Supporting Information

Bidirectional Near-Infrared Regulation of Motor Behavior by Orthogonal Emissive Upconversion Nanoparticles

Jingxuan Guo,[1,2,#] Lili Chen,[1,#] Feihong Xiong,[1,2,#] Yongning Zhang,[1,#] Ruipeng Wang,[1,#] Xuefei Zhang,[1,2] Quan Wen, [3] Shangbang Gao,[1,*] and Yan Zhang,[1,2,*]

1. College of Life Science and Technology, Huazhong University of Science and Technology, Wuhan 430074, China

2. National Engineering Research Center for Nanomedicine, Huazhong University of Science and Technology, Wuhan 430074, China

3. Hefei National Laboratory for Physical Sciences at the Microscale, School of Life Sciences, University of Science and Technology of China, Hefei 230027, China

Experimental

Materials and Reagents. Gadolinium acetate hydrate (99%), ytterbium acetate hydrate (99%), and thulium acetate hydrate (99%) were all purchased from Alfa Aesar. Erbium acetate hydrate (99%), yttrium acetate hydrate (99%) and neodymium acetate hydrate (99%) were purchased from Sigma-Aldrich. 1-octadecene (90%) and oleic acid (90%) were purchased from Aladdin. Sodium hydroxide (>98%), ammonium fluoride (98%), HCl, hexane (AR), ethanol (AR) and chloroform (AR) were all purchased from Sinopharm Chemical Reagent Co., Ltd. All reagents were used as received without further purification.

Synthesis of OUCNPs. The core, core/shell, core/shell/shell, core/shell/shell/shell, and OUCNPs were synthesized according to our previous method with slightly modification.[1] Briefly, 0.5 mmol $Ln(Ac)_3 \cdot xH_2O$ (Ln=Y, Yb, Tm) were added into the three neck bottles with 2 mL deionized water. Then, the mixture was dissolved by ultrasonication. Afterwards, 3 mL OA and 7 mL ODE were added and the solution was heated at 140 °C for 1 h. After the mixture was cooled down to room temperature, 5 mL of methanol solution containing 1.25 mmol NaOH and 2 mmol NH_4F were added, and the mixture was kept at 50 °C for 30 min. Then, the solution was heated to 100 °C for 10 min under vacuum. Subsequently, the solution was raised to 290 °C with the rate of 10 °C/min, and maintained for 1 h. All the above procedures were conduct under Ar atmosphere and kept stirring with a moderate speed. The product was precipitated with ethanol, collected by centrifugation at 8000 rpm for 10 min, washed with ethanol/hexane, and the final product were dispersed in 4 mL of hexane. The shell was grown on the core nanoparticles with the same protocols.

Preparation of ligand-free OUCNPs. 2 mL of hydrophobic OUCNPs were precipitated with ethanol, and redispersed in 4 mL of ethanol by ultrasonication. Then, the same volume of 2 M HCl solution were mixed with the suspension and ultrasonication for 10 min. The ligand-free OUCNPs nanoparticles were collected by centrifugation at 12800 rpm for 30 min, washed with ethanol, and redispersed in DMF with 40 mg/mL and stored at 4 °C for further use.

Characterization. Transmission electron microscopy (TEM) images were recorded on a HITACHI HT7700 microscope operating at 120 kV. High-resolution TEM (HR-TEM) imaging and energy-dispersive X-ray (EDX) spectroscopy were done using a transmission electron microscope (Tecnai G2 F30, FEI) equipped with an X-ray energy dispersive spectrometer. The crystal structure of UCNPs was studied by X-ray diffraction (XRD) using a diffractometer (X'Pert PRO, PANalytical B. V., Almelo, Netherlands) using Cu Kα radiation at $\lambda = 0.15406$ nm and operating at 40 kV and 40 mA. Each sample was recorded from 10° to 70° with a step size of 0.0131 and a step time of 9.945 s. To obtain the upconversion photoluminescence spectra, samples were dispersed in hexane/water (2 mg/ml) in a standard quartz cuvette at room temperature, and fluorescence spectra were acquired on a Fluoromax-Plus, Horiba Jobin Yvon

spectrofluorometer with a commercial 808 nm NIR laser (Changchun Laser Optoelectronics Technology Co., Ltd. China). The hydrodynamic diameter of nanoparticles was determined by a dynamic light scattering instrument (DLS, Zetasizer Nano ZS90, Malvern Instruments Ltd, UK) equipped with 4 mW He–Ne laser source (λ = 633 nm, scattering angle: 90°). FTIR spectra were acquired on Fourier Transform Infrared spectroscopy (VERTEX 70, Bruker Corp., Germany).

Strain Maintenance. Strains were maintained as described.[2] All *C. elegans* were cultured on standard Nematode Growth Medium (NGM) plates seeded with OP50 and maintained at 20 °C incubators. Unless otherwise stated, the wild-type animal refers to the Bristol N2 strain. Unless otherwise stated, all transgenic strains that contain BiPOLES were cultured in darkness on OP50 NGM plates supplemented with 0.5 mM ATR.[3] Transgenic lines used in this study include: SGA803 *gaaIs65* (P*unc-17*::BiPOLES::3'UTR-*lin-44*::GFP); KP3814 *nuIs152* (P*unc-129*::GFP::SNB-1 + P*ttx-3*::mRFP). All were backcrossed at least 4 times against N2 prior to analyses.

NIR Tm,Er-OUCNPs Optogenetic Behavioral Analysis. L4 to young adult *C. elegans*, maintained in standard culture conditions, was transferred to M9 solution in a 60 mm imaging plate with or without Tm,Er-OUCNPs. OUCNPs were diluted into the M9 solution to the final concentration. One minute after the transfer, a two-minute video of the swimming animal was recorded on a stereoscopic fluorescence microscope (Axio Zoom V16, Zeiss) equipped with a digital camera (acA2500-60um, Basler). OUCNPs The NGM plate was irradiated with NIR laser coupled with a φ200 μm fiber with a condenser collimator (focal length 8 cm, focal spot diameter 1 mm). The NGM plate was placed at the focal point of the condenser collimator. All images were captured with a 15X objective (Apo, Zeiss) at 10 frames per second by Pylon 4 software. Data recorded on the same day were pooled and quantified. Post-imaging analyses utilized WormLab 4.0 and in-house written MATLAB scripts. A Wormlab or homemade Matlab script was used to divide a virtual image of each *C. elegans* body into 33 segments. The angle between three joint points was used to calculate the curvature of the middle point loci. The curvature values for each body segment from the head to tail over time were plotted, and were converted to a heat map of movements.

Confocal Imaging. The strain *nuIs152* (P*unc-129*::GFP::SNB-1) of *C. elegans*, which expressed the synaptic protein SNB-1 synaptobrevin to cholinergic DA-class of motorneurons under the P*unc-129* promoter, was used to detect the potential toxicity of OUCNPs and NIR to neuronal development. For OUCNPs + NIR experiments, animals were cultured with OUCNPs (5 mg/ml) and then were irradiated by 808 nm NIR (0.64 W/mm^2) and 980 nm NIR (0.64 W/mm^2) every 5 s, repeat for three times. After resting for 30 min, the entire synaptic fluorescence of the animal was recorded. The puncta from the dorsal middle body were then used to quantify fluorescence intensity and distribution (Figure 4a). Fluorescence signals were captured from live L4 larva using a Plan-Apochromatic 60X objective on a confocal microscope (FV3000, Olympus) in the same conditions. Worms were immobilized by 2.5 mM

levamisole (Sigma-Aldrich) in M9 buffer. Straightened dorsal nerve cords were extracted from the raw images using the "straightened to line function" from Image J. Background fluorescence was subtracted by the average intensity in a region devoid of SNB-1::GFP puncta. Two main parameters were determined: maximal fluorescence intensity and puncta number. Maximal fluorescence intensity along the cord was calculated using the "plot profile" function of Image J. Synaptic puncta numbers were calculated using the "analyze particles" function, with an intensity threshold of 400.[4] Sodium selenite (Na_2SeO_3, Sigma-Aldrich) was diluted in distilled water to the 70 mM stock and then was added to NGM agar with final 5 mM concentration. Mock-exposure plates had an equivalent volume of water added. L4 stage animals were picked on plates culturing for 12 h.

Electrophysiology. Dissection and recording of *C. elegans* were carried out using same protocols described in previous reports.[5-7] Briefly, 1- or 2-day-old hermaphrodite adults were glued (Histoacryl Blue, Braun) to a Sylgard-coated cover glass covered with bath solution (Sylgard 184, Dowcorning) under a stereoscopic microscope (M50, Leica). After clearing the viscera by suction through a glass pipette, the cuticle flap was turned and gently glued down using WORMGLU (GluStitch Inc.) to expose the neuromuscular system. Body wall muscle cells were patched using 4-6 MΩ-resistant borosilicate pipettes (1B100F-4, World Precision Instruments). Pipettes were pulled by micropipette puller (P-1000, Sutter) and fire-polished by microforge MF-830 (Narishige). mPSCs and action potentials were recorded in the whole-cell configuration by EPC9 amplifier (HEKA, Germany), using the Pulse and processed with Igor 6 (WaveMetrics) and Clampfit 10 software (Axon Instruments, Molecular Devices). mPSCs were recorded at -60 mV under voltage clamp configuration, while action potentials were recorded at 0 pA under current clamp configuration. Data were digitized at 10–20 kHz and filtered at 2.6 kHz. The pipette solution contains (in mM): K-gluconate 115; KCl 25; $CaCl_2$ 0.1; $MgCl_2$ 5; BAPTA 1; HEPES 10; Na_2ATP 5; Na_2GTP 0.5; cAMP 0.5; cGMP 0.5, pH7.2 with KOH, ~320mOsm. The bath solution consists of (in mM): NaCl 150; KCl 5; $CaCl_2$ 5; $MgCl_2$ 1; glucose 10; sucrose 5; HEPES 15, pH7.3 with NaOH, ~330 mOsm. Chemicals were obtained from Sigma unless stated otherwise. Experiments were performed at room temperature (20-22 ^oC). The frequency, amplitude and discharge of mPSCs and action potentials were analyzed using Clampfit 10.

Thrashing Assay. Different concentrations of Tm,Er-OUCNPs mixed in M9 buffer were dropped (50 μl each) in 12 well plates with NGM beforehand. One minute after the animals (4-6 hr post early L4 stage) were transferred, thrashing behaviors were recorded by a digital camera (acA2500-60um, Basler). Images were captured at 10 fps with proper magnification (2X). For NIR irradiation experiments, each animal was exposed to NIR for 3-5 s to reduce possible heating effects. The thrashing score measured the frequency of body bending for every 1 s.

Brood Size Assay. Eggs before hatch from *nuIs152* were picked to OP50 NGM plates without or with Tm,Er-OUCNPs (5 mg/ml). For each group, two eggs were put into

one plate until they became young adults. After the first day of egg laying, they were transferred to fresh Tm,Er-OUCNPs mixed OP50 plates every 24 hr to avoid food shortages. The number of laid eggs in each group was then counted manually. The total number of eggs was calculated by accumulating all data. Experiments were repeated at least 15 times for each group.

Statistical Analysis. The Mann-Whitney U test, two-tailed Student's *t* test, and one-way ANOVA test were used to compare data sets. $P < 0.05$ was considered to be statistically significant (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). Graphing and subsequent analysis were performed using Igor Pro (WaveMetrics), Clampfit (Molecular Devices), Image J (National Institutes of Health), Matlab (MathWorks), GraphPad Prism 5 (GraphPad Software Inc.) and Excel (Microsoft). For electrophysiology, behavior analysis and fluorescence imaging, unless specified otherwise, each recording trace was obtained from a different animal; data were presented as the Mean ± SEM.

Supporting Figures:

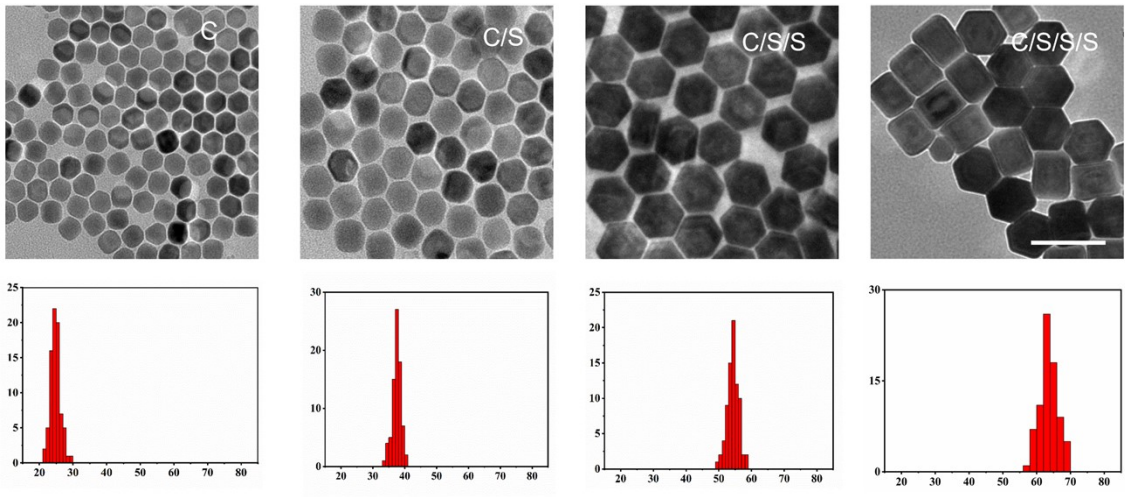

Figure S1. TEM images (upper panels) and distribution (bottom panels) of as-synthesized core (C, $NaYF_4$:Yb,Tm), core-shell (C/S, $NaYF_4$:Yb,Tm@ NaNd,YbF_4), core-shell-shell (C/S/S, $NaYF_4$:Yb,Tm@NaNd,YbF_4@$NaYF_4$), core-shell-shell-shell (C/S/S/S, $NaYF_4$:Yb,Tm@NaNd,YbF_4@$NaYF_4$@$NaErF_4$).

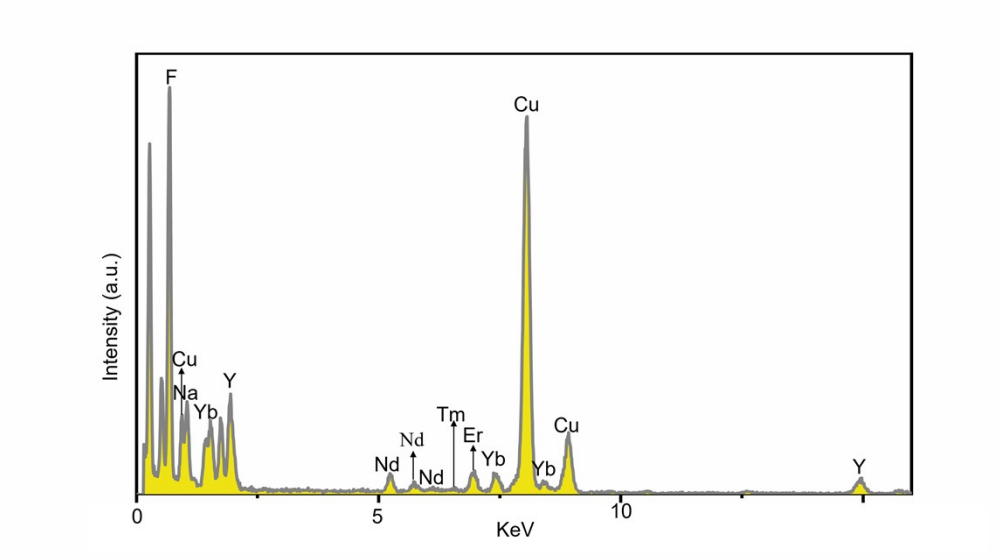

Figure S2. EDX mapping of OUCNPs.

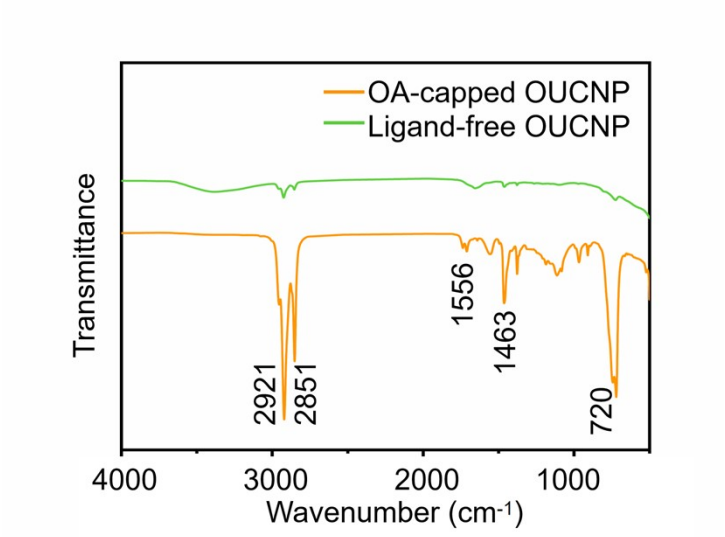

Figure S3. Fourier transform infrared (FTIR) spectroscopy of oleic acid-capped and ligand-free OUCNPs.

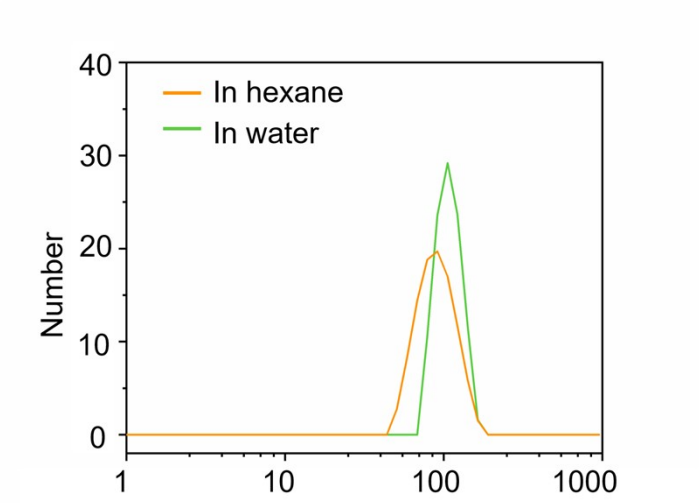

Figure S4. Dynamic light scattering (DLS) measurement of Tm,Er-OUCNPs in hexane and water, respectively.

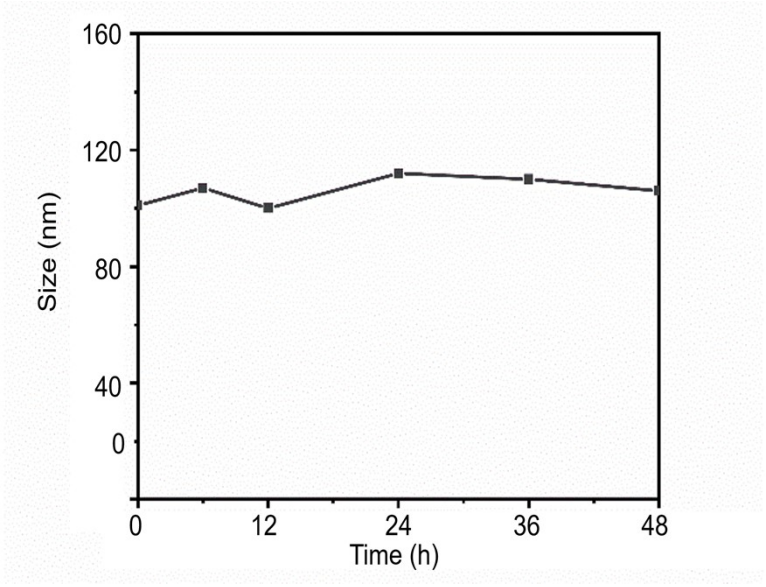

Figure S5. Hydrodynamic stability of Tm,Er-OUCNPs in water.

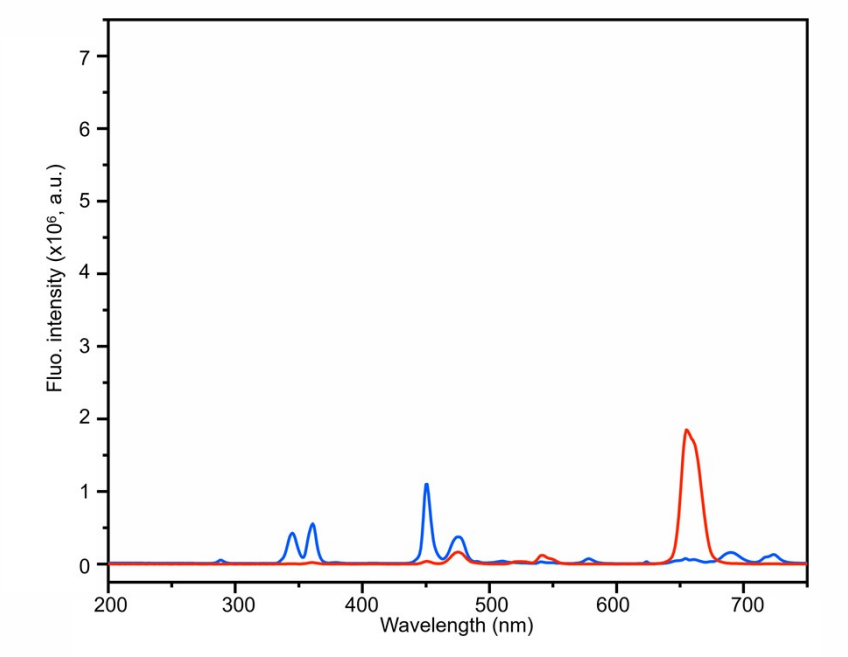

Figure S6. Photoluminescence spectra of Tm,Er-OUCNPs in water upon 808 nm (blue line) and 980 nm (red line) excitations, respectively.

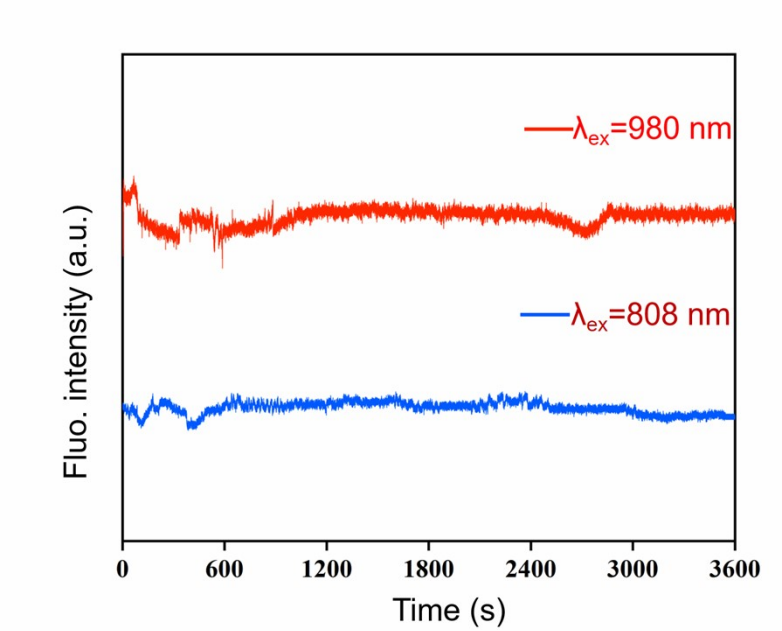

Figure S7. Photostability of Tm,Er-OUCNPs in water upon 808 nm (blue line) and 980 nm (red line) excitations, respectively.

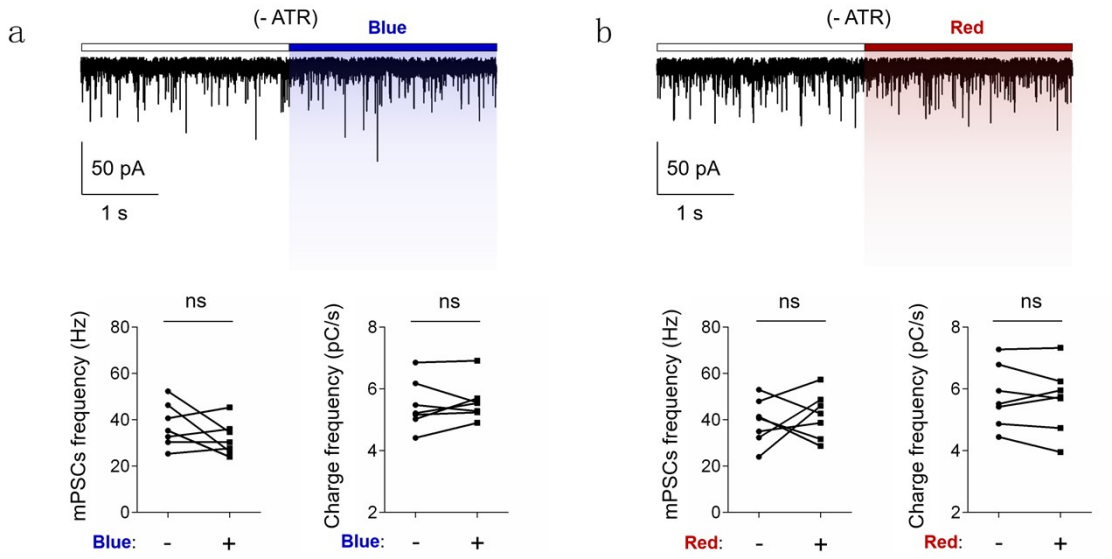

Figure S8. No response of BiPOLES to lights stimulation without ATR. (a， b) Upper, representative spontaneous miniature postsynaptic currents (mPSCs) recorded from the body wall muscle before and during the illumination of blue and red lights, respectively. Bottom, quantification of the mPSCs frequency and charge frequency before and during the illumination. No response of mPSCs was observed to the lights stimulations.

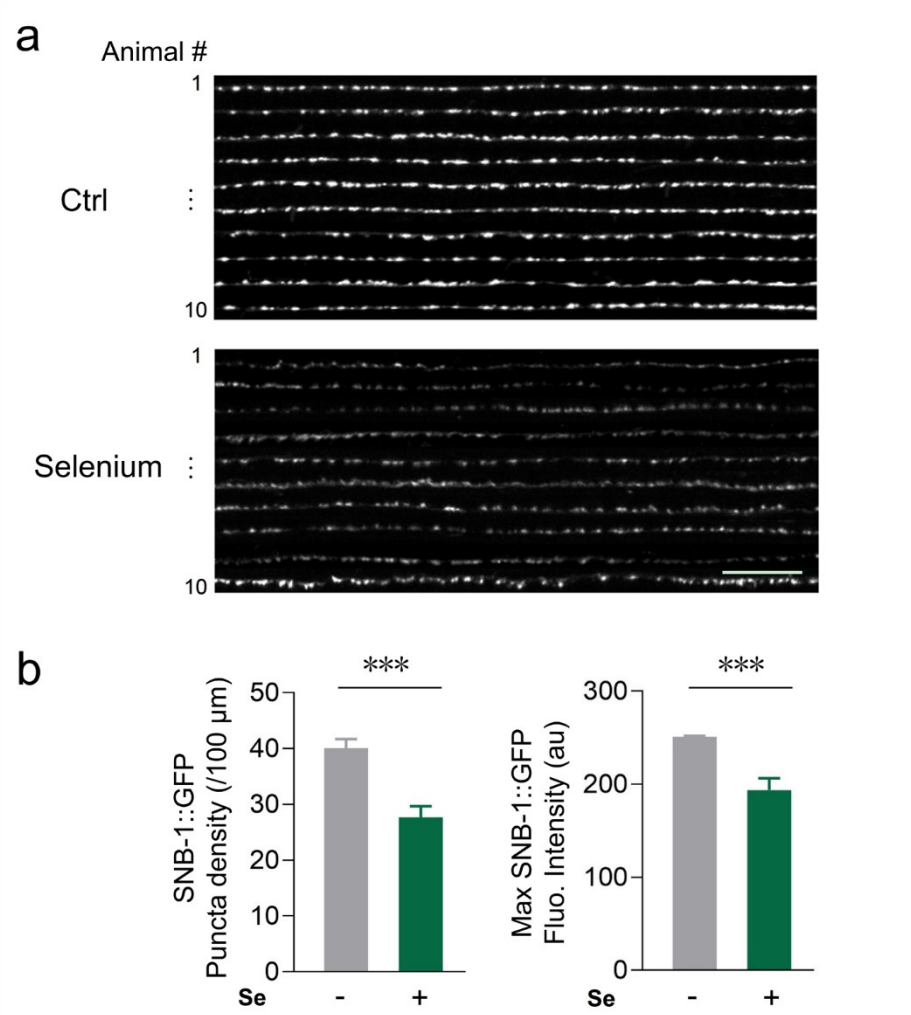

Figure S9. Synaptic morphology effect of Selenium. (a) The distribution of SNB-1::GFP puncta of 10 individuals without (Ctrl) or with 5 mM Selenium. Scar bar: 10 µm. (b) Quantification of SNB-1::GFP puncta density and maximum SNB-1::GFP fluorescence intensity in animals under different conditions.

REFERENCES

1. Zhang, Y.; Yu, Z.; Li, J.; Ao, Y.; Xue, J.; Zeng, Z.; Yang, X.; Tan, T. T., Ultrasmall-Superbright Neodymium-Upconversion Nanoparticles via Energy Migration Manipulation and Lattice Modification: 808 nm-Activated Drug Release. *ACS Nano* **2017,** *11* (3), 2846-2857.

2. Brenner, S., The genetics of Caenorhabditis elegans. *Genetics* **1974,** *77* (1), 71-94.

3. Liewald, J. F.; Brauner, M.; Stephens, G. J.; Bouhours, M.; Schultheis, C.; Zhen, M.; Gottschalk, A., Optogenetic analysis of synaptic function. *Nat. Methods* **2008,** *5* (10), 895-902.

4. Maro, G. S.; Gao, S.; Olechwier, A. M.; Hung, W. L.; Liu, M.; Ozkan, E.; Zhen, M.; Shen, K., MADD-4/Punctin and Neurexin Organize C. elegans GABAergic Postsynapses through Neuroligin. *Neuron* **2015,** *86* (6), 1420-32.

5. Gao, S.; Zhen, M., Action potentials drive body wall muscle contractions in Caenorhabditis elegans. *Proc. Natl. Acad. Sci. U S A* **2011,** *108* (6), 2557-62.

6. Richmond, J. E.; Jorgensen, E. M., One GABA and two acetylcholine receptors function at the C. elegans neuromuscular junction. *Nat. Neurosci.* **1999,** *2* (9), 791-7.

7. Mellem, J. E.; Brockie, P. J.; Madsen, D. M.; Maricq, A. V., Action potentials contribute to neuronal signaling in C. elegans. *Nat. Neurosci.* **2008,** *11* (8), 865-7.