Supplementary Material

Photostimulable Near-Infrared Persistent Luminescent Nanoprobes for Ultrasensitive and Longitudinal Deep-Tissue Bio-Imaging

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This file contains Figure S1 to Figure S13

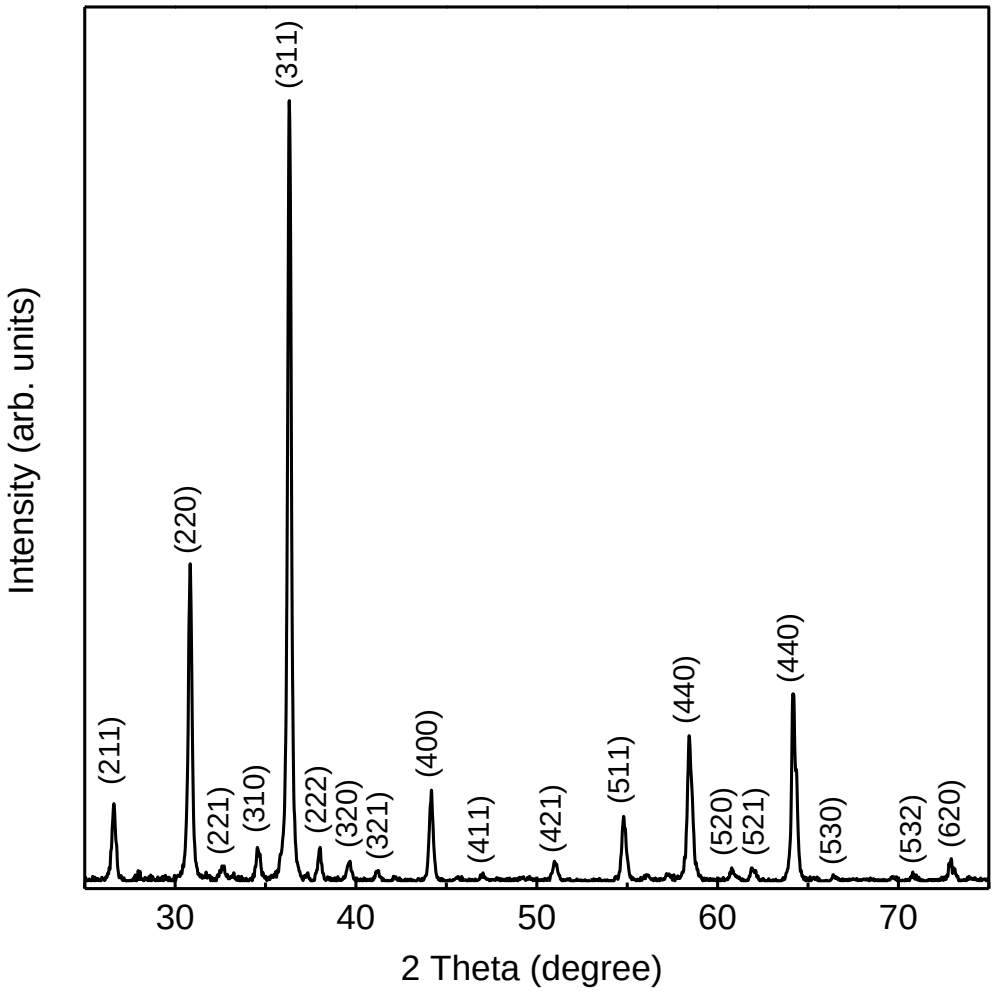


Figure S1. XRD pattern of $LiGa_5O_8:Cr^{3+}$ nanoparticles. The material is identified as cubic $LiGa_5O_8$ (PDF No. 76-199).

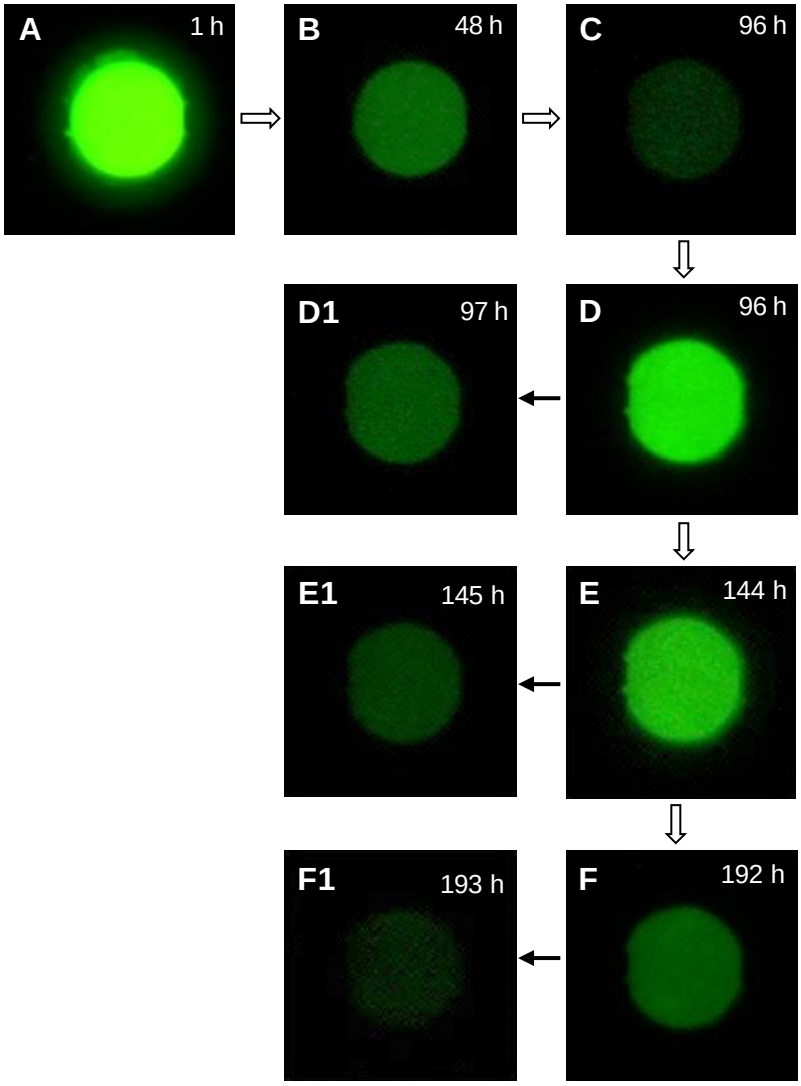


Figure S2. NIR images for persistent luminescence and photostimulated persistent luminescence (PSPL) in LGO:Cr nanoparticles. 250 mg LGO:Cr nanoparticles were pressed into a sample holder with diameter of 20 mm. Before imaging, the sample was irradiated by a 254 nm UV lamp for 15 min. (A–C) Natural decay of persistent luminescence to 96 h. (D–F) Starting from the 96 h time point, the sample was stimulated daily by a white LED flashlight (Olight SR51) for 15 s. All the PSPL images were taken at 10 s after the stimulation. Images (D1), (E1), and (F1) were taken at 1 h after the stimulation. The images were taken by a Pentax digital SLR camera which was connected to an ATN PVS-14 Generation III night vision monocular. The imaging parameter is manual/ISO 400/10 s. The imaging experiment was conducted in a dark room.

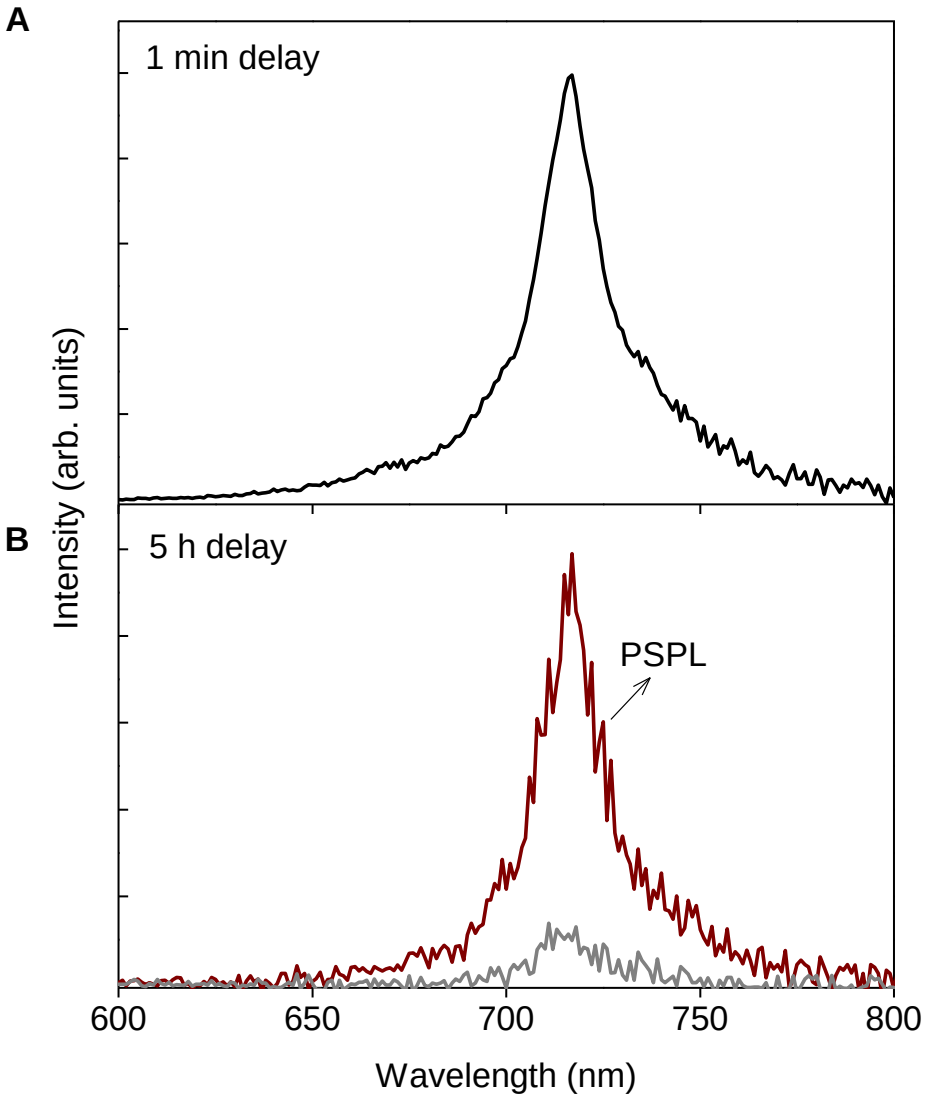


Figure S3. Persistent luminescence and PSPL properties of LGO:Cr nanoparticles in aqueous colloidal solution. The concentration of LGO:Cr nanoparticles in solution is 1 mg/mL. The solution was irradiated with a 254 nm UV lamp for 5 min. (A) Persistent luminescence emission spectrum recorded at 1 min after the UV irradiation. (B) The grey curve is the persistent luminescence emission spectrum recorded after 5 h decay. The 5-h decay solution was then stimulated with a white LED flashlight for 10 s. The brown curve is the resulting PSPL emission spectrum. The profiles of the persistent luminescence and PSPL emission spectra of the LGO:Cr nanoparticles in colloidal solution are almost identical to those of the materials in air (see Figure 1).

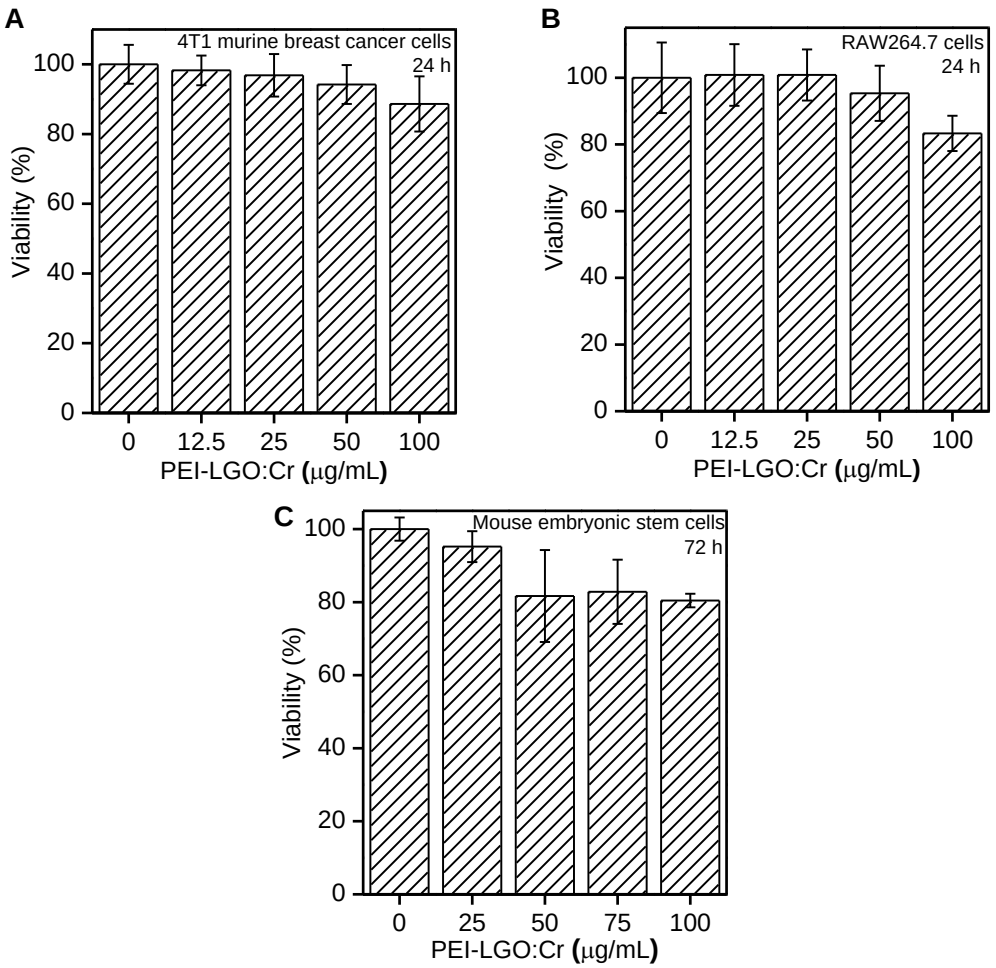
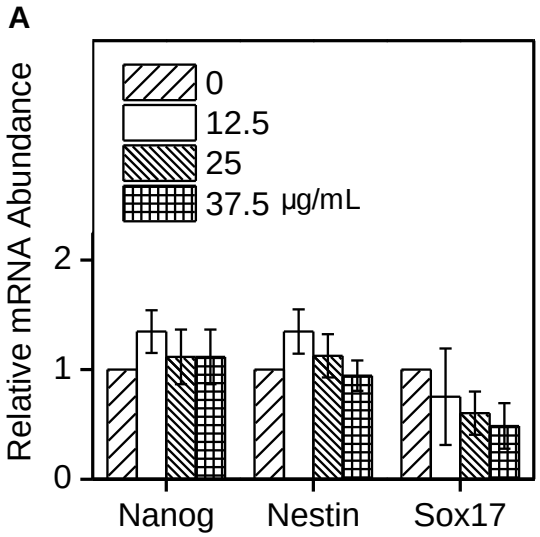


Figure S4. Viability assay with (A) 4T1 murine breast cancer cells, (B) RAW264.7 murine macrophage cells, and (C) murine embryonic stem cells (ESCs). In preparing the 4T1 cells and RAW264.7 cells assay, the cells (2×10^4/well) were added to the stock solution of PEI-LGO:Cr nanoparticles with concentrations varying from 0 to 100 μg/mL, and co-incubated in 24-well plate for 24 h in RPMI-1640 medium with 10% fetal bovine serum (FBS). The incubated cells were collected by trypsin treatment, followed by centrifugation and re-suspension in 0.01M PBS (1x, pH7.4). Trypan blue solution (0.4%, w/v) was then added into the suspension and the dead cells were counted under a microscope using a hemacytometer. In preparing the mouse ESCs assay, the cells (0.5×10^4/well) were cultured in 0.1% gelatin-coated 96-well plates using DMEM (Hyclone) medium supplemented with 10% FBS, 10% KSR (Gibco), 1% non-essential amino acids (NEAA), 4 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, 0.1 mM β-mercaptoethanol, and 1000 U/mL leukemia inhibitory factor (LIF, ESGRO, Chemicon). The cells were maintained in a humidified cell culture incubator at 37°C under 5% CO_2. After 12 h of incubation, the wells were replenished with fresh medium and treated with various concentrations of PEI-LGO (0−150 μg/mL) for 72 h, followed by addition of WST8 solution for 1 h. The plates were read at 450 nm using an OPTImax microplate reader (Molecular Devices), and the percentage of cell viability was then calculated. For all the three types of cells, the measurement at each PEI-LGO:Cr concentration was repeated 3 times.

A



B

Primer	Primer Sequence: Forward (F) and Reverse (R)	Product size (bps)	Purpose of Maker
Nanog	F: GAGGAAGCATCGAATTCTGG R: TGCAGAGCATCTCAGTAGCAG	100	Self renewal
Nestin	F: AGGCGCTGGAACAGAGATT R: TTCCAGGATCTGAGCGATCT	131	Differentiation to neural cells
Sox17	F: CTTTATGGTGTGGGCCAAAG R: GCTTCTCTGCCAAGGTCAAC	122	Differentiation to endoderm

Figure S5. The impact of PEI-LGO:Cr nanoparticles on the renewal and differentiation of mouse embryonic stem cells (ESCs). Stock solution of PEI-LGO nanoparticles was first sterilized in an autoclave. Mouse ESCs (0.5×10^4/well) were cultured in 0.1% gelatin-coated 96-well plates using DMEM (Hyclone) medium supplemented with 10% FBS, 10% KSR (Gibco), 1% non-essential amino acids (NEAA), 4 mM L-glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin, and 0.1 mM β-mercaptoethanol. The cells were maintained in a humidified cell culture incubator at 37 ºC under 5% CO_2. After 12 h of incubation, the wells were replenished with fresh medium and treated with various concentrations of PEI-LGO:Cr (0−37.5 μg/mL) for 72 h. The cells were then collected and subjected to RNA isolation (RNeasy min kit, QIAGEN), cDNA synthesis (iScript cDNA synthesis kit, BioRad), and qRT-PCR amplification (iQ SYBR Green Supermix, BioRad) using three specific primers, i.e., Nanog, Nestin and Sox17, to identify different mRNAs. Normalized qPCR-run CT values (iCycler, BioRad) were analyzed to yield relative mRNA abundance compared to that of the untreated samples. **(**A) Relative mRNA abundance using Nanog, Nestin and Sox17 primers. (B) Summary of information of the three primers.

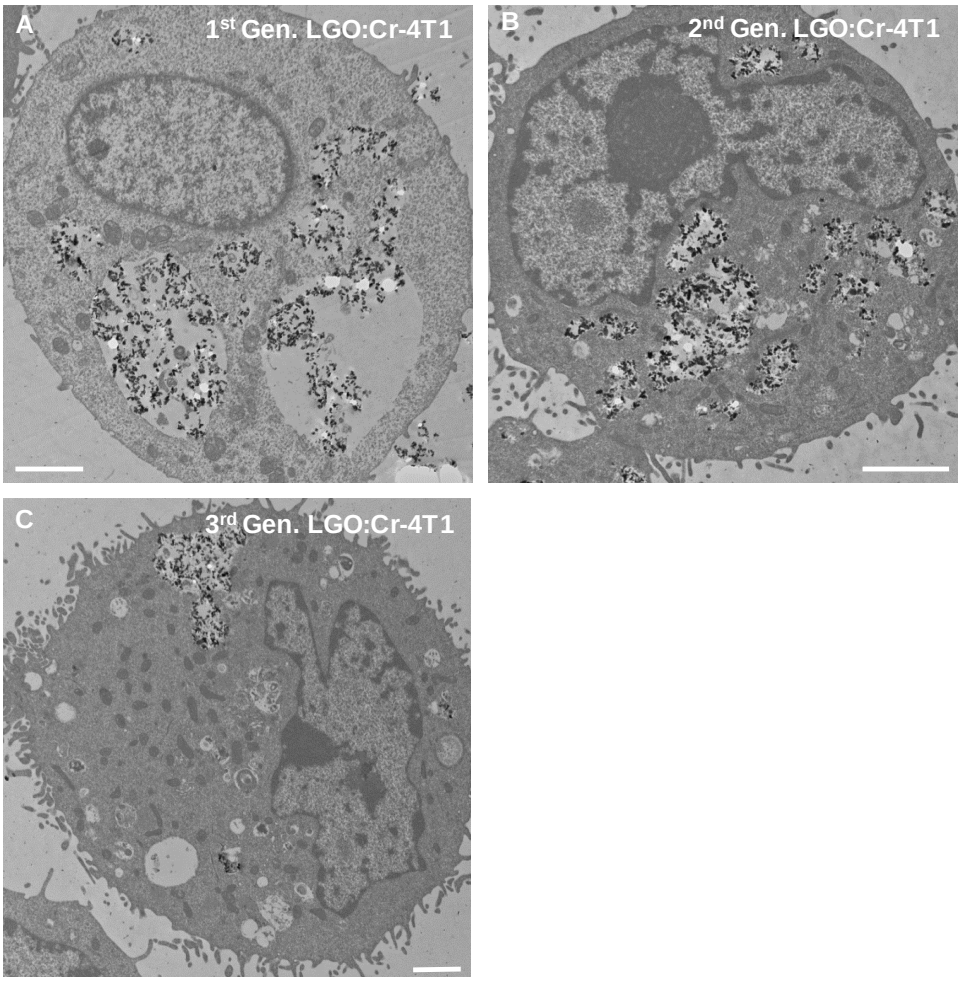


Figure S6. Thin-section TEM images of PEI-LGO:Cr nanoparticle labeled 4T1 cells from different passages. 4T1 cells (1.0×10^6 cells/mL) were added to PEI-LGO:Cr nanoparticles suspension (100 μg/mL) and co-incubated in cell culture flask for 2 days using RPMI-1640 medium (with 10% fetal bovine serum). Part of the cells (1st generation) were collected for TEM imaging and the rest were continuously cultured in medium till the 3rd generation. Image in (A), (B) and (C) are the thin-section TEM images of the 1st, 2nd and 3rd generation of 4T1 cells loaded with PEI-LGO:Cr nanoparticles. Scale bars, 2 μm. In preparation of the thin-sections, the three generations of cells were fixed with Karnovsky's EM fixative (2.5% glutaraldehyde and 2% paraformaldehyde in 80 mM phosphate buffer, pH 7.3–7.4) for 12 h at 4 °C. Secondary fixation was conducted in 1% osmium tetroxide with 1.5% potassium ferrocyanide in double distilled H_2O for 1 h at 4 °C. Dehydration was done in ethanol. Pure Epon-Araldite resin was added and infiltrated overnight at room temperature. All resin was removed the next day, and fresh resin was added to the appropriate depth. The sample was polymerized for 18 h. Ultrathin sections of cells were obtained by cutting *en face* (parallel to the surface on which the cells were grown) using a ultramicrotome, and then stained with uranyl acetate and lead citrate before viewing in the TEM.

It is worth noting that no detectable NIR photoluminescence and NIR persistent luminescence signals were recorded from the culture buffers using our FluoroLog-3 spectrofluorometer, indicating that the release of LGO:Cr nanoparticles from the tagged cells during cell culture was negligible.

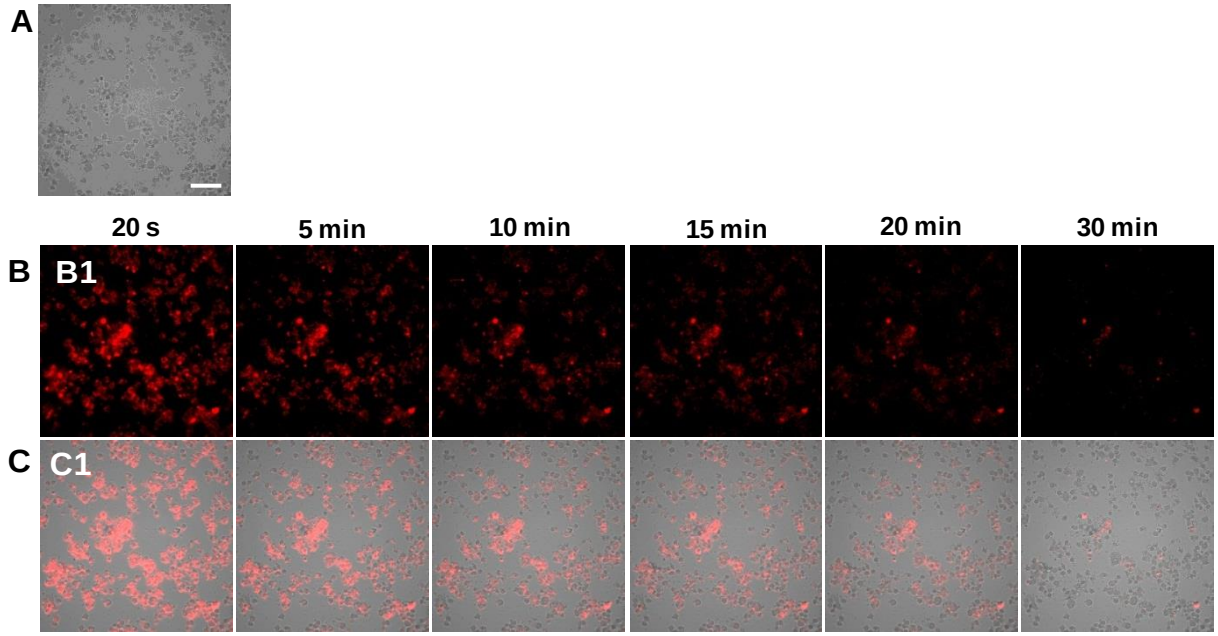
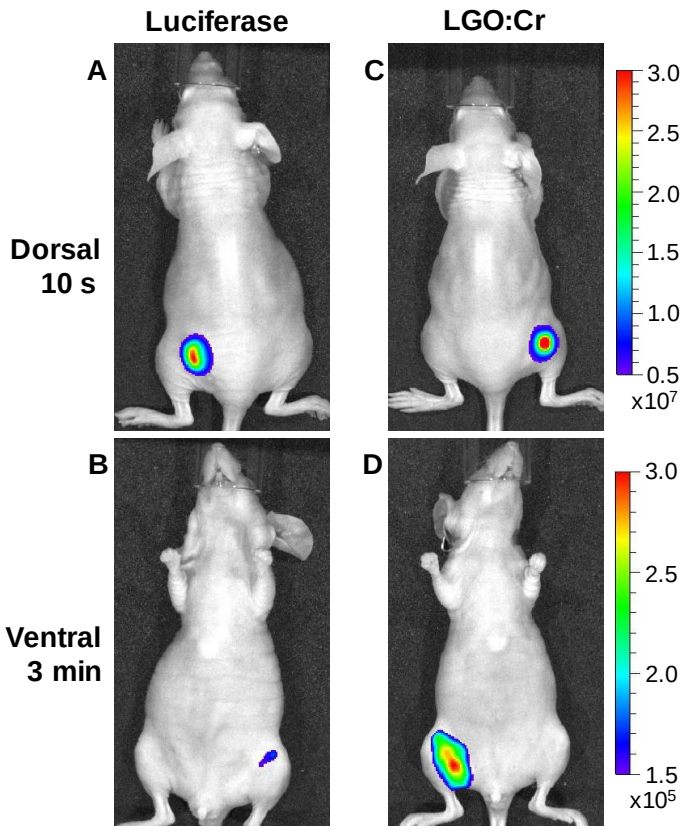


Figure S7. NIR persistent luminescence microscopic images of fixed 4T1 cells labeled with PEI-LGO:Cr nanoparticles. The imaging was conducted on an Olympus LV200 bioluminescence microscope. (A) Bright-field optical image of PEI-LGO:Cr labeled 4T1 cells. Scale bar, 100 μm. The cells were then irradiated by a 254 nm UV lamp for 5 min. (Row B) Persistent luminescence images of the PEI-LGO:Cr labeled 4T1 cells taken between 20 s and 30 min after the irradiation. The exposure time is 1 min. (Row C) Merged images by overlapping (A) with the images in (Row B). Images (A), (B1) and (C1) are the same as those in Figures 2B, 2C and 2D, respectively.



E

	Time	View	Total Flux (p/s)
Luciferase	10 s	Dorsal	9.726×10^7
	3 min	Ventral	1.740×10^5
LGO:Cr	10 s	Dorsal	8.025×10^7
	3 min	Ventral	2.547×10^6

Figure S8. Comparison of the luminescence between firefly luciferase and LGO:Cr nanoparticles. 4T1 cells stably expressing firefly luciferase (f-luc-4T1) were labeled with PEI-LGO:Cr nanoparticles. The dually labeled 4T1 cells were divided into two equal parts with each part containing about 1.0×10^6 cells. One part was incubated with D-luciferin (150 μg/ml), and 10 min later was subcutaneously injected onto the back of the left hind limb of a nude mouse for imaging from (A) dorsal and (B) ventral sides. The other part was irradiated by a 254 nm UV lamp for 15 min, and then subcutaneously injected onto the back of the right hind limb of a nude mouse for imaging from (C) dorsal and (D) ventral sides. The dorsal ((A) and (C)) and ventral ((B) and (D)) view images were taken at 10 s and 3 min after the injection, respectively. All the images were acquired on an IVIS Lumina II imaging system in the bioluminescence mode with the emission filter set as "open", and with an exposure time of 2 min. (E) Summary of total flux (in photons per second) in (A–D). The signals of bioluminescence and persistent luminescence are comparable when the animals were viewed from the dorsal side. From the ventral side, however, the intensity of LGO:Cr persistent luminescence is about one order of magnitude higher.

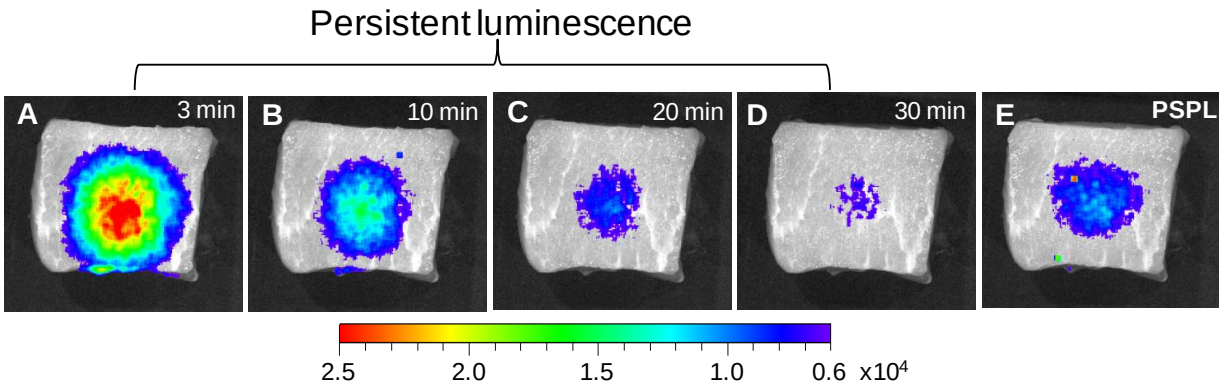


Figure S9. Phantom study of the penetrating power of the NIR persistent luminescence and NIR PSPL signals using pork as a model tissue. LGO:Cr nanoparticles were dispersed in water to form a suspension with nanoparticle concentration of 0.3 mg/mL. The suspension was irradiated with a 254 nm UV lamp for 5 min. (A–E) 50 µL of the 0.3 mg/mL suspension (containing about 15 µg LGO:Cr nanoparticles) was injected into a piece of pork at 2.5 cm deep. The images in (A–D) shows the NIR persistent luminescence images taken at 3–30 min after the injection. The image in (B) is the same as the one in Figure 3G. At the 30 min time point, the pork was illuminated by an Olight SR51 white LED flashlight (900 lumens) for 15 s to elicit NIR PSPL signals. The image in (E) shows the NIR PSPL signals taken at 2 min after the LED stimulation. All the images were taken on an IVIS Lumina II imaging system in the bioluminescence mode with an exposure time of 2 min.

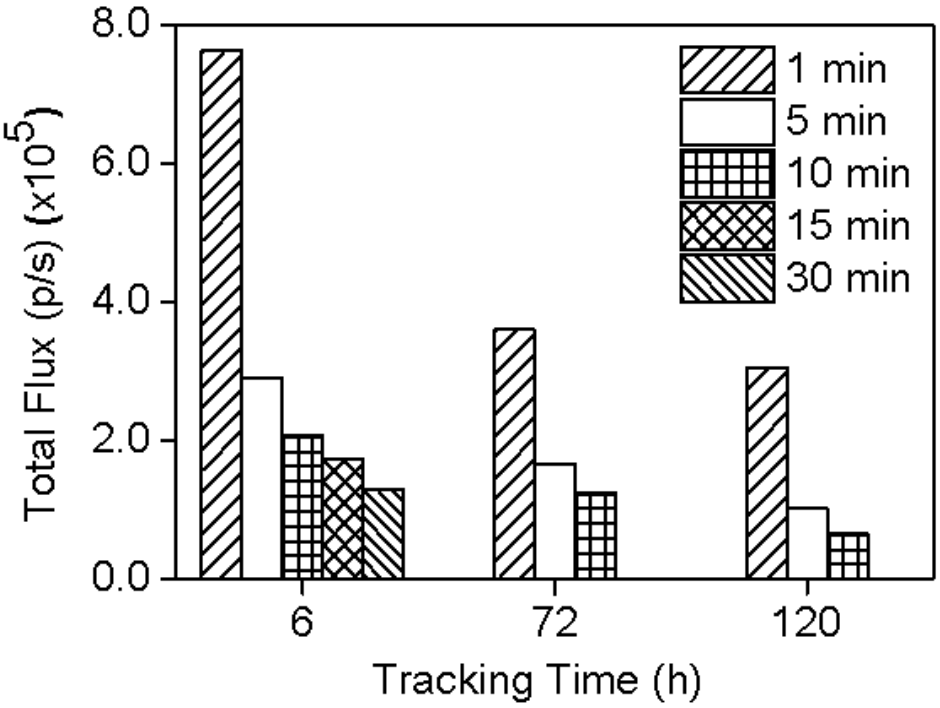
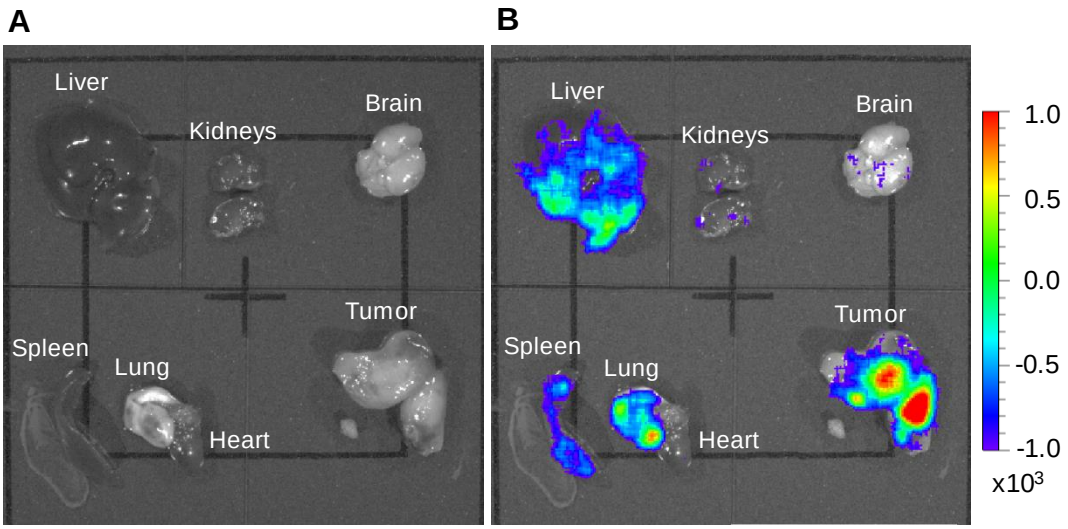


Figure S10. Quantitative analysis of the PSPL signals in Figure 5. In Figure 5, only images taken at 1 min after the stimulation were shown (the lower row). In the actual experiment, PSPL images were taken at 1, 5, 10, 15 and 30 min after the stimulation at the 6 h time point, and 1, 5 and 10 min after the stimulation at the 72 h and 120 h time points. The quantification was achieved by analyzing signals within the region of interests (ROIs) using vendor provided software Living Image (Version 4.3.1 SP1).



C

Organs	Normalized Percentage of Net Flux (%)	Relative Standard Derivation (RSD) (%)
Liver	100.0	2.5
Tumor	111.3	0.8
Lung	30.0	6.2
Spleen	13.6	2.2
Kidney	3.5	9.9
Heart	1.6	17.8
Brain	2.2	6.9

Figure S11. *Ex vivo* images and biodistribution of NIR persistent luminescence signals among tumor and major mouse organs for cell tracking with PEI-LGO:Cr nanoparticles labeled RAW264.7 cells in subcutaneous 4T1 tumor model. The tumor and organs were collected from the mouse in Figure 6C. (A) Background image of major organs, including liver, kidneys, brain, spleen, lung and heart, and tumor before UV irradiation. (B) Image taken after the organs and tumor were irradiated by a 254 nm UV lamp for 15 min. The signals were attributed to the NIR persistent luminescence from the LGO:Cr nanoparticles. Both images were acquired on an IVIS Lumina II imaging system in the bioluminescence mode with the emission filter set as "open", and with an exposure time of 5 min. (C) Biodistribution of the NIR persistent luminescence signals from the organs and tumor. The fluxes (in photons per second) of luminescence from the organs and tumor before and after the UV excitation was recorded on the IVIS system. The net flux of each organ as well as the tumor was obtained by subtracting the flux obtained before UV excitation from the flux obtained after UV excitation. The net flux of each organ and tumor was then normalized to that of the liver.

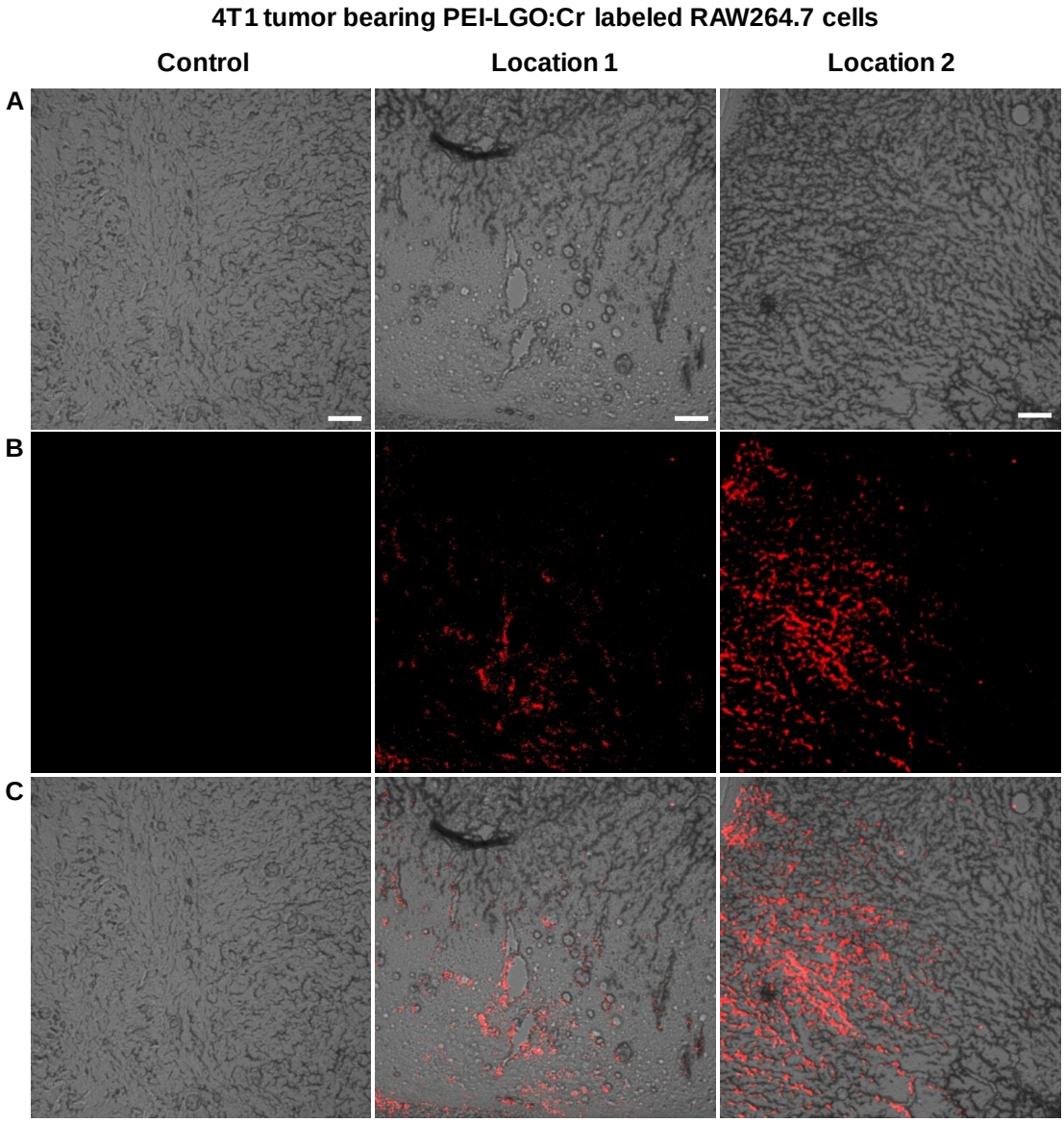
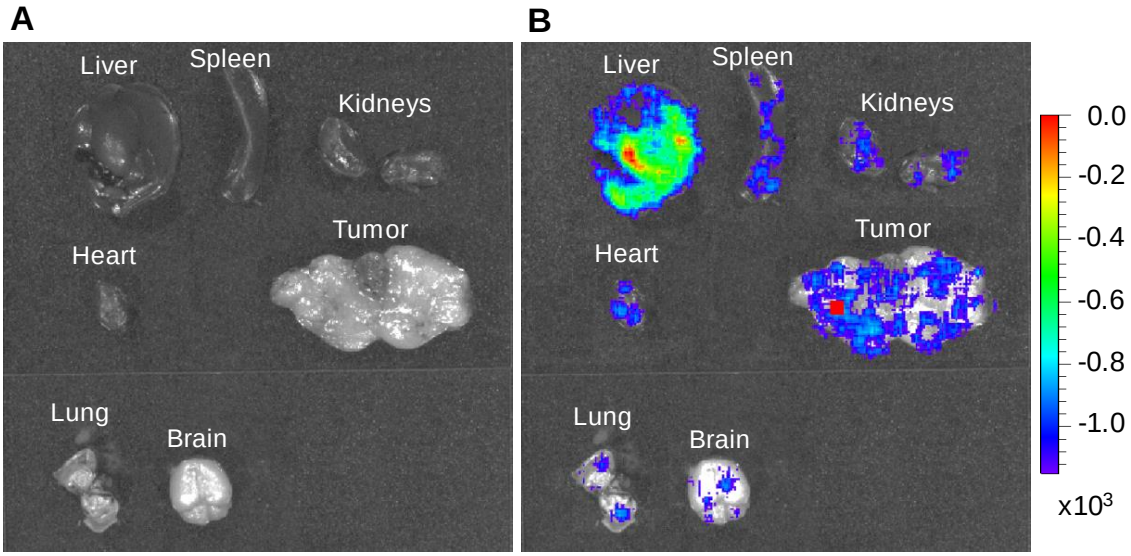


Figure S12. Microscopic study of 4T1 tumors with/without PEI-LGO:Cr labeled RAW264.7 cells. The labeled 4T1 tumor was collected from the cell tracking experiment in Figure 6. The tumors were cryo-cut into 8-μm-thick slices. The imaging study was conducted on an Olympus LV200 bioluminescence microscope. (Row A) Bright-field images of control 4T1 tumor and labeled 4T1 tumor (in two locations). Scale bars, 200 μm. The tumors were then irradiated by a 254 nm UV lamp for 5 min. (Row B) Bioluminescence images of 4T1 tumors. The signals in the labeled tumor were attributed to the NIR persistent luminescence from the LGO:Cr nanoparticles. The exposure time is 5 min. (Row C) Merged images by overlapping the same column images in (Row A) and (Row B).



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Organs	Normalized Percentage of Net Flux (%)	Relative Standard Derivation (RSD) (%)
Liver	100.0	2.6
Tumor	122.0	0.6
Lung	9.5	18
Spleen	13.4	20.4
Kidney	11.9	15.6
Heart	4	13.9
Brain	6.3	3

Figure S13. *Ex vivo* images and biodistribution of NIR persistent luminescence signals among tumor and major mouse organs for active tumor targeting with c(RGDyK)-conjugated LGO:Cr nanoparticles in subcutaneous 4T1 tumor model. The tumor and organs were collected from the mouse in Figure 6E. (A) Background image of major organs, including liver, spleen, kidney, heart, lung and brain, and tumor before UV irradiation. (B) Image taken after the organs and tumor were irradiated by a 254 nm UV lamp for 15 min. The signals were attributed to the NIR persistent luminescence from the LGO:Cr nanoparticles. Both images were acquired on an IVIS Lumina II imaging system in the bioluminescence mode with the emission filter set as "open", and with an exposure time of 5 min. (C) Biodistribution of the NIR persistent luminescence signals from the organs and tumor. The fluxes (in photons per second) of luminescence from the organs and tumor before and after the UV excitation was recorded on the IVIS system. The net flux of each organ as well as the tumor was obtained by subtracting the flux obtained before UV excitation from the flux obtained after UV excitation. The net flux of each organ and tumor was then normalized to that of the liver.