Supplementary Material

Three-Photon Luminescence of Gold Nanorods and Its Applications for High Contrast Tissue and Deep *In Vivo* Brain Imaging

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Table S1. Growth conditions for GNRs with different longitudinal LSPR peaks.

LSPR (nm)	$AgNO_3$ (ml)	HCl (ml)	Seed (ml)
840	12	1.1	0.2
900	12	1.5	0.4
1000	12	2.4	0.4
1036	12	2.6	0.4
1060	16	2.7	0.4
1100	18	2.7	0.4

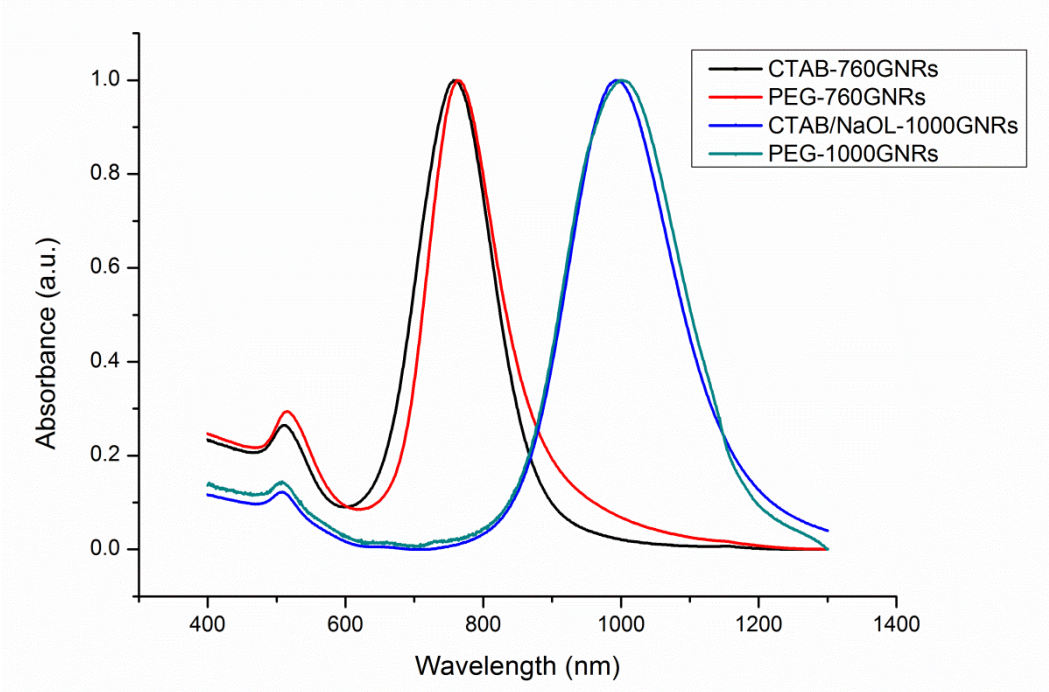

Figure S1. UV-Vis-NIR absorption spectra of GNRs before and after PEG modification.

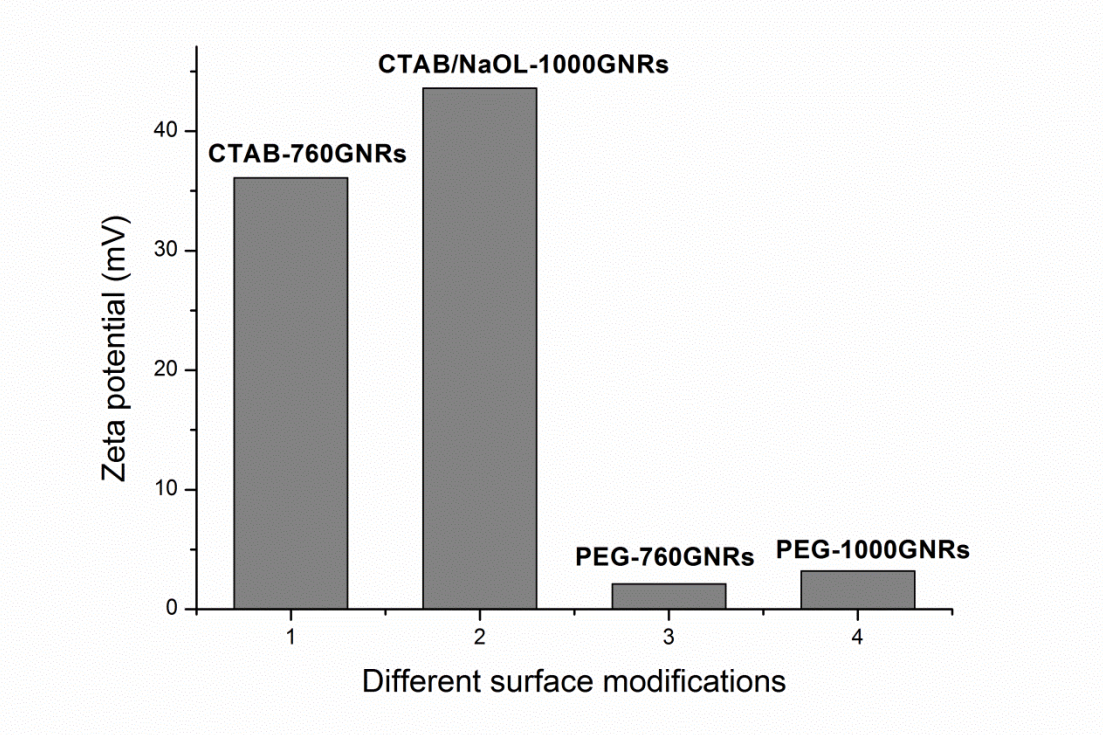

Figure S2. Zeta potentials of GNRs with different surface modifications. CTAB-GNRs and CTAB/NaOL-GNRs showed positive charge. After the PEG modification, their zeta-potentials were nearly neutral, which confirmed successful displacement of CTAB/NaOL molecules by PEG polymer.

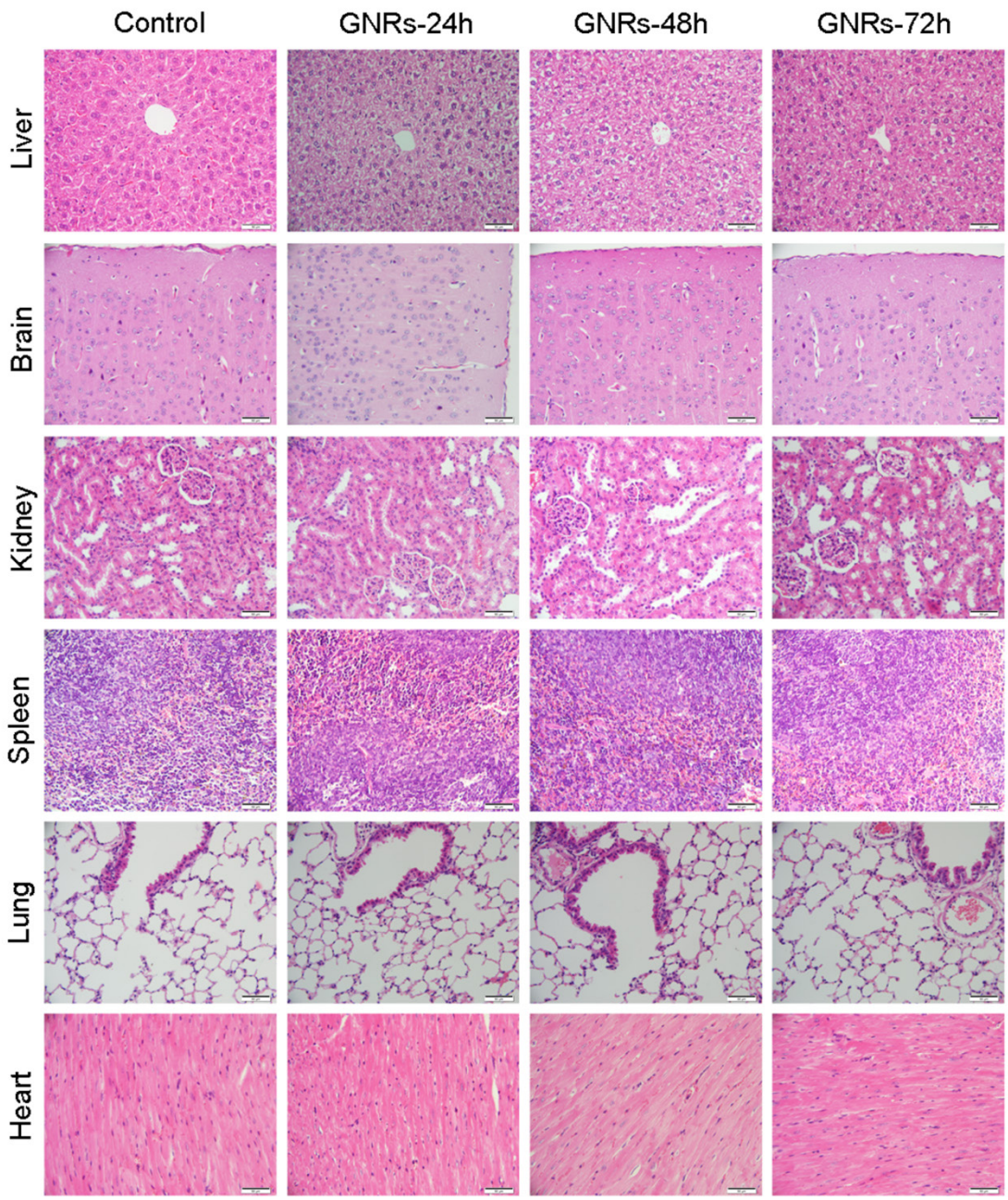

Figure S3. Histological examination of liver, brain, kidney, spleen, lung and heart stained with haematoxylin and eosin. All tissues were excised from BALB/c mice euthanized 24 h, 48 h and 72 h after the administration of 50 nM of PEG-GNRs (200 μl in 1 × PBS). The control mice were treated with 200 μl 1 × PBS. Scale bars: 50 μm.

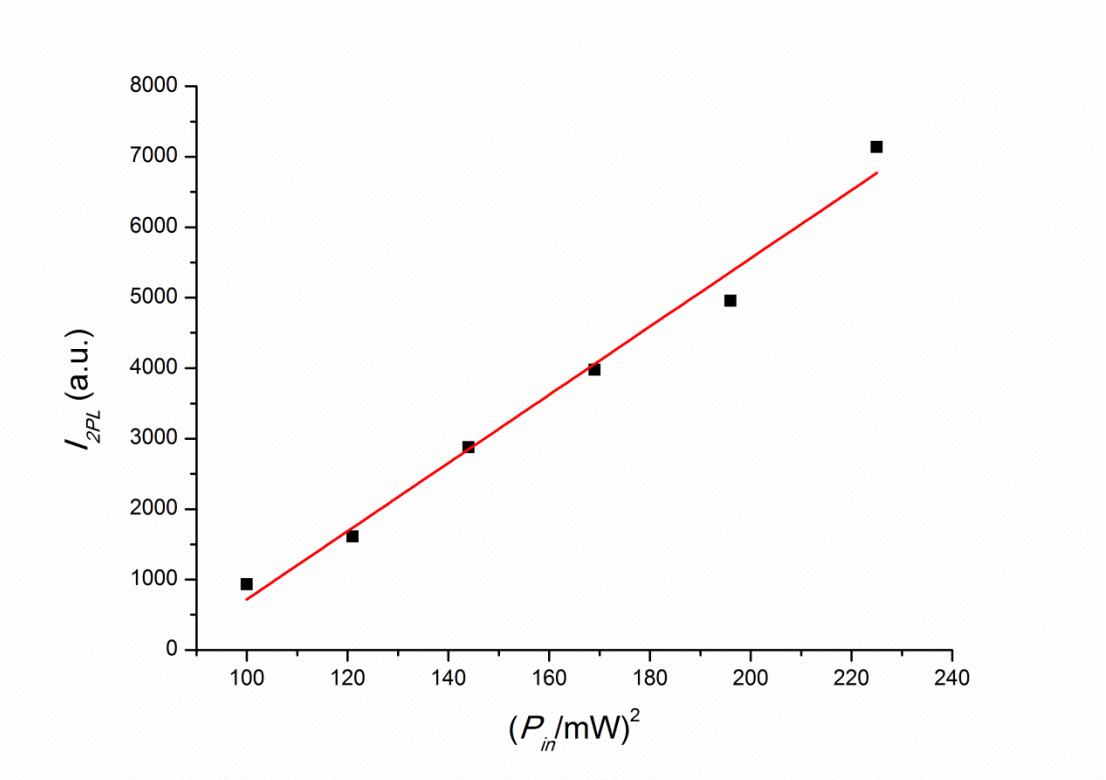

Figure S4. Square dependence of 2PL from 760GNRs on the excitation intensity of the 760 nm fs laser.

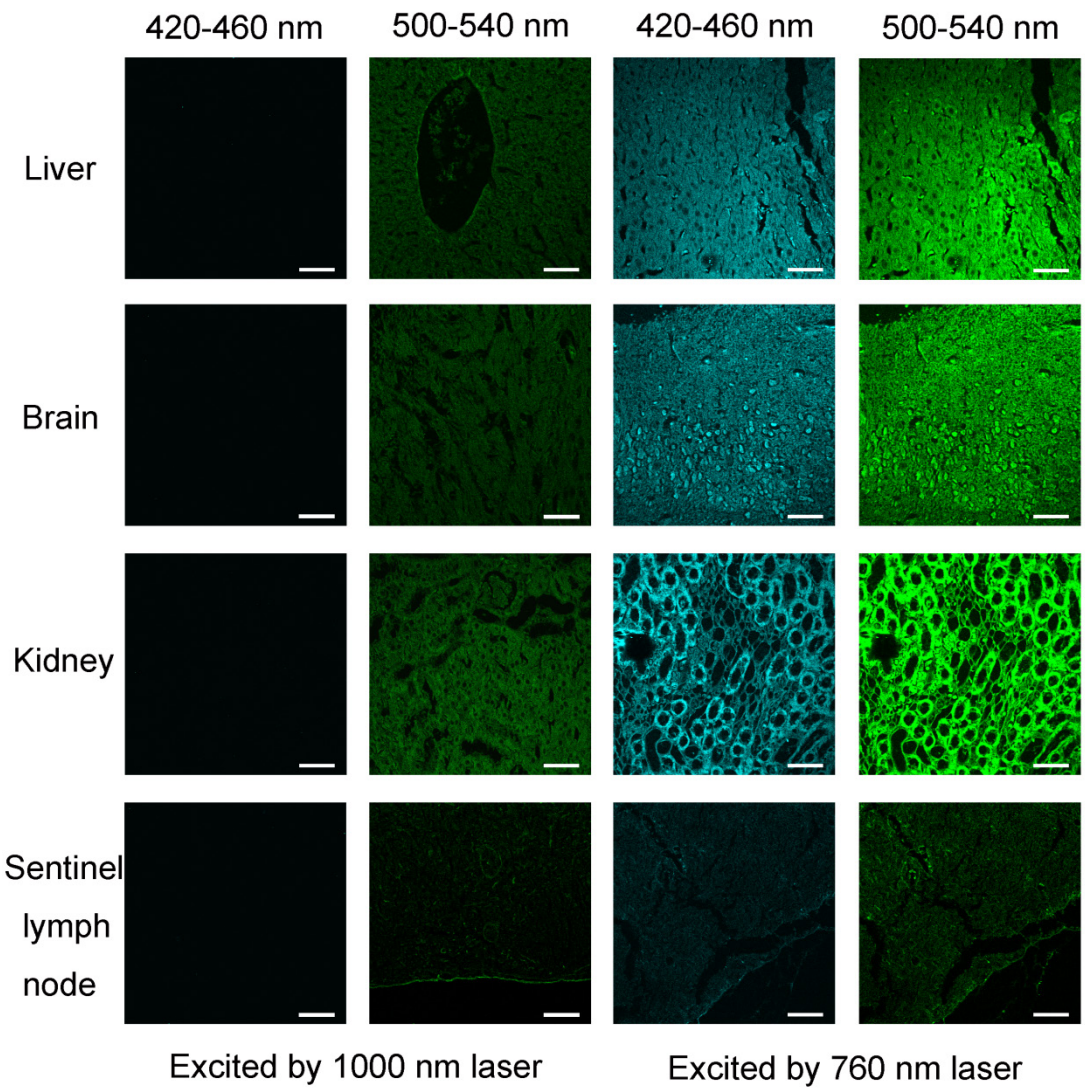

Figure S5. MPL images of tissue slices harvested from mice injected with saline.

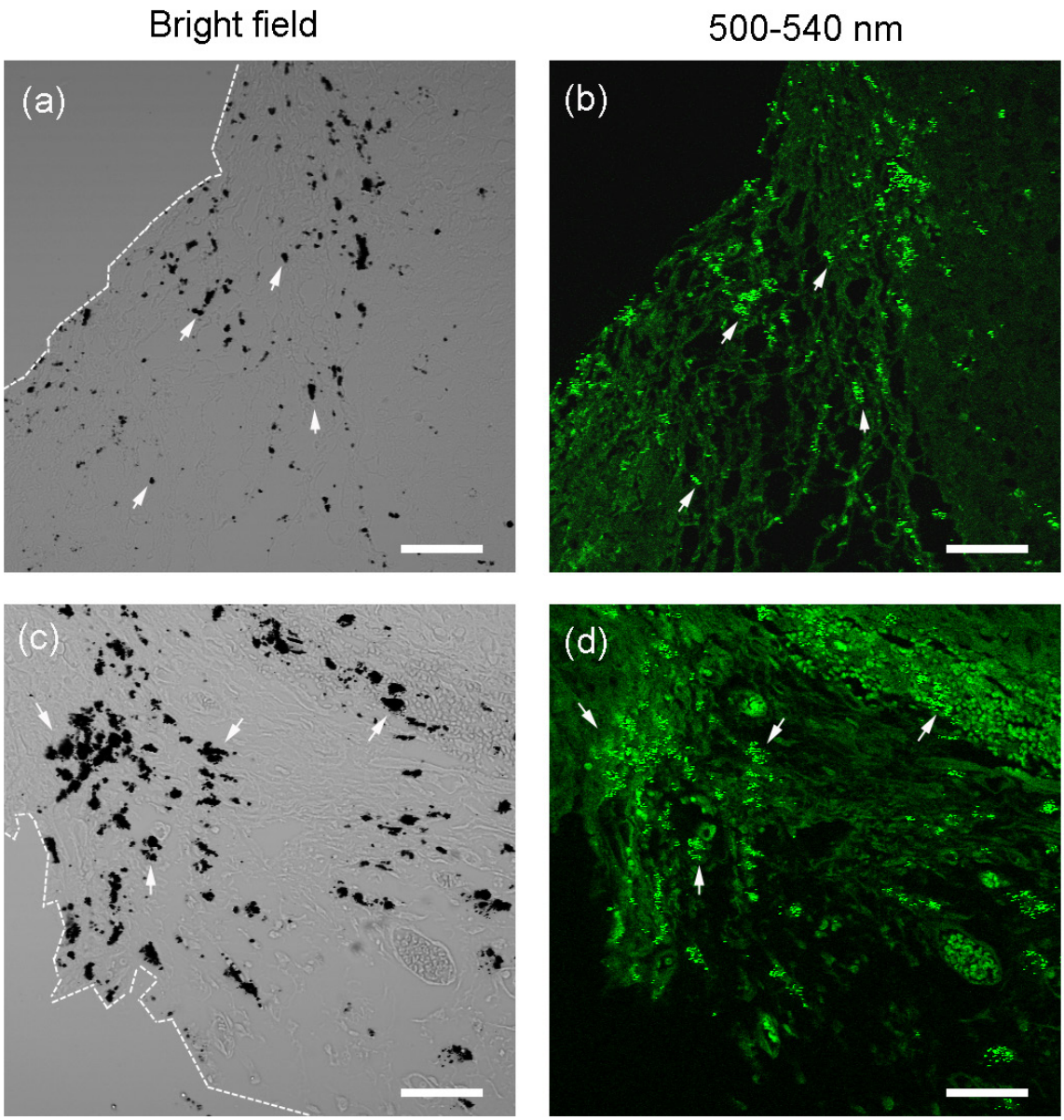

Figure S6. Bright field and 2PL images of tumor tissue slices harvested from mice injected with PEG-1000GNRs, PEG-760GNRs. (a), (b), (c), (d) correspond to Figure 5a, 5c, 5e and 5g in the main text. The white dotted lines indicate the boundaries of the tumor tissue. The white arrows indicate the black dots in the bright field images, which are correlated with the bright dots in 2PL images. Scale bars: 50 μm.

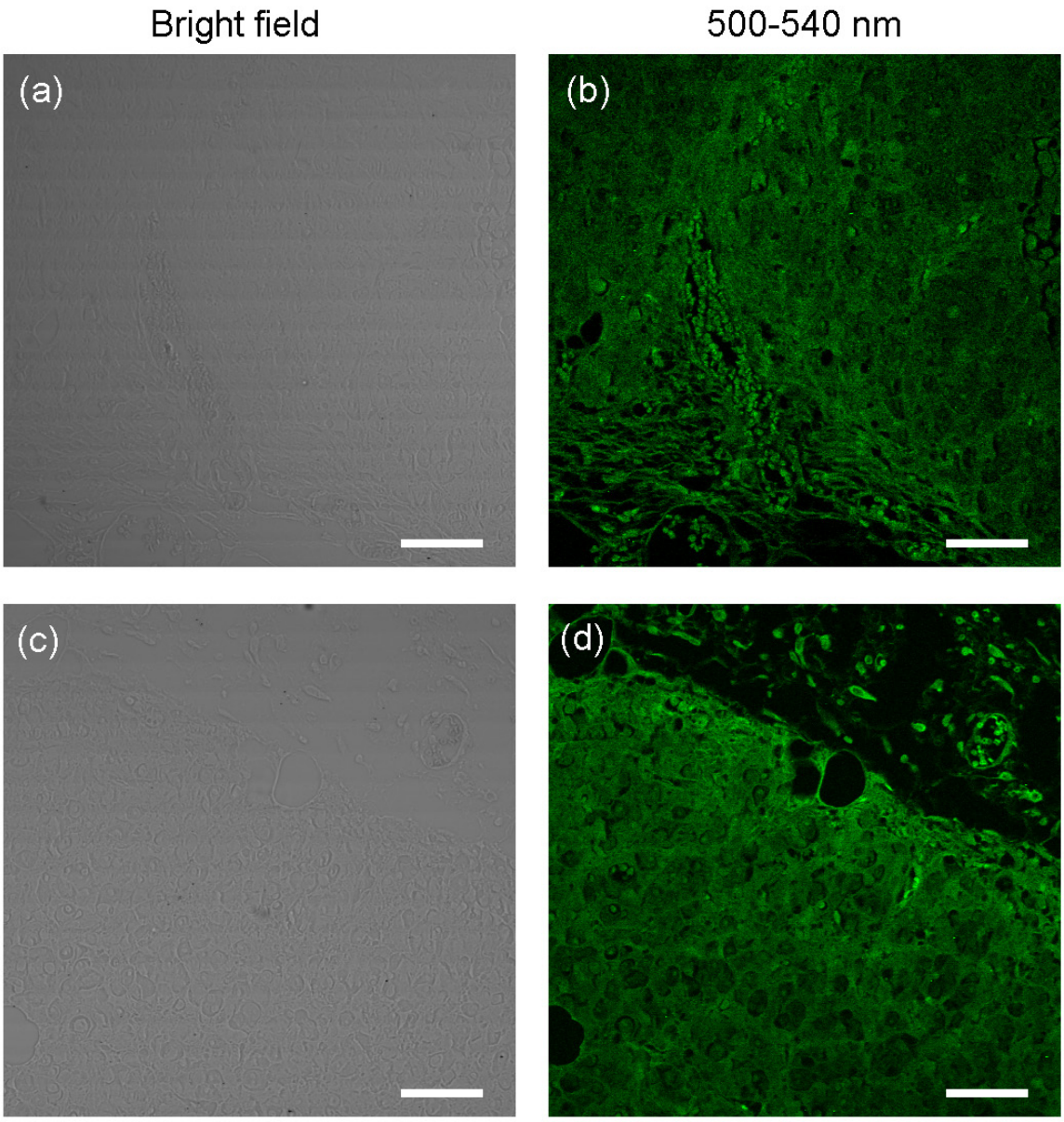

Figure S7. Bright field and 2PL images of tumor tissue slices harvested from mice injected with saline. (a), (b), (c), (d) correspond to Figure 5i, 5k, 5m and 5o in the main text. Scale bars: 50 μm.

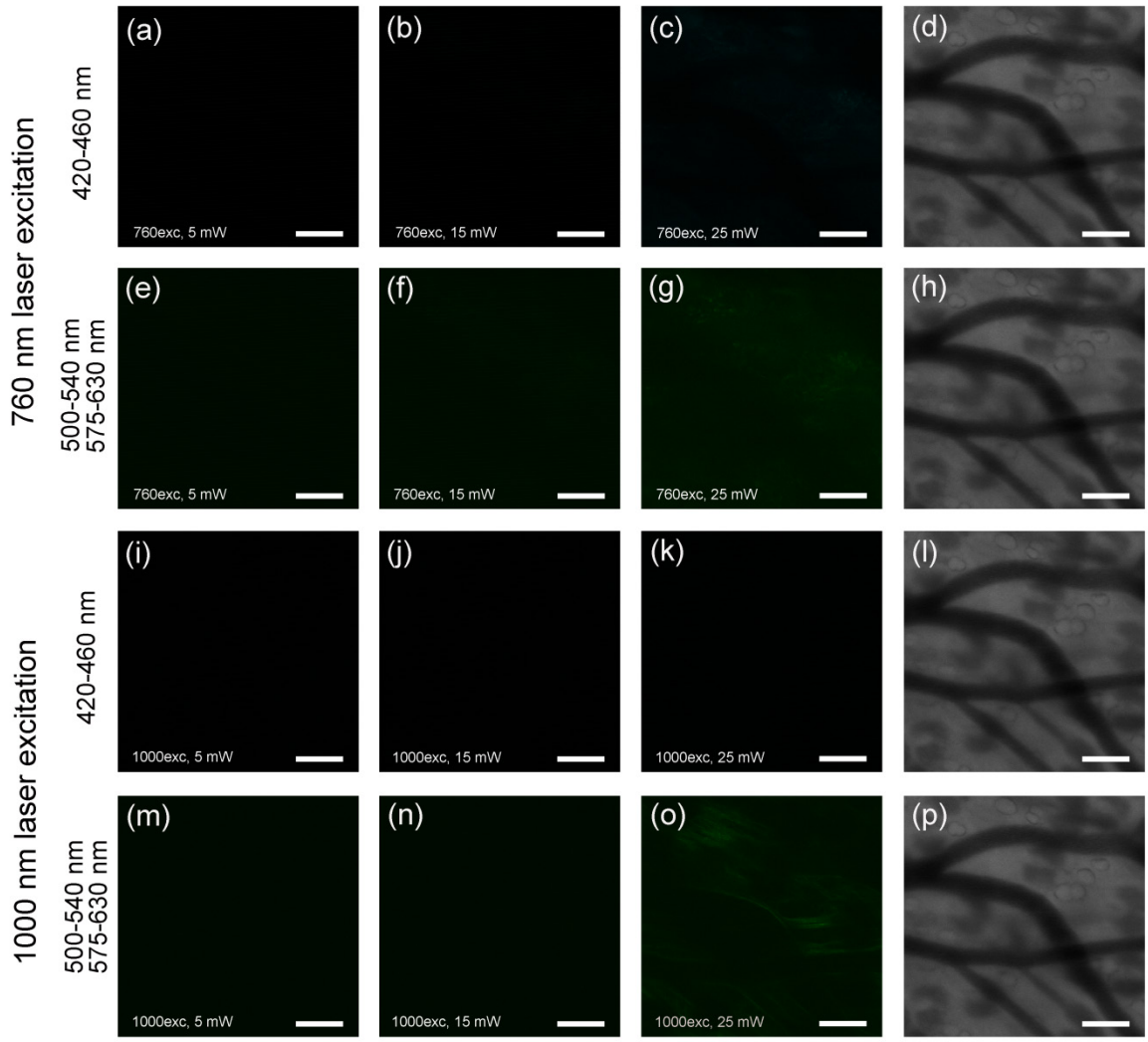

Figure S8. Intravital MPL images of ear blood vessels of mice, which were treated with saline. The bright field images in the right column indicate the ear blood vessels, and the same areas were excited by 760 nm and 1000 nm fs lasers with different powers. The autofluorescence from ear blood vessels was hardly observed even when the fs excitation power was very high (25 mW). Scale bars: 100 μm.

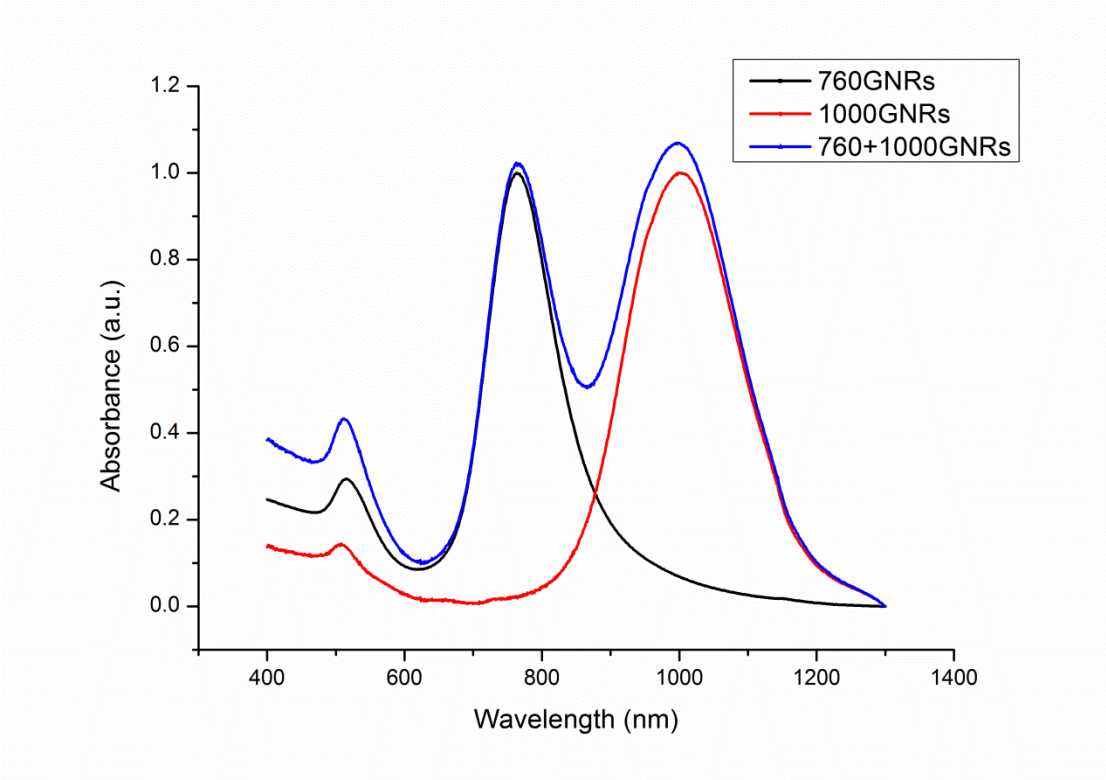

Figure S9. Normalized absorption spectra of PEG-1000GNRs, PEG-760GNRs and their mixture.

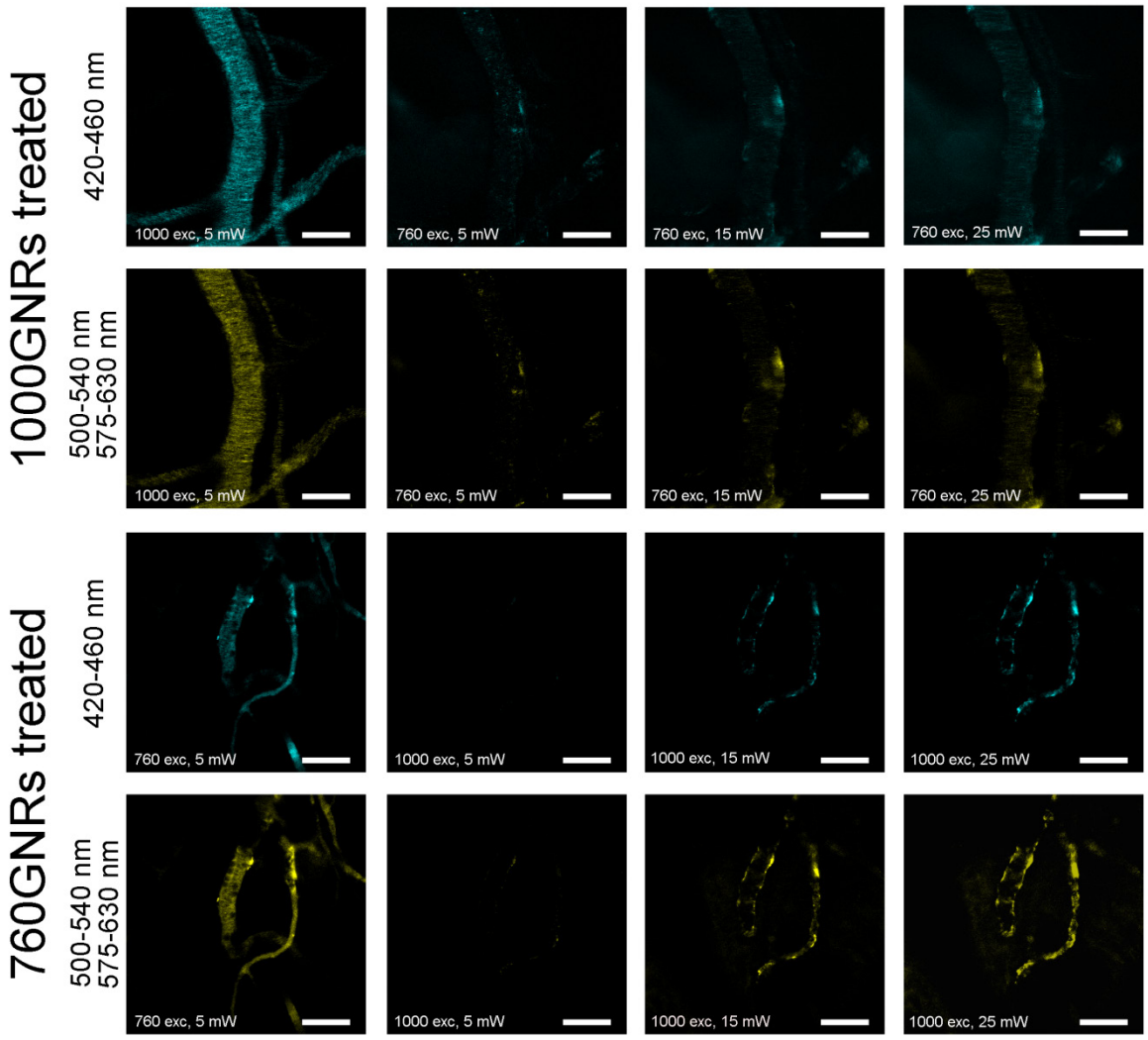

Figure S10. Intravital MPL imaging of PEG-1000GNR-stained and PEG-760GNRs-stained mice ear blood vessels, excited by 1000 nm or 760 nm fs lasers. Bright MPL of GNRs in mice ear blood vessels *in vivo* can be excited by a fs laser at a low power when its wavelength is coupled with the longitudinal LSPR band of the GNRs. However, higher power is required to excite MPL of the GNRs when fs excitation wavelength is far away from their absorbance band. Scale bars: 100 μm.

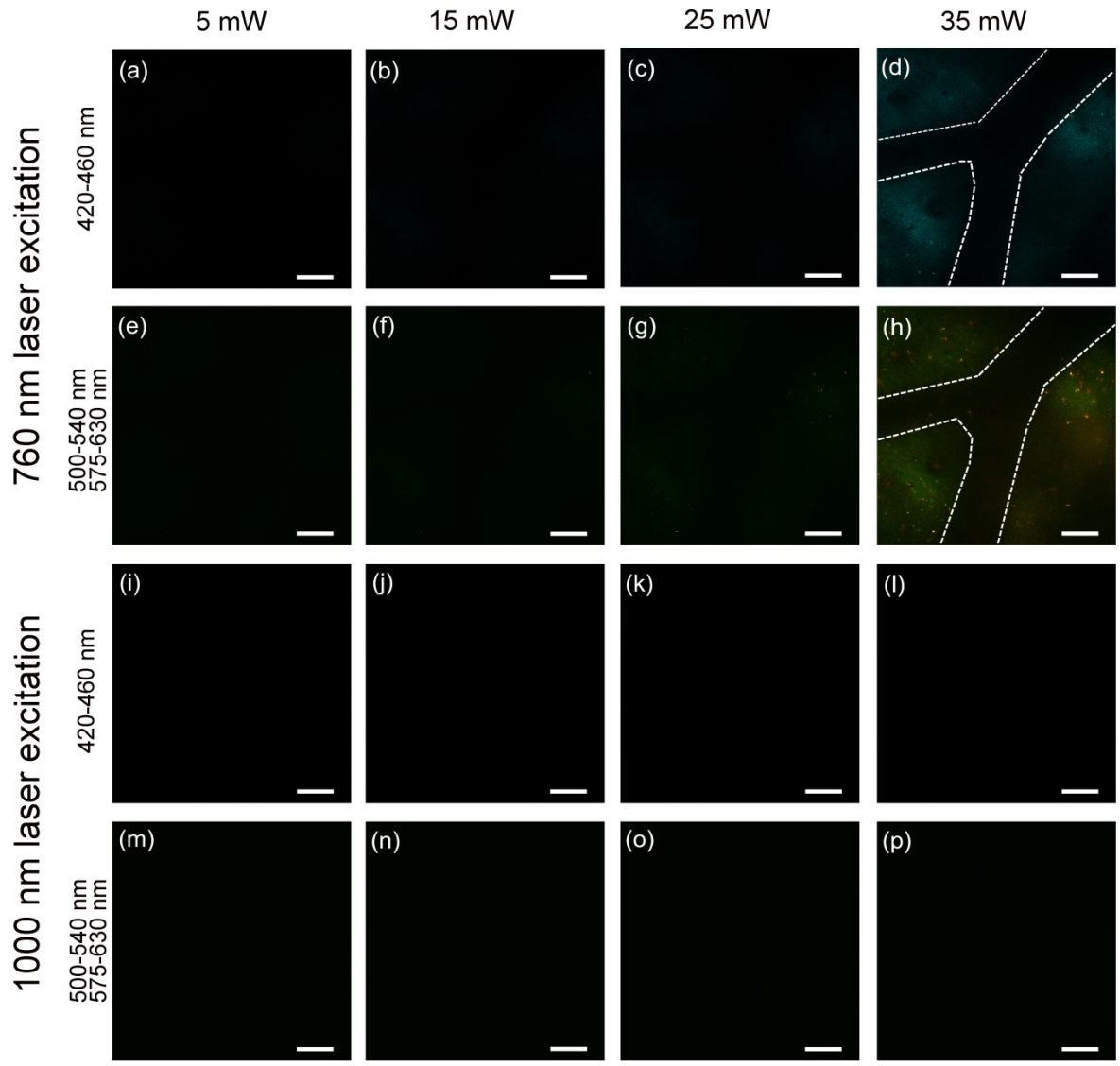

Figure S11. Intravital MPL images of brain blood vessels of mice, which were treated with saline. The dotted lines in (d) and (h) indicate the contour of blood vessels and the same areas were excited by 760 nm and 1000 nm fs lasers with different powers. The autofluorescence from brain blood vessels was hardly observed even when the fs excitation power was very high (35 mW), and only autofluorescence from tissue nearby the vessels could be observed. Scale bars: 100 μm.

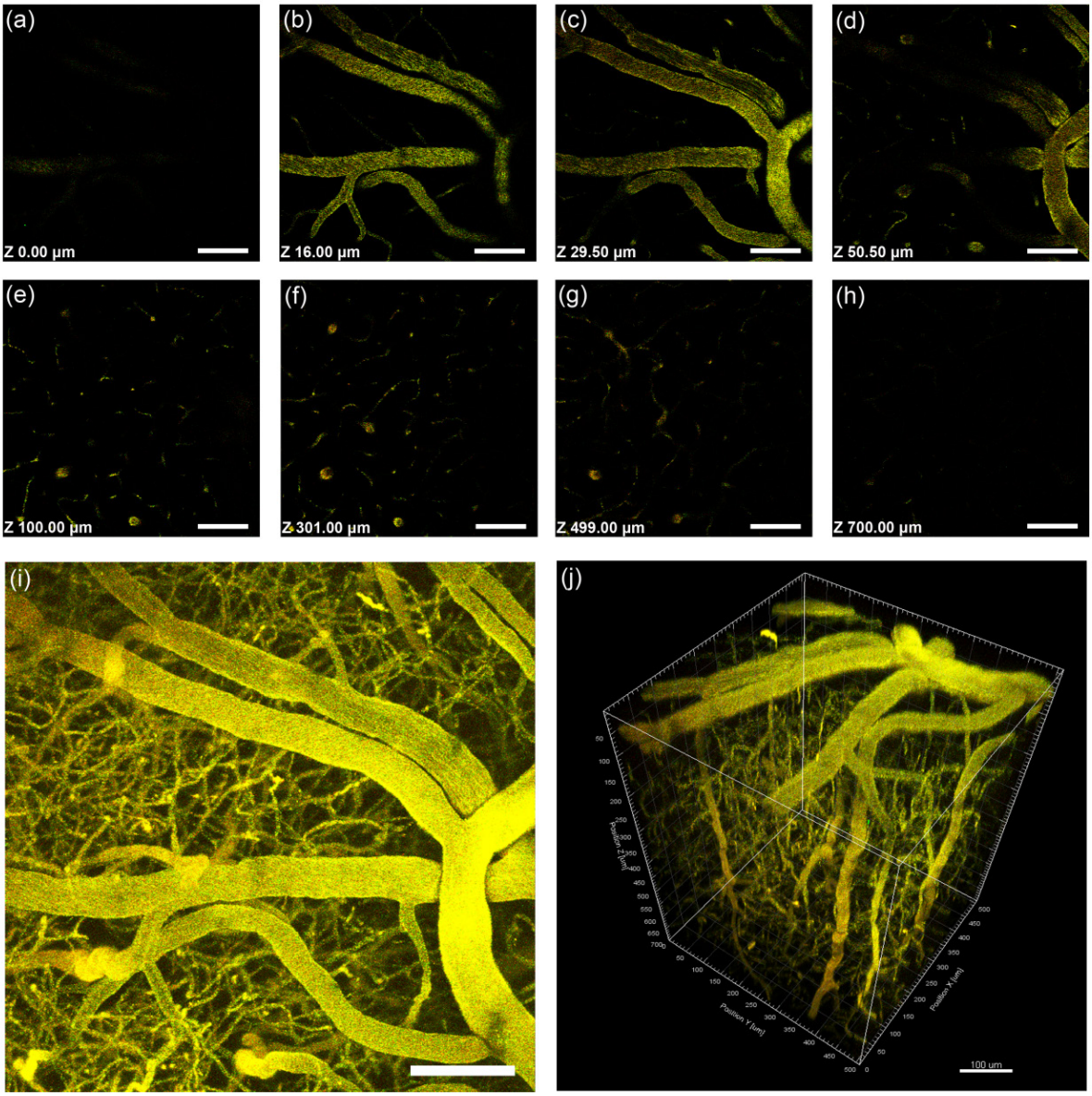

Figure S12. Intravital 2PL images of PEG-1000GNRs-stained mouse brain blood vessels. (a)-(h) Images at various vertical depths of mouse brain. (i) Stacked 2PL images from a depth of 0 µm to 700 µm. (j) 3D reconstructed image showing blood vessels in mouse brain. λ_{ex} = 1000 nm. Signal collected within 500-540 nm and 575-630 nm ranges. Scale bars: 100 µm.