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COMMUNICATION

Shortwave infrared fluorescence in vivo imaging of nerves for minimizing the risk of intraoperative nerve injury

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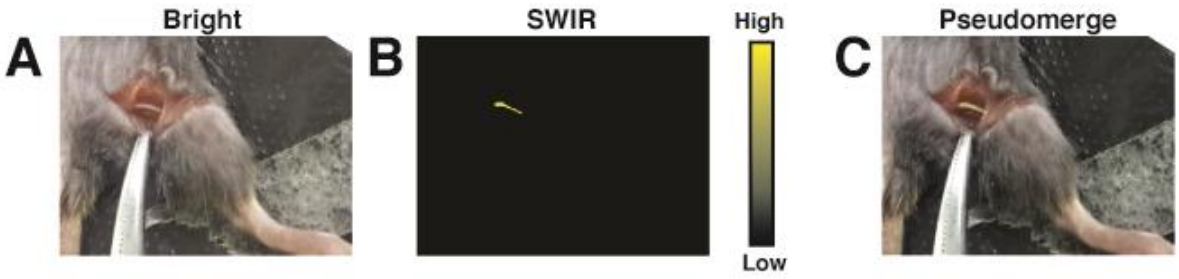

Figure.S1, The in vivo imaging of nerve in C57bl/6 mice.

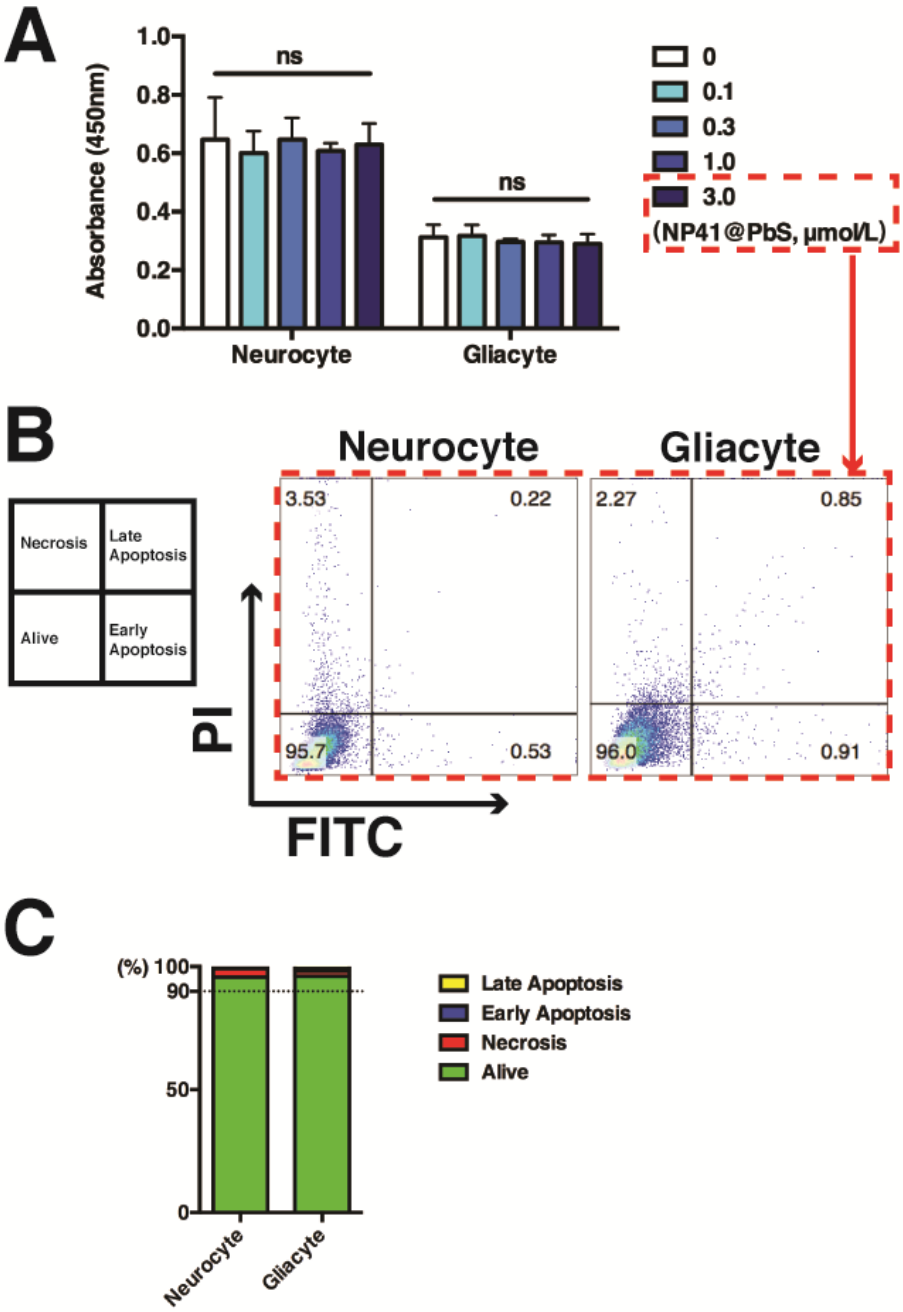

Figure.S2, The cell evaluation of NP41@PbS

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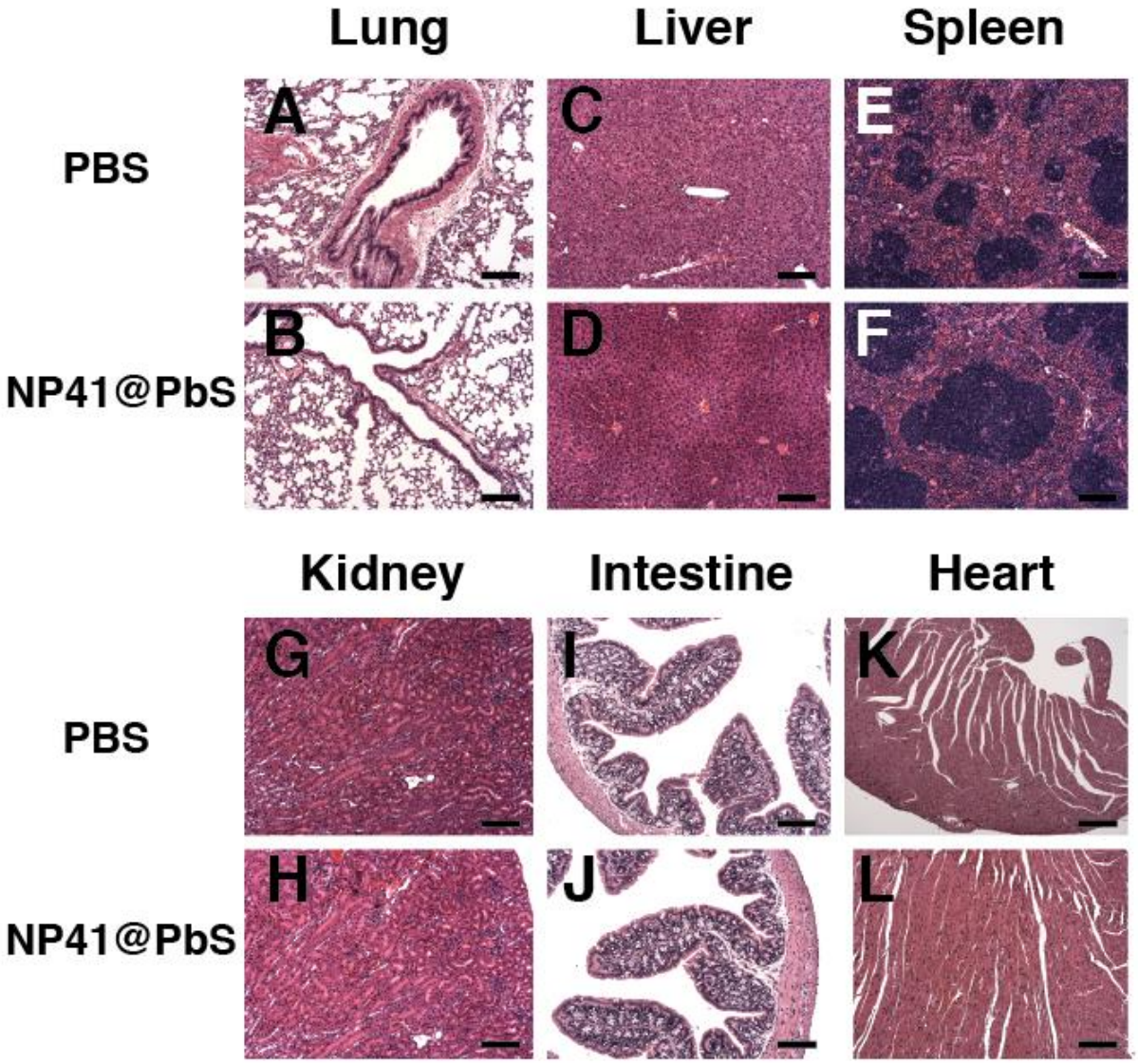

Figure.S3, The representing HE images of six organs.

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Methods：

Preparation of RNase-A-Encapsulated PbS QDs: The synthesis route is based on our previous method [1]. Briefly, 50 µL NaOH solution (1 M) was added dropwisely into 1 mL of RNase-A/Pb^{2+} precursor solution, prepared by mixing 500 µL of 50 mg/mL RNase-A with 500 µL of 10 mM $Pb(OAc)^2$, to basify the system pH to ~11. After 5 min stirring, 200 µL of 10 mM Na_2S solution was added, and then the mixed solution was heated at 80°C for 30 s in a microwave reactor with an input power of 30 W. The mixture rapidly changed from colorless into dark brown, suggesting the formation of PbS QDs. The as-prepared QDs were dialyzed against 1×PBS buffer (pH=7.4) overnight to neutralize system pH and remove unreacted reagents (e.g., Pb^{2+}, Na^+, OAc^-, etc). Thereafter, the resulting stock solution was stored at 4°C in darkness.

NP41 Peptide Synthesis and Conjugation with PbS QDs: An lysinamide was added at the C-terminal of NP41 (NTQTLAKAPEHT, C-terminal lysinamide). PbS QDs, NP41, EDC and sulfo-SMCC (4.4 mg; Pierce Premium Grade Sulfo-SMCC, Thermo Scientific, IL, USA) were suspend in MES buffer (50 nM, 1 mL, 6.5<pH<7.0; EDC: Sulfo-SMCC=2.56: 1 mol). The reaction mixture was vortexed at 37℃ for 30 mins and room-temperature for 60 min, at last the mixture was stored in 4℃ for 46 hrs. The NP41@PbS QDs were purified by centrifugation and redispersed in PBS for 3 times. Then the pH of buffer was adjusted to 7.4 for biological studies. [2]

In Vivo Imaging: All nude mice were raised under filtered air (temperature: 22±2°C; humidity: 55±5%) in plastic cages with sterilized wood shavings for bedding, fed with a standard pellet diet and tap water. For imaging applications, the animals had been anesthetized with 5% (v/w) chloral hydrate. After that, a 200 µL aliquot of NP-41@PbS QDs (~5.0µmol/ml, dosage: ~1 µmol per mouse) was intravenously injected into tail vein. Immediately after the injection, the signal was recorded at various time points over a 24 h period. Surgery was performed by single surgeon (X.H.)- Sciatic nerve: An incision was performed along femur to separate adipose & muscle tissues underlying and expose sciatic nerve; The brachial plexus: Incise the skin along humerus and resect the pectoralis major to expose the brachial plexus. Transected-resuture model: The brachial plexus was cut off at the level of mid-point of humerus, and using 11-0 nylon sutures to resuture the nerves for 3 stitches under 10× microscope. At last the mice were sacrificed to harvest their major organs for ex vivo imaging and pathohistology stain. An 808 nm diode laser with 850 and 1000 nm shortpass filters was used as the excitation source, and the power density was set at 6000mA with exposure time of 700 ms. In order to collect NIR-II fluorescence images with an InGaAs CCD camera, the emission signal was filtered by an 1100 nm long-pass filter coupled.

Otsu's Method: Two-dimensional Otsu's method was performed in this study as described previously by using MATLAB software. [4, 5] In brief, the algorithm (fluorescence image in this study) was reset to a single intensity threshold and separated into two classes-foreground (Nerve fluorescence signal) and background (the other fluorescence signals). The threshold was determined by maximizing inter-class variance.

Histopathology &Immunohistochemistry:

H&E staining. Specimens were fixed in 10% neutral buffered formalin, decalcified in Formical-2000, and embedded in paraffin. Histologic sections (6 µM) were stained with hematoxylin and eosin (H&E). [6]

Immunostaining was performed on longitudinal 5-µm-thick sections of nerve. After blocking with albumin, mouse polyclonal antibodies against S100 protein (1:1,000; Dakopatts AB) and mouse

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monoclonal antibodies against beta-tubulin III (1:1,000, Sigma-Aldrich) were applied for 12 hrs at 4℃. After rinsing in 0.01 M phosphate-buffered solution (PBS), secondary goat anti-mouse antibodies Alexa Fluor 568 and Alexa Fluor 488 (1:300; Molecular Probes, Europe BV, Leiden, the Netherlands) were applied for 1 hr at room temperature in the dark. The slides were cover-slipped with Vecta-shield mounting medium following counterstaining of the nuclei with 40-6-Diamidino- 2-phenylindole (DAPI; Vector Laboratories, Burlingame, CA).
The staining specificity was tested by omission of primary antibodies and examined under the fluorescence microscope (10× magnification, IX81; Olympus, Center Valley, PA).

Cell viability assay: Cells were seeded at 2.0×10^4 per well into 96-well plates. After adhesion, cells were treated with different concentrations of M802 or Herceptin. The CCK-8 cell proliferation kit (Dojindo, Kumamoto, Japan) was used to measure cell viability on 6 consecutive days. For each test, 10 µL of the CCK-8 reagent was added to the cells, the cells were incubated at 37 °C for 3 h, and the absorbance at 450 nm was then measured using a microplate reader (Molecular Devices). [7]

Apoptosis assay: Cells were seeded in triplicate into 24-well plates at a concentration of 2.0×10^5 cells/well, and then treated with 3 different concentrations of M802 or Herceptin. After 48 h at 37 °C and 5% CO^2, cells were dissociated with trypsin/EDTA (Gibco), washed twice with cold PBS, and then labeled with Annexin V/PI (BioLegend) as described previously. [7]

Behaviour Tests: Mechanical pain sensory test. The reaction time of mechanical foot contraction reflex was measured to test the pain sensory recovery after NP41@PbS QDs injection. The reaction time was recorded by electronic von-Frey pain meter. The mice were placed on a metal screen and covered with a plexiglass box. After 30 minutes of adaptation, the mice' forelimb paws were stimulated from 0 g, and added 0.5 g per time until 10.0g. The time from the beginning of stimulation to the appearance of fore-paws lifting was recorded. Each animal was stimulated three times at intervals of 10 min, and the average of the results of three times was obtained. Temperature sensory test

The temperature sensory test. It was measured weekly NP41@PbS injection by using plantar thermal stimulation meter. In a quiet environment, keeping the indoor temperature constant at 25℃. Mice were put in a plexiglass box with a 6 mm thick glass plate underneath. After 30 min of adaptation, the mice were stimulated by infrared beam on the middle of the plantar. The latency period was recorded from the beginning to the appearance of forelimb lifting. Automatic cut-off time was 20 seconds to prevent skin burns. Each animal was measured three times at intervals of 10 min, and the average value was obtained.

Sciatic Functional Index. By using a footprint method, the sciatic functional index (SFI) of each group was determined. Briefly, the paws of mice were contaminated by ink and then, let the mice walk through a narrow channel (8 ×60 cm) with a white paper covering. The bilateral hinder foot footprints of the mice were measured and calculated by using the following formula: SFI = (109.5×(ETS – NTS)/NTS)-(38.3×(EPL – NPL)/NPL)+(13.3×(EIT – NIT)/NIT) – 8.8.
*E is experimental; N is normal, and in this study, NP41@PbS were injected into mice systematically, so the left and right paw were calculated instead of E& N. PL is the print length (distance between second toes and heel); TS is toe spread (distance between first and fifth toes); and IT is intermediate toe spread (the distance between the second and fourth toes). [3]

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