Electronic Supplementary Information

Plasmonics-enhanced and optically modulated delivery of gold nanostars into brain tumor

Hsiangkuo Yuan,[a†] Christy M. Wilson,[b†] Jun Xia,[c†] Sarah L. Doyle,[d†] Shuqin Li,[b] Andrew M Fales[a], Yang Liu,[e] Ema Ozaki,[d] Kelly Mulfaul,[d] Gabi Hanna,[f] Gregory M. Palmer,[f] Lihong V. Wang,[c] Gerald A. Grant,[b] Tuan Vo-Dinh[a,e]*

Experimental Section

*PEG-GNS synthesis***.** All chemicals were purchased from Sigma-Aldrich (St. Louis, MO) and used as received unless noted otherwise. Detailed surfactant-free GNS synthesis and characterization has been presented elsewhere.[1] Briefly, citrate gold seeds were prepared by adding 15 ml of 1% trisodium citrate to 100 ml of boiling $HAuCl_4$ (1 mM) under vigorous stirring for 15 minutes. GNS were prepared using a seed-mediated method by quickly mixing $AgNO_3$ (1 ml, 3 mM) and ascorbic acid (500 µl, 0.1 M) together into 100 ml of $HAuCl_4$ (0.25 mM) containing HCl (100 µl, 1M) and citrate gold seeds (1 ml, OD_{520}: 2.8) followed by filtration using 0.22 µm nitrocellulose membrane. PEG-GNS was prepared by adding final 2 µM of $SHPEG_{5000}$ (O-[2-(3-mercaptopropionylamino)ethyl]-O'-methylpolyethylene glycol, MW 5000) to freshly synthesized GNS for 10 minutes followed by two centrifugal washes. NPs were concentrated by centrifugation to desired concentration (1~5 pmole, 150 µl in PBS) before animal use. NPs were examined by TEM (Fei Tecnai G^2 Twin, 200 kV) and VIS-NIR spectroscopy (Shimadzu UV-3600; Shimadzu corporation, Japan). The NPs' hydrodynamic size and concentration were assessed by nanoparticle tracking analysis (NTA 2.3, Build 0025, NanoSight NS500; Nanosight Ltd. UK).

Cranial window implantation and orthotopic brain tumor animal model. The animal experiments were carried out in accordance with Duke University Institutional Animal Care and Use Committee guidelines. For cerebral microangiogram and photothermal experiment, a cranial window chamber was implanted. Female CD-1 nu/nu mice were anesthetized (ketamine/xylazine 100/10 mg kg^{-1} i.p. or isoflurane inhalation) and placed in a stereotaxic frame with their body temperature maintained at 37 °C. The scalp and underlying soft tissue over the parietal cortex were removed bilaterally. A drill was used to create a rectangular cranial window centered on the midsagittal suture that extended from the bregma to the lambdoid sutures. The dura mater was excised followed by implantation of human xenograft D270 cell suspension (10^8 cells ml^{-1}, 10 µl) in basement membrane matrix (BD Matrigel Matrix, BD Bioscience, Bedford, MA). Following implantation, a glass plate was glued to the bone surrounding the cranial window. For non-window tumor animals, mice were implanted with human D270-GBM cell line stereotactically through a small burr hole (2 mm right side the mid-sagittal suture, 2-3 mm back of the lambdoid suture, 5 mm below skull surface) 1~2 weeks before the nanoparticle injection. During the imaging, anesthetized mice were placed on a heating frame to maintain body temperature at 37 °C. For histology, mice were sacrificed with sodium phenobarbital (200 mg kg^{-1}, i.p.). Excised whole brains were placed in Hoeschet 33342 solution (Life Technology, Grand Island, NY) or snap frozen over liquid nitrogen and OCT compound. Cryosectioned samples (7 µm) were acetone fixed and stained with H&E (Sigma-Aldrich, St. Louis, MO) or CD-31 antibodies (BD Biosciences, San Jose, CA), fluorophore-labeled secondary antibodies (Life Technology, Grand Island, NY), and covered with a DAPI mounting medium (Vector Laboratories, Burlingame, CA) followed by imaging under MPM.

*Multiphoton microscopy imaging and irradiation***.** The MPM images were recorded using a commercial multiphoton microscope (Olympus FV1000; Olympus America, Center Valley, PA) with 3 detection channels (420-460 nm, 495-540 nm, 575-630 nm) on photomultiplier tubes (Hamamatsu, Bridgewater, NJ). A femtosecond Ti:Sapphire laser (Chameleon Vision II; Coherent, Santa Clara, CA) with tunable range 680-1080 nm, 140 fsec pulse width and 80 MHz repetition rate was used. The laser beam was focused through a 25× 1.05 NA water-immersion objective (XLPL25XWMP). Depth images were 3D-reconstructed using Fiji or Imaris (Bitplane, South Windsor, CT). Tile images were acquired via a motorized stage (ProScan III, Prior Scientific, Inc., Rockland, MA) and stitched using FluoView (FV10-ASW). For laser irradiation, anesthetized mice were kept on a 37 °C heating stage and exposed to 800

nm pulsed laser irradiation (35 mW) on the same MPM. The laser power was measured with a thermopile power meter (Newport, Irvine, CA). The treatment was performed by depth scanning the area (spot size 500×500 μm^2, 10 μs $pixel^{-1}$, 5 μm per slice).

*Photoacoustic computer tomography***.** The procedures were approved by the Washington University at St. Louis Institutional Animal Care and Use Committee. The photoacoustic computer tomography (PACT) experiment was performed on a 512-element full-ring ultrasonic transducer array.[2] The central frequency of the array was 5 MHz and the bandwidth was more than 80%. A Ti:Sapphire laser (10 ns pulse) 800 nm 15 mJ cm^{-2} was used. Detailed description of the system can be found elsewhere.[3] Anesthetized mouse (Swiss Webster, 3-4 months old) was depilated and secured to the PACT system. The cortex surface was positioned flat and lined up with the imaging plane. Following the PEG-GNS injection, continuous monitoring was performed over 2.5 hours. Signal was normalized to the signal of the 1st frame. Image series were taken at 1.6 s per frame for the initial 600 seconds followed by 60 s per frame until 10000 seconds.

Inflammasome activation study. Bone marrow derived Macrophages (BMDMs) were isolated and cultured according to standard procedures. Briefly, bone marrow was isolated from the femurs of C57/Bl6 mice and cultured with conditioned DMEM containing 10% FCS and Macrophage colony stimulating factor for 6 days before treatment. Cells were seeded onto 96 well plates and rested overnight. Cells were primed with LPS (100 ng ml^{-1}) for 3 hours and treated with nanoparticles for a further 24 h. Supernatants were harvested after which and MTS assay (Promega) was used to examine cell viability. ELISA was used to quantify cytokines in the supernatants from the various experimental groups used throughout this study. IL-1β (R&D Systems), IL-6 (R&D Systems), and TNFα (R&D Systems) were analyzed throughout. All ELISAs were conducted a minimum of three times in triplicate.

1. H. Yuan, C. G. Khoury, C. M. Wilson, G. A. Grant and T. Vo-Dinh, *Nanotechnology*, 2012, **23**, 075102.
2. J. Xia, M. R. Chatni, K. Maslov, Z. Guo, K. Wang, M. Anastasio and L. V. Wang, *J Biomed Opt*, 2012, **17**, 050506.
3. J. Yao, J. Xia, K. I. Maslov, M. Nasiriavanaki, V. Tsytsarev, A. V. Demchenko and L. V. Wang, *NeuroImage*, 2013, **64**, 257-266.

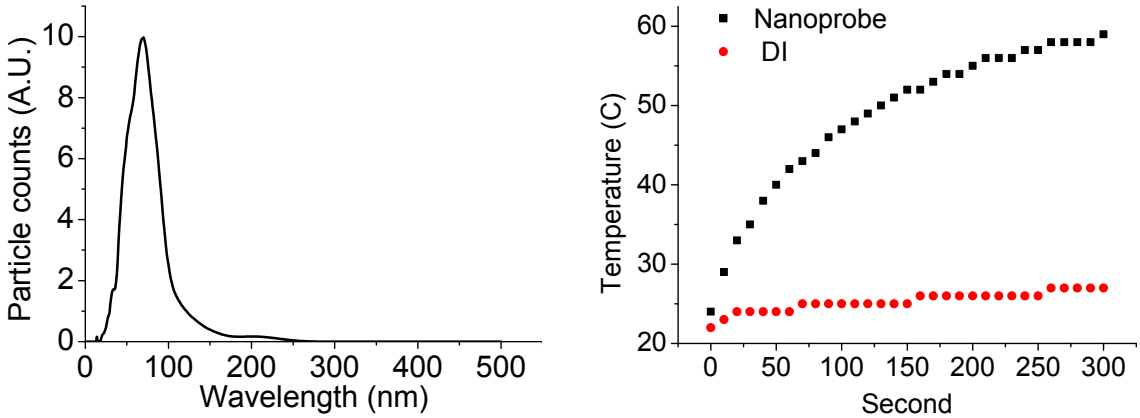

Figure S1. (Left) Hydrodynamic size measured by nanoparticle tracking analysis (NTA) found a mode diameter of 80 nm. (Right) Photothermal response of 0.1 nM nanoprobes and DI irradiated by 785 nm laser 490 mW.

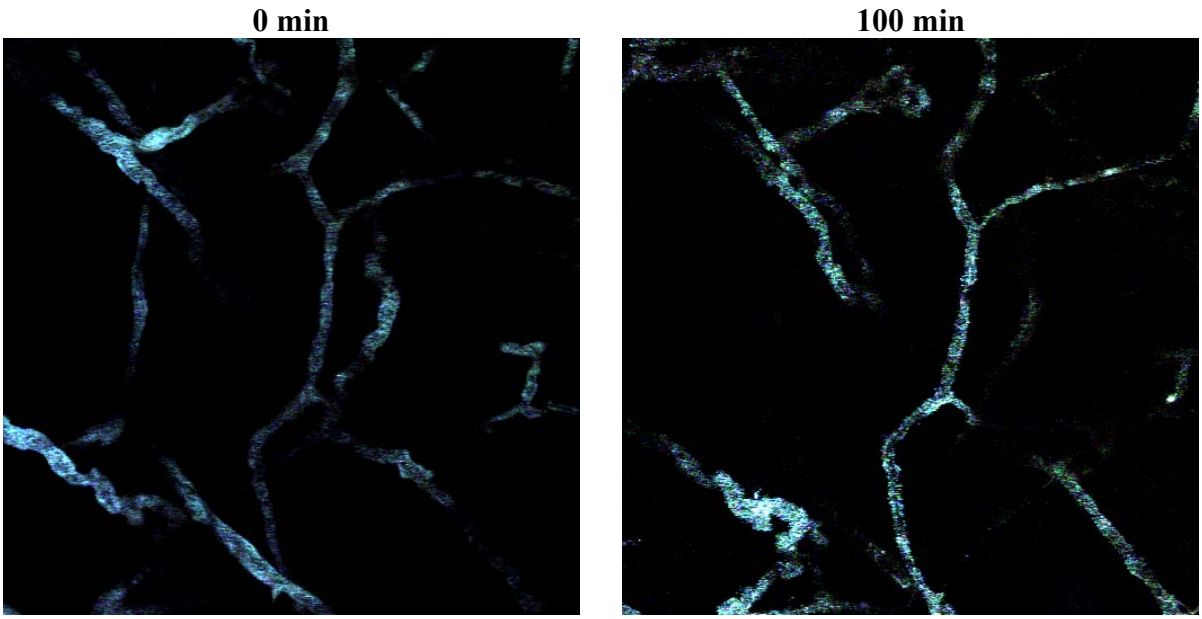

Figure S2. MPM images showing similar intravascular PEG-GNS intensity at 0 min and 100 min following PEG-GNS (1 pmole) tail vein injection. Size: 0.5x0.5 mm^2. 800 nm, transmission 0.6%.

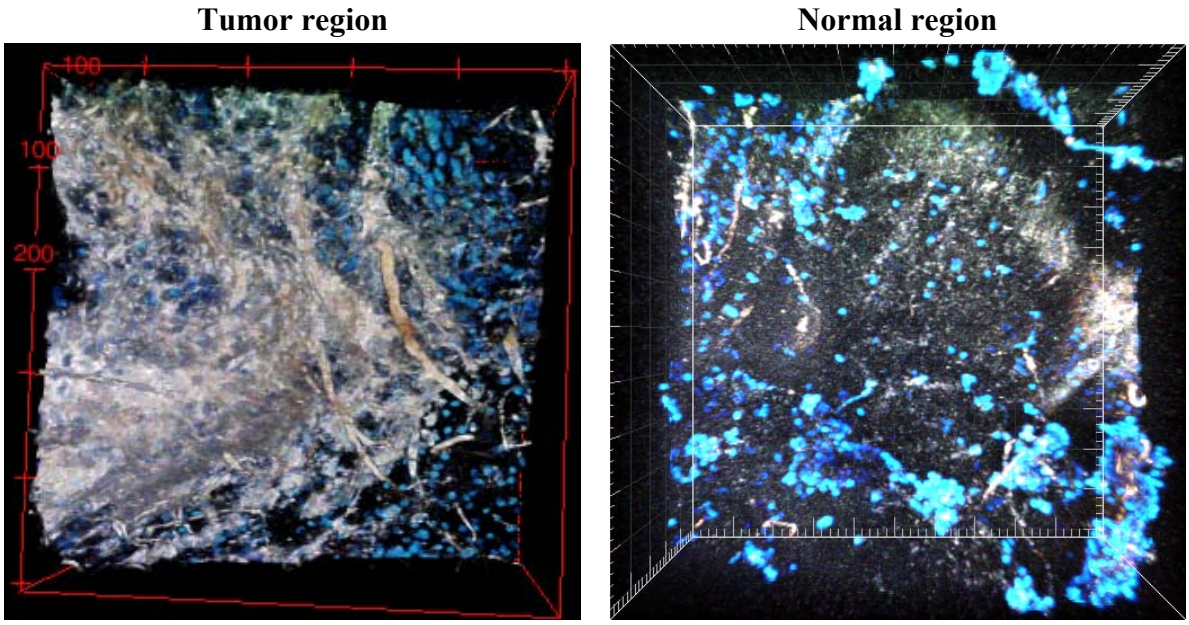

Figure S3. MPM images of excised whole brain with orthotopic tumor (post-injection 3h). Hoescht 33342-stained nuclei are blue. Extensive PEG-GNS (white pattern) distribution can be seen in the tumor region but much less in the normal region. Size: 0.5x0.5 mm^2.

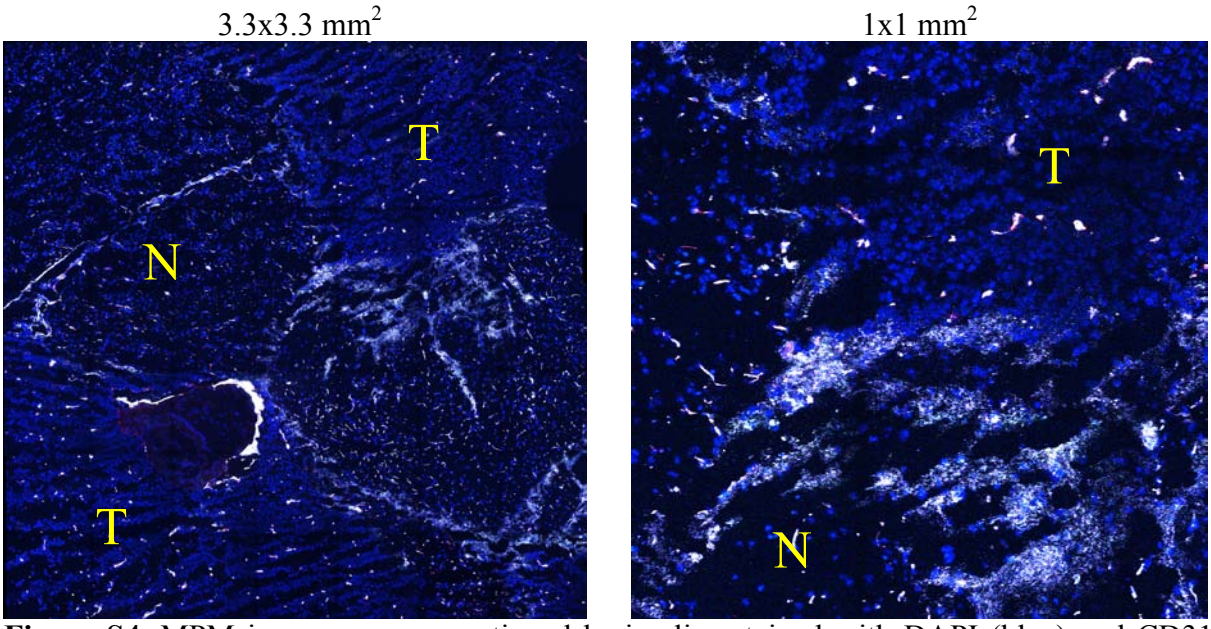

Figure S4. MPM images on cryosectioned brain slice stained with DAPI (blue) and CD31 (red) from a profused tumor mouse post 48 hours PEG-GNS injection (1 pmole) . (Left) Tile image (3.3x3.3 mm^2) showing PEG-GNS (white) accumulation at tumor periphery and in blood vessels (small white/red punctates). (Right) Zoom-in image (1x1 mm^2). T: tumor. N: normal.

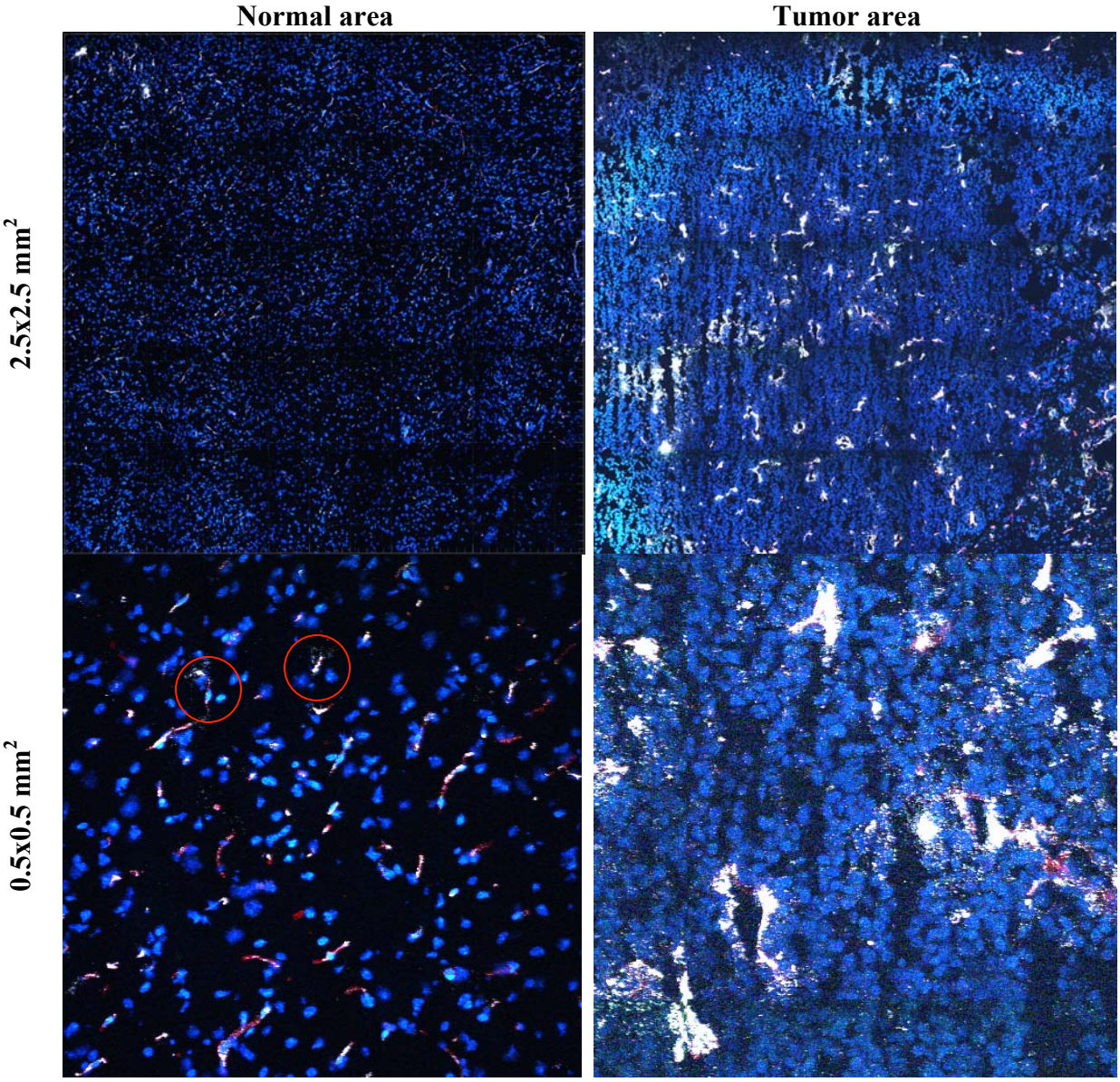

Figure S5. MPM images of DAPI and CD31-stained cyrosectioned brain slices from a perfused tumor mouse post-48hr PEG-GNS injection (5 pmole). In normal brain, PEG-GNS was found mostly in the endothelial cells (white colocalizes with red) with minute extravasation in the larger vessels (red circles). In tumor brain, PEG-GNS can be seen not only in the endothelial cells but also in the perivascular spaces. High concentration PEG-GNS may saturate the RES clearance hence accumulating more through the leaky brain tumor vasculature.

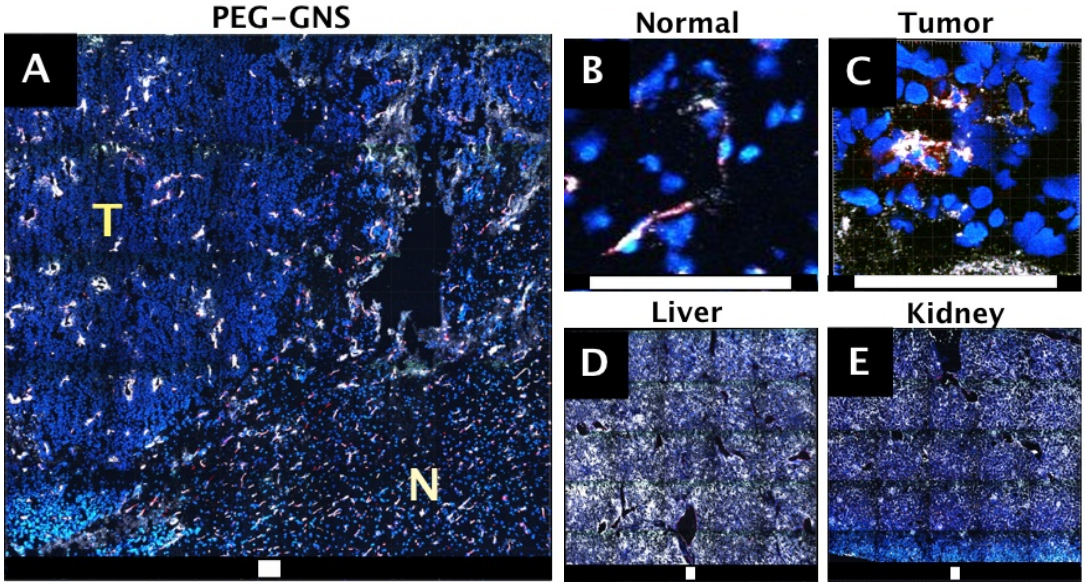

Figure S6. MPM images of DAPI (blue) and CD31 (red)-stained cryosectioned specimens from perfused brain excised 48-hr after neutral PEG-GNS (A-E) injection (5 pmole). Large-area tile images showing distribution of PEG-GNS (A) preferentially in the brain tumor. T: tumor. N: normal. Zoom-in images of normal areas (B) and tumor areas (C) showing increased extravastion in the tumor regions. Tile images showing accumulation of PEG-GNS in liver (D) and kidney (E). Scale bar: 100 μm.

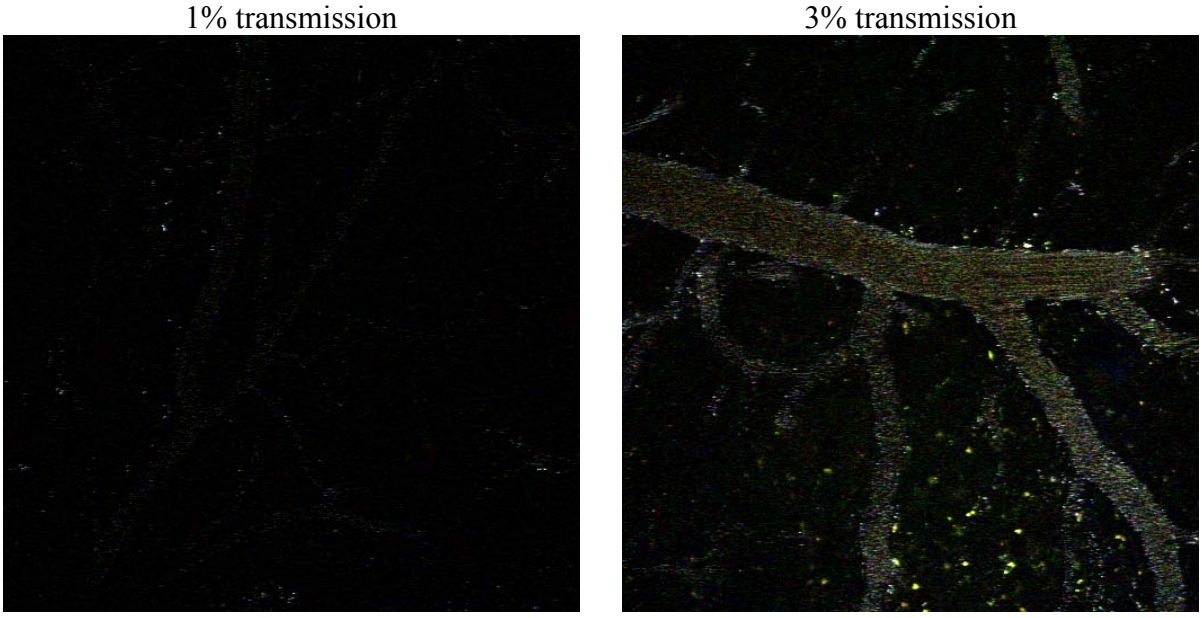

Figure S7. MPM images of normal brain side under two different transmission settings 2 days after photothermal treatment through a cranial window on live mouse. Under the same power setting as the tumor side (1% transmission power), only very faint signal can be seen. 3% transmission power revealed very little PEG-GNS (white) on the vascular wall. This suggestion very minimal intravascular or endothelial retention from low dose PEG-GNS injection. Image size 500×500 μm^2.

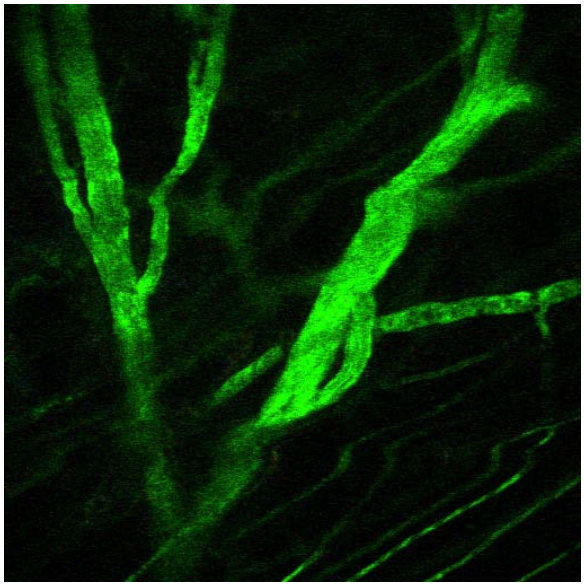

Figure S8. MPM image of the brain vessels in a tumor mouse (no PEG-GNS injection) immediately after the laser irradiation. FITC-dextran (250 kDa, 10mg/kg) was injected prior to the laser treatment to visualize the blood vessels. Image size 500×500 μm^2.

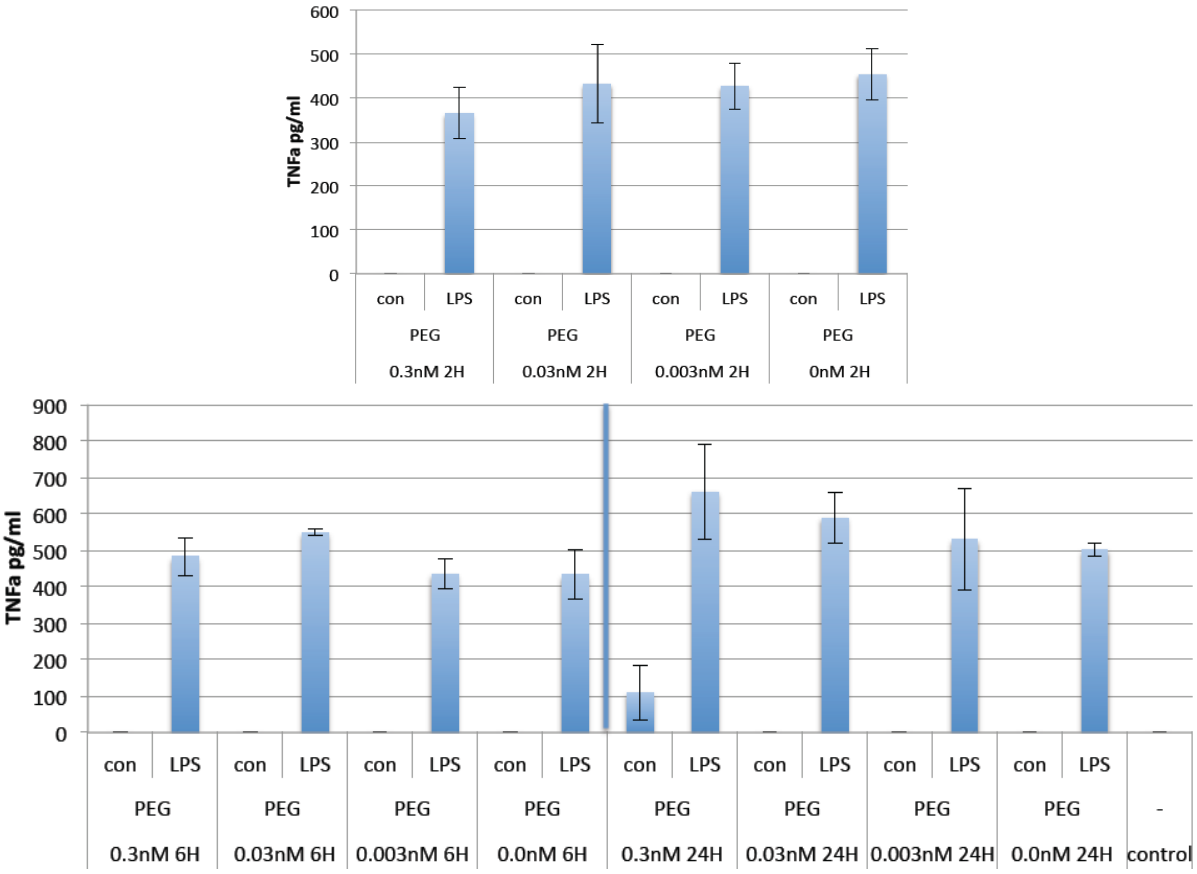

Figure S9. Induction of TNFα at 3 different time points under 3 different PEG-GNS concentrations. With primed macrophages, TNFα level were induced in a concentration dependent fashion at 24h incubation. At 2h and 6h, the difference was not apparent.