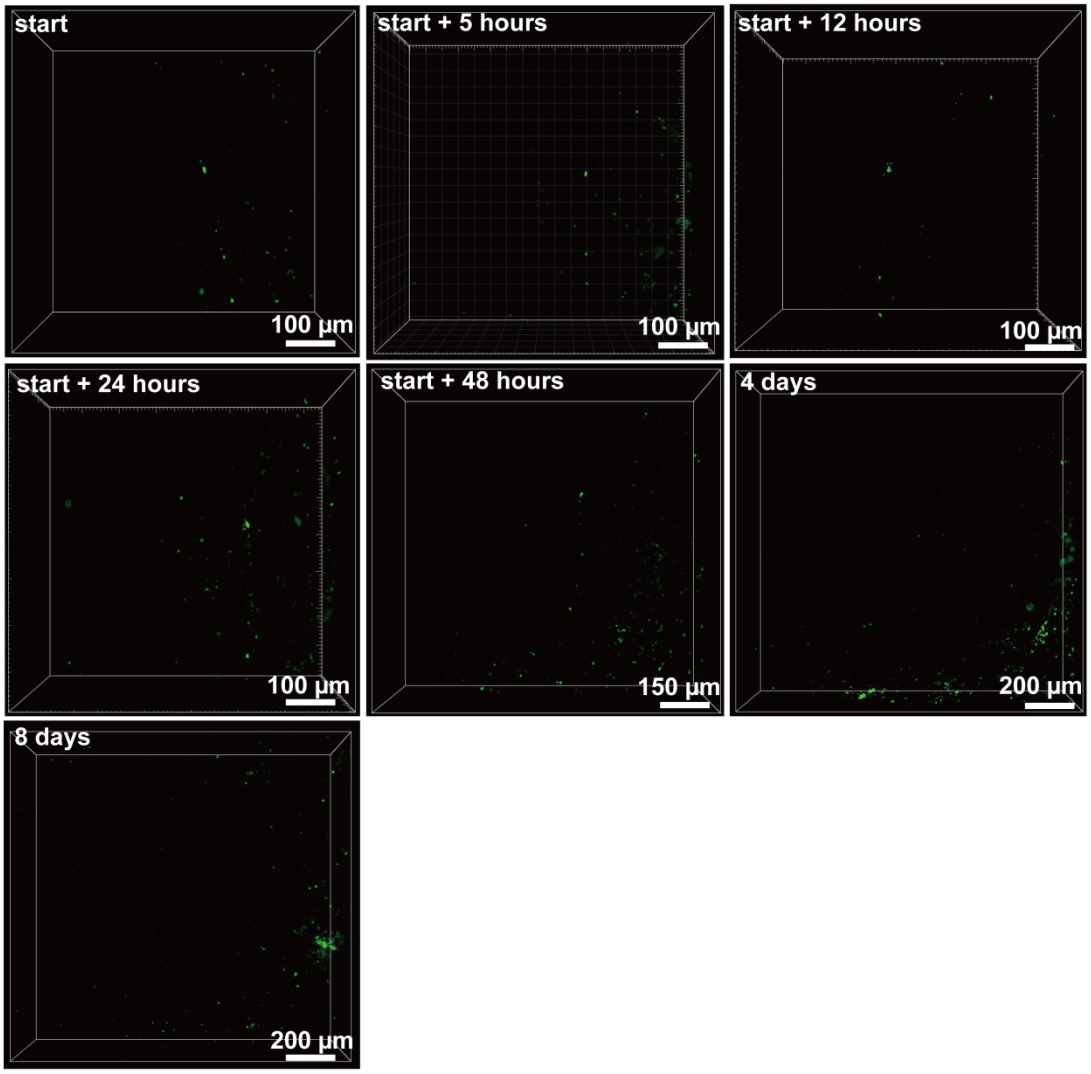
B



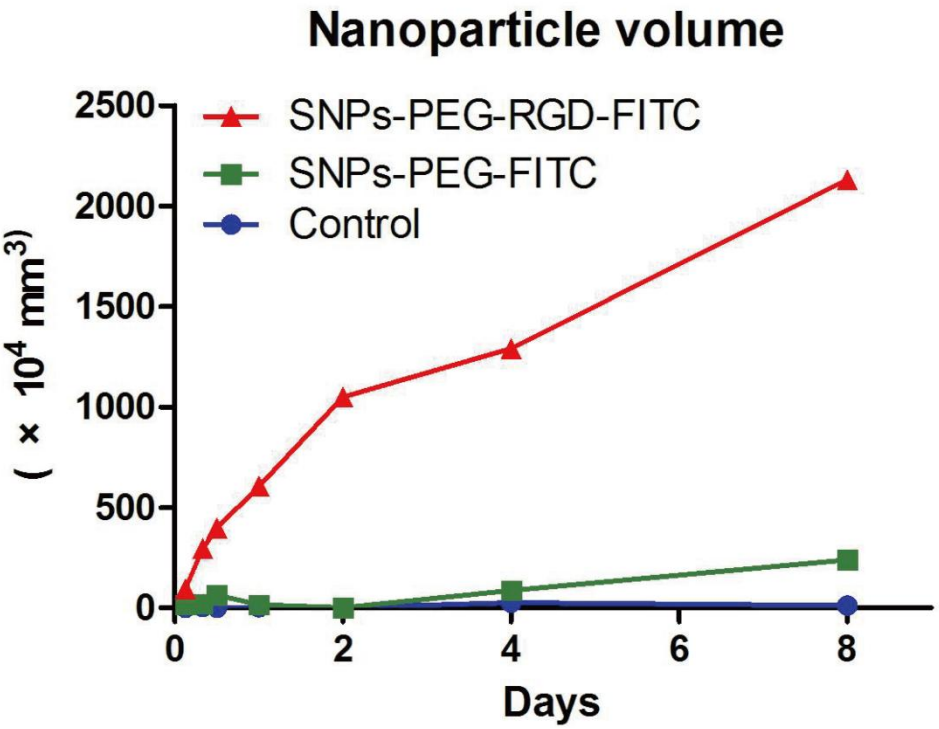
Supplementary Figure 4



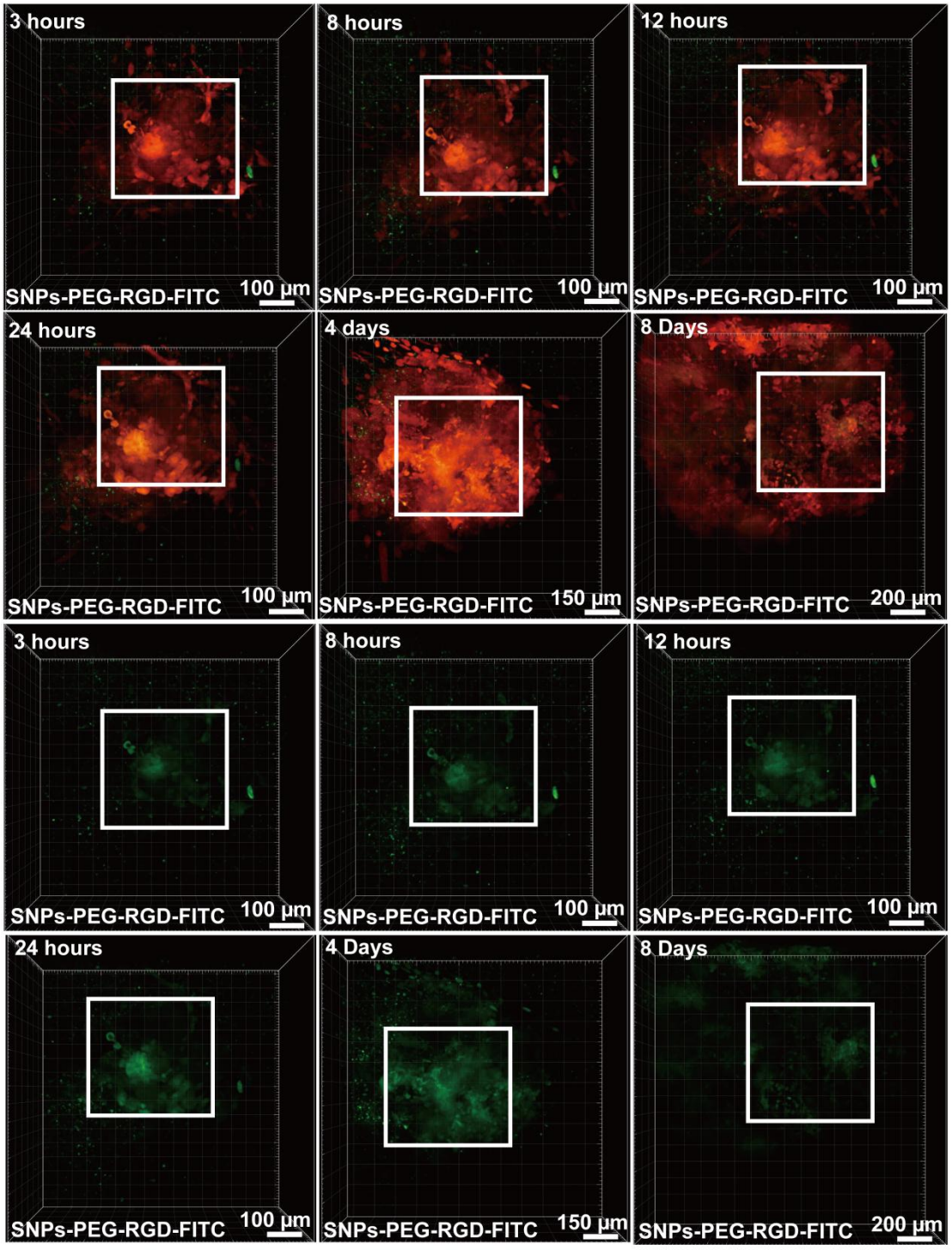
Supplementary Figure 5



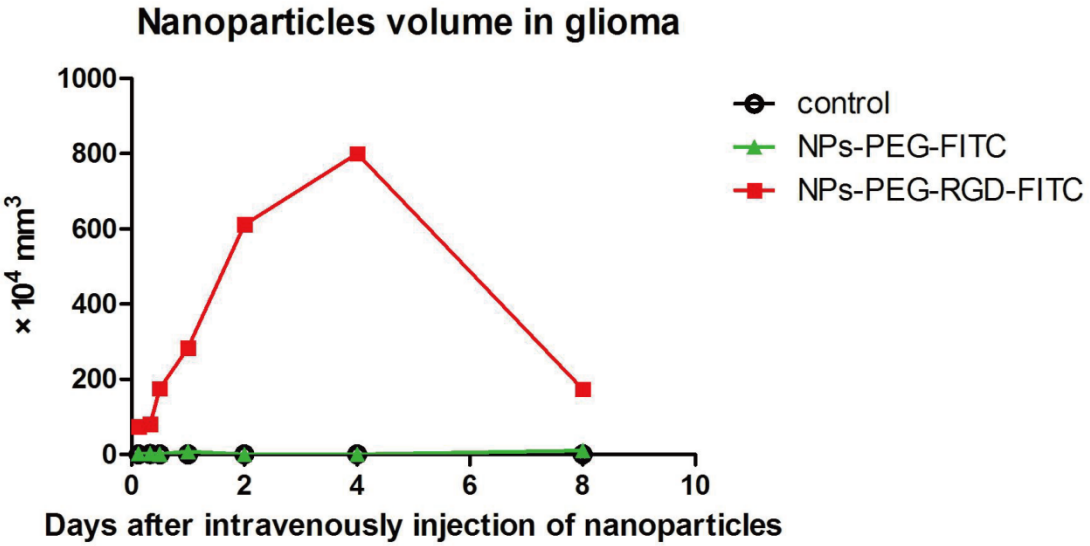
Supplementary Figure 6



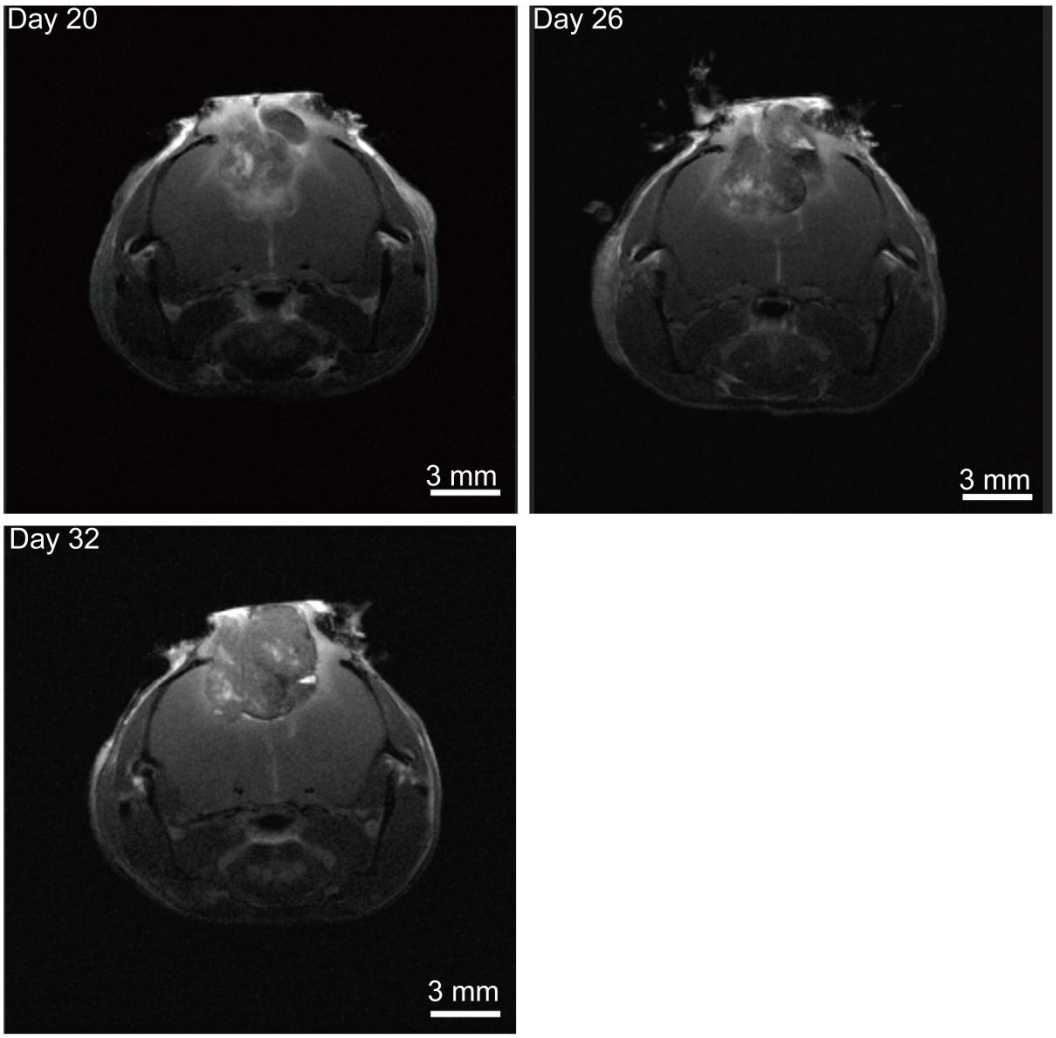
Supplementary Figure 7



Supplementary Figure 8



Supplementary Figure 9



Supplementary Figure 10