Supporting Information

Organelle-Specific Detection of Phosphatase Activities with Two-Photon Fluorogenic Probes in Cells and Tissues

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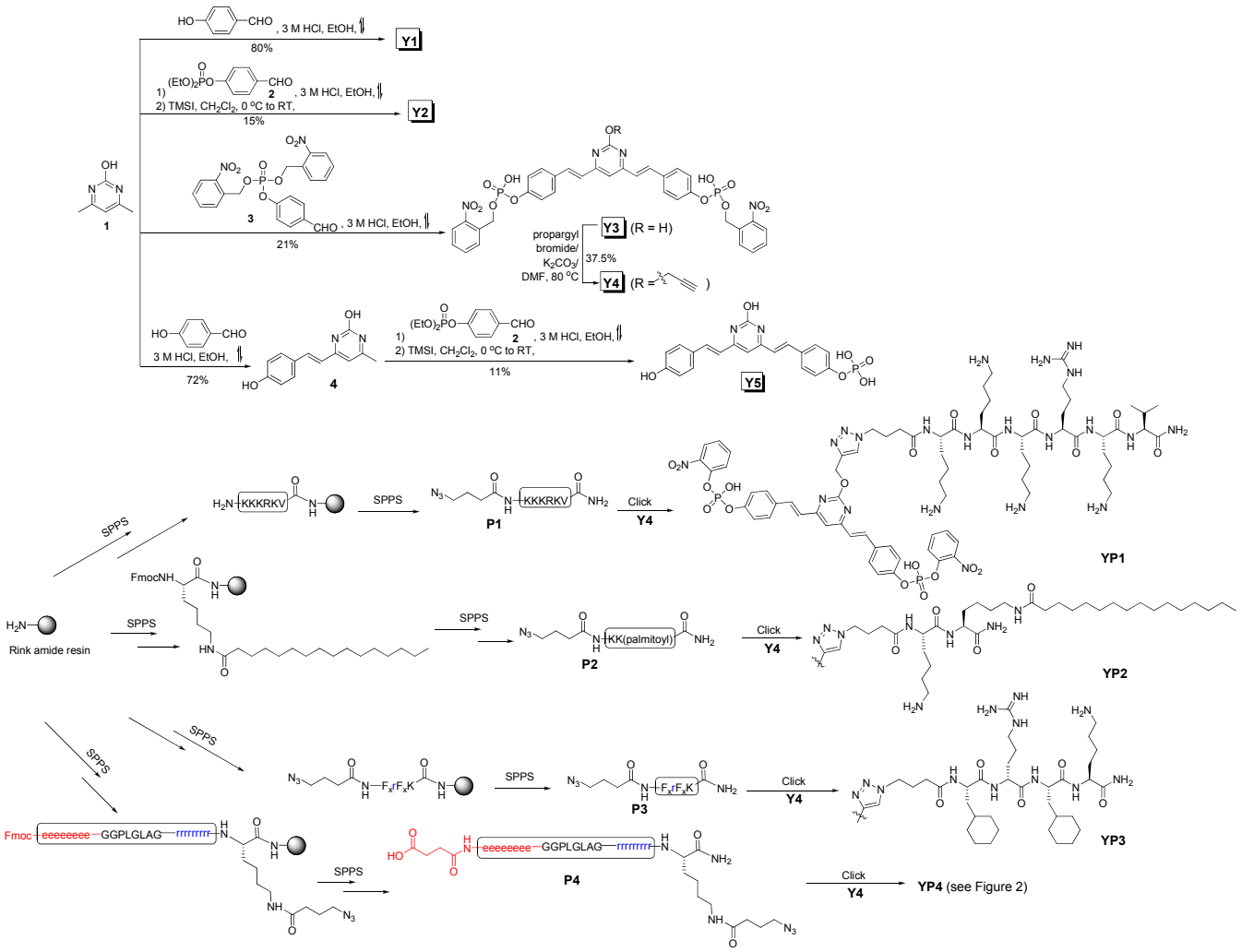


Scheme S1. Synthesis of the 2P fluorogenic probes **Y1-5** and their peptide conjugates, **YP1-4.**

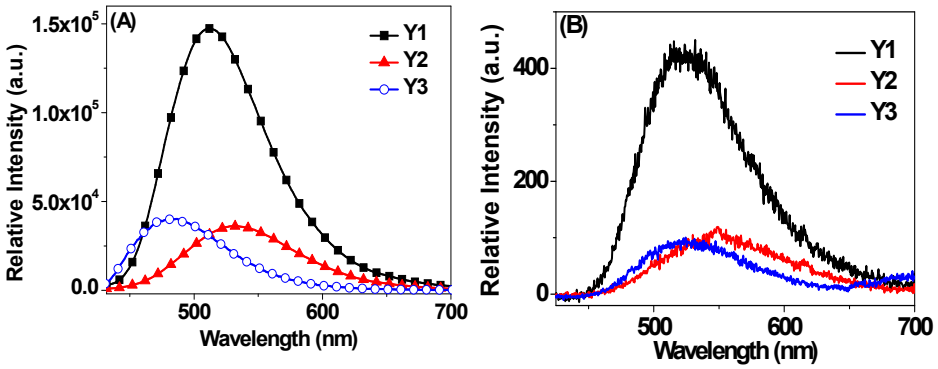


Figure S1. (A) One-photon and (B) two-photon excited fluorescence spectra of **Y1, Y2** and **Y3** in Hepes buffer (*p*H = 7.5, supplemented with 50 mM NaCl, 2.5 mM EDTA, 2 mM DTT and 0.08 % Triton X-100). C = 1.0 ×10^{-6} mol L^{-1}

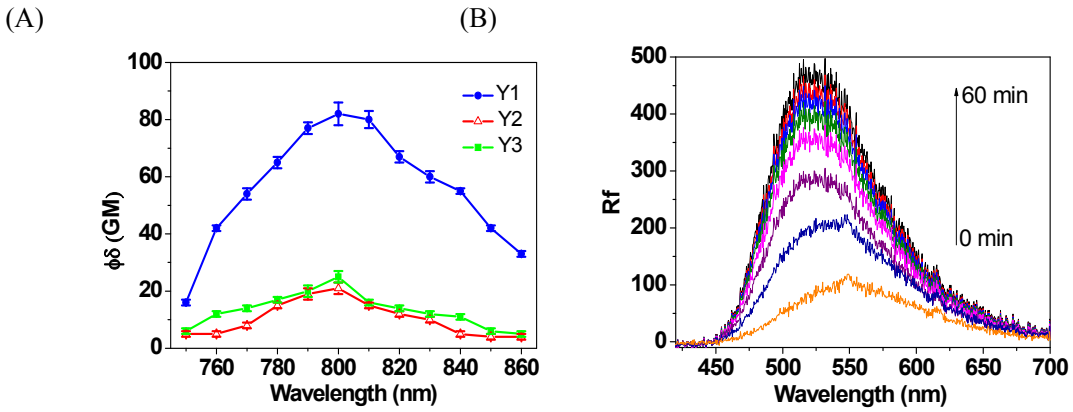
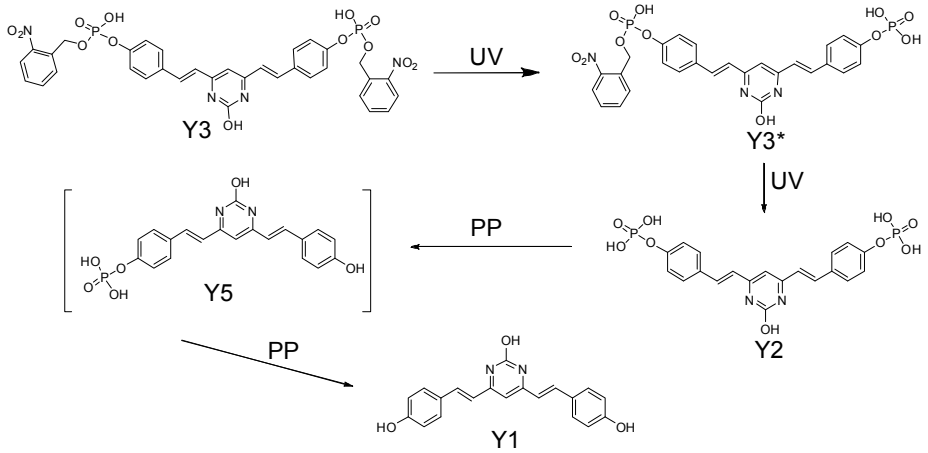


Figure S2. (A) Two-photon action cross-section spectra of **Y1, Y2** and **Y3** in Hepes buffer. (B) Time-dependent two-photon excited fluorescence emission spectra of **Y2**→**Y1** dephosphorylation by PTP1B (60 min). PTP1B/probe concentration ratio = 1:30. Reactions were carried out at room temperature in Hepes buffer (supplemented with 50 mM NaCl, 2.5 mM EDTA, 2 mM DTT and 0.02 % Triton X-100, *p*H = 7.5; probe concentration: 1.6 μM).

(A)



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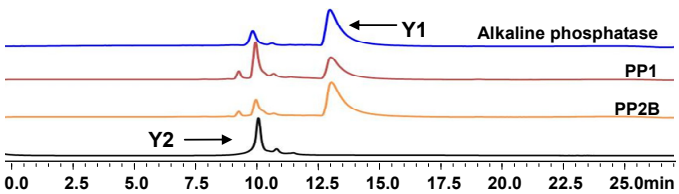


Figure S3. (A) Schematic showing of UV-assisted photo cleavage of **Y3**, giving **Y3*** (detected in the reaction; see maintext), then **Y2**. Upon phosphatase (PP) cleavage, **Y2** was converted to **Y1** via the mono-phosphorylated intermediate **Y5** (not detected in the

reaction). (B) PP cleavage of **Y2** (in Hepes Buffer at RT for 60 min; see maintext) monitored by LC-MS. Results indicated **Y2** were active towards all three phosphatases (alkaline phosphatase, PP1 and PP2B). **Y5** was not detected throughout the duration of the reaction.

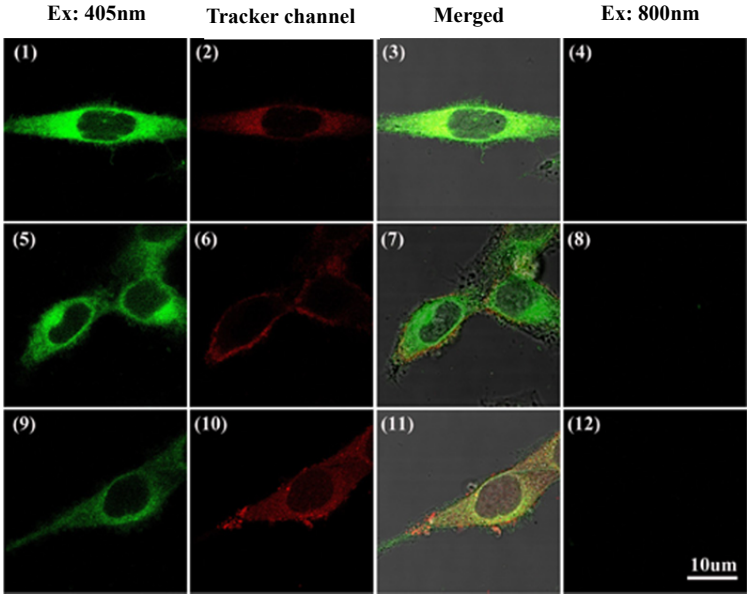


Figure S4. (A) Schematic showing of ELF 97™ dephosphorylation and detection of phosphatase activities. (B) One- and two-photon excited fluorescence images of HeLa cells showing endogenous phosphatase activities detected by treatment with ELF 97™ phosphatase substrate (100-fold dilution from commercial kit). Scale bar = 10 µm.

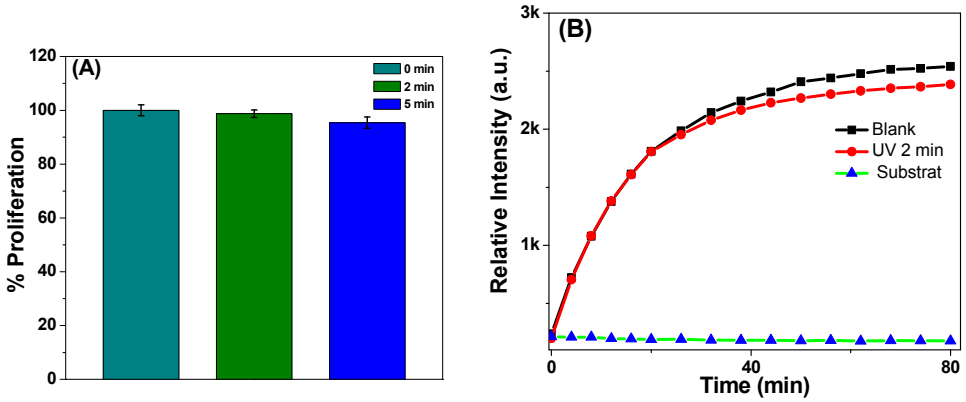


Figure S5. (A) *XTT* assay profiles of HepG2 cells upon 2 and 5 min of UV irradiation. Cell viability was determined using the XTT colorimetric cell proliferation kit (Roche) following manufacturer's guidelines. Briefly, HepG2 cells were grown to 20-30% confluency (since they will reach ~ 90% confluency within 48 to 72 h in the absence of UV irradiation) in 96-well plates under the conditions described above. The medium was aspirated, and then washed with PBS, and then treated, in duplicate, with UV irradiated 2 or 5 min. Staurosporine (STS, 200 nM) was used as a positive control. After a total treatment time of 24 h, proliferation was assayed using the XTT colorimetric cell proliferation kit (Roche) following manufacturer's guidelines (read at 450 nm, ref. at 650 nm). (B) Microplate-based PTPs enzymatic assay of UV effect of HepG2 cells. Cells were seeded on 10 cm dishes and grown till 80 ~ 90% confluency in corresponding growth media. The medium was aspirated, washed with PBS, theh UV-irradiated for 2-5 min. The growth medium was removed and cells were harvested. Cell pellets were sonicated and Dounce-homogenized in 50 mM Tris buffer (*p*H 7.5) to provide soluble total proteome fractions (supernatant). The phosphatase assays were performed in 384 wells microplate. Total (final total protein conc. 5.0 mg/mL) proteome fractions were dispensed into 2 different wells. Subsequently, to these wells were added DiFMUP (4 mM final conc.) as the enzyme substrate. The reactions were monitored continuously over 80 min using a BioTek multi-mode fluorescence microplate reader (ex = 360 ± 10 nm; em = 460 ± 10 nm). Experiments were done in duplicate.

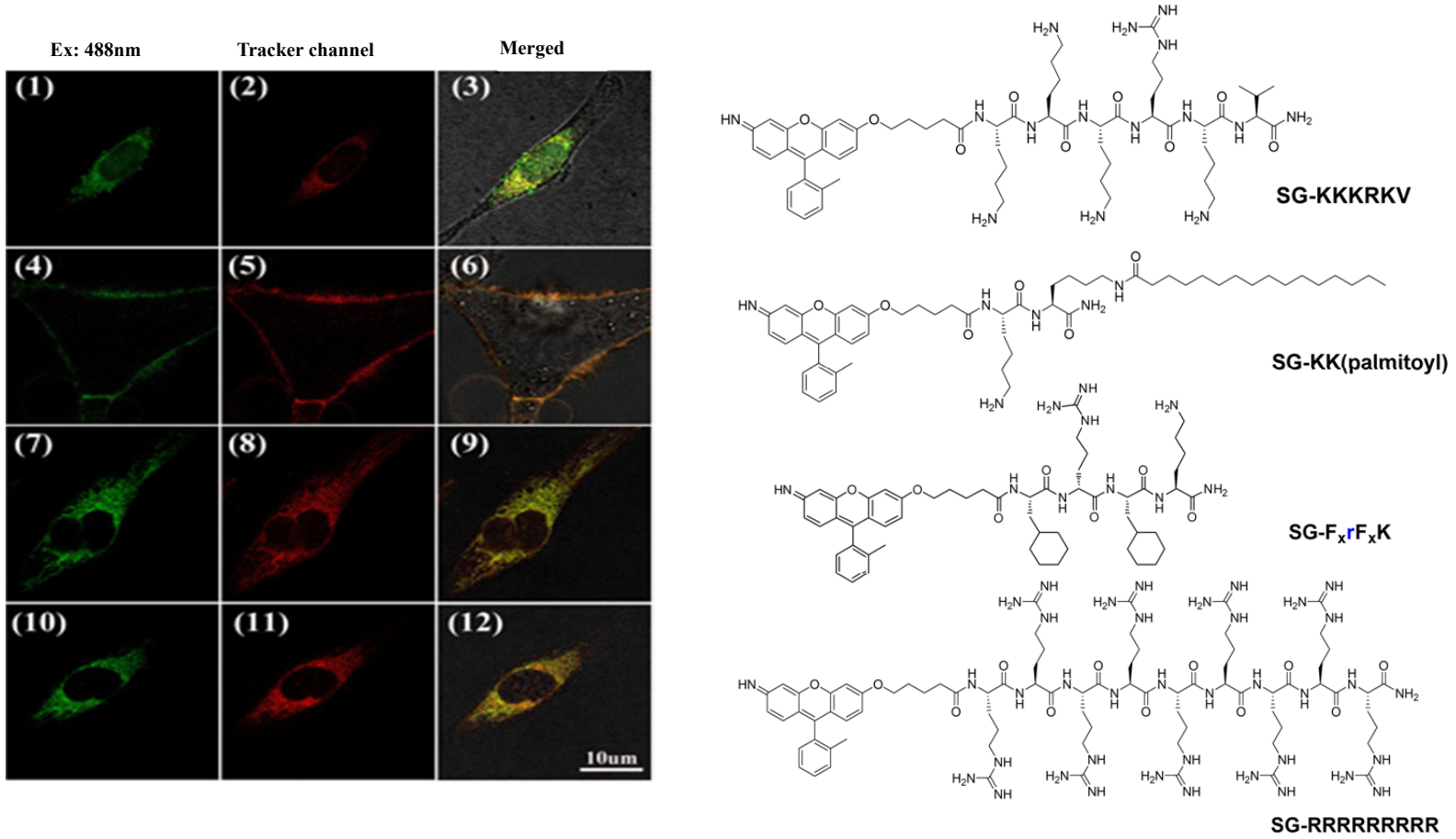


Figure S6. One-photon excited fluorescence images of HeLa cells first treated with SG-conjugated CPPs (1 μM for **SG-KKKRKV**, **SG-F_xrF_xK** and **SG-RRRRRRRRR**, 0.5 μM for **SG-KK(palmitoyl)**), Ex = 488 nm (Argon ion laser, PMT range: 500 ~ 550 nm), followed by incubation with 0.25 μg/mL of a Tracker. Excitation tracker channel (white laser): **(1-3): SG-KKKRKV**; **(4) ~ (6) SG-KK(palmitoyl**; **(7) ~ (9) SG-F_xrF_xK**; **(10) ~ (12) SG-RRRRRRRRR**. Scale bar = 10 μm.

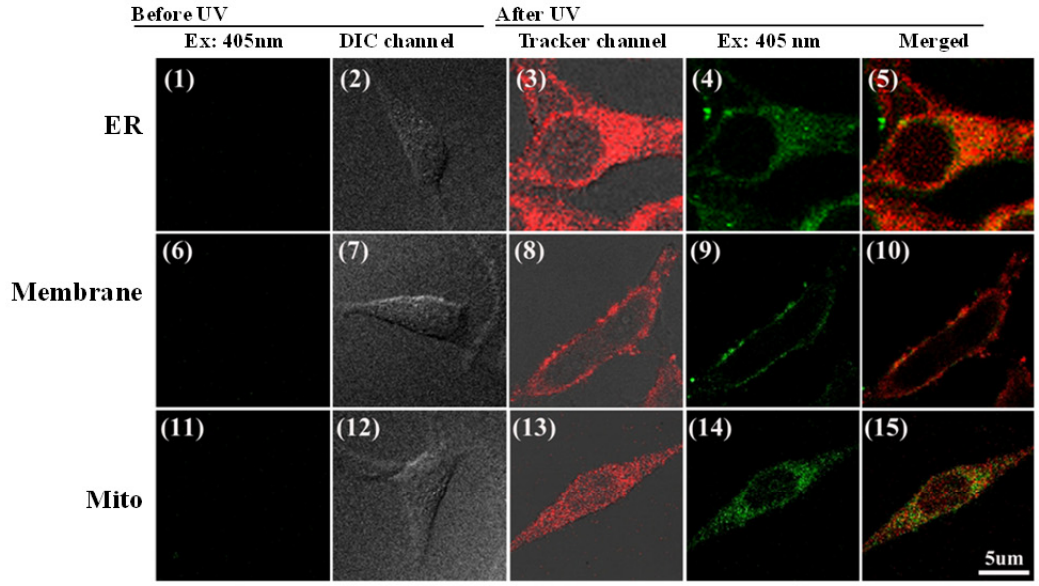


Figure S7. One-photon excited fluorescence images of HeLa cells were first treated with **YP1** to **YP3**, Ex = 405 nm (PMT range: 460 ~ 550 nm), followed by incubation with 0.25 μg/mL of a Tracker before and after UV irradiation. (1) to (5): **YP1**; (6) to (10): **YP2**; (11) to (15): **YP3**. Scale bar = 5 μm.

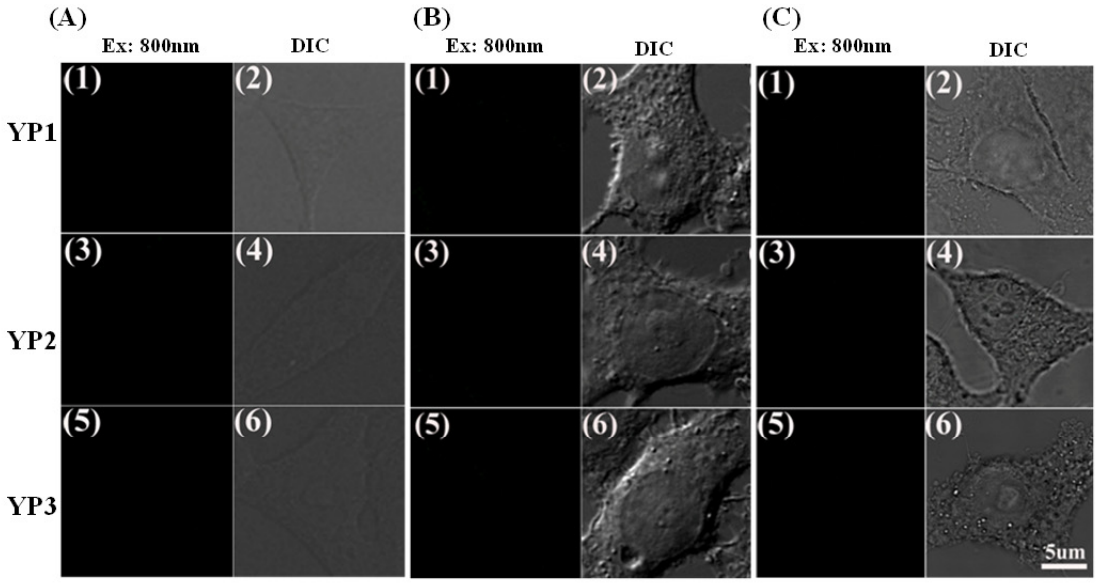


Figure S8. Two-photon excited fluorescence images of HeLa (A), and HepG2 (B) cells treated with **YP1**~**YP3**. Without UV irradiation, the cells were directly imaged with the Leica TCS SP5X Confocal Microscope System. (C) Two-photon excited fluorescence images of HepG2 treated with Na_3VO_5 (100 μM) 30 mins before incubating with **YP1**~**YP3** and UV irradiation. Scale bar = 5 μm.

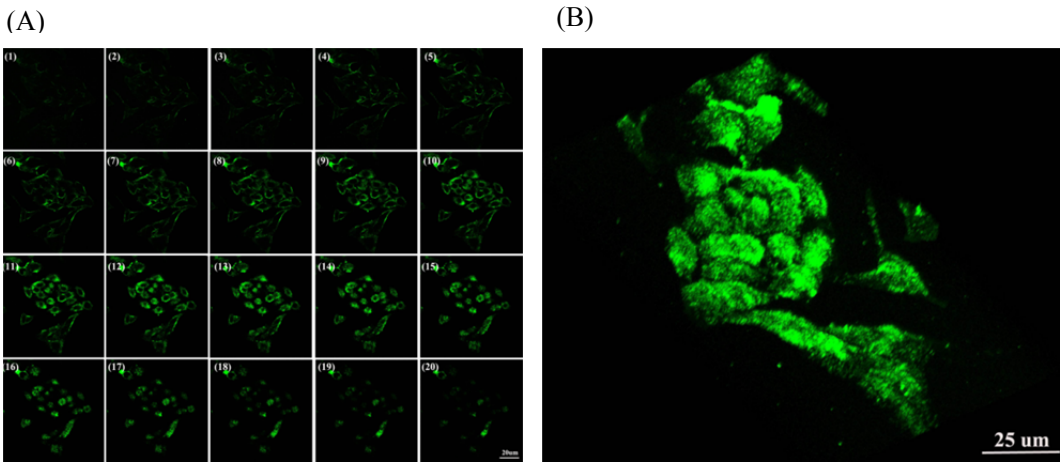


Figure S9. (A) Two-photo excited fluorescence 3D images of HepG2 cells obtained by treatments with **YP2** and UV irradiation (2 min). The images were taken at an 11.96 μm Z-axis thickness (0.63 μm for each picture), Ex = 800 nm. **(1)** to **(20)** is from the bottom to top of the cells, respectively. Scale bar = 20 μm. (B) Two-photon excited fluorescence 3D images of HepG2 cells reconstructed from (A). Scale bar = 25 μm.

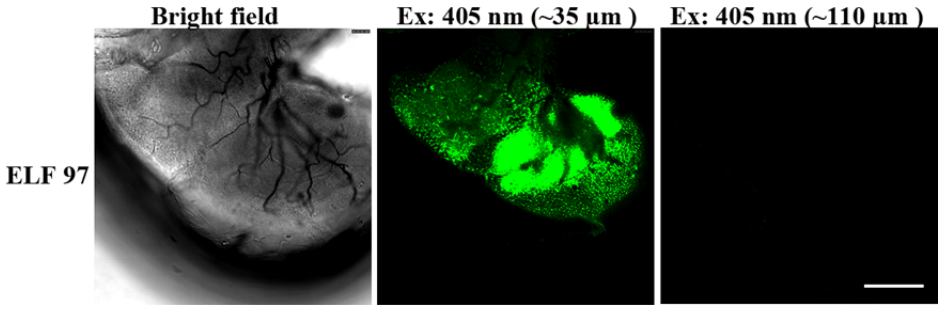
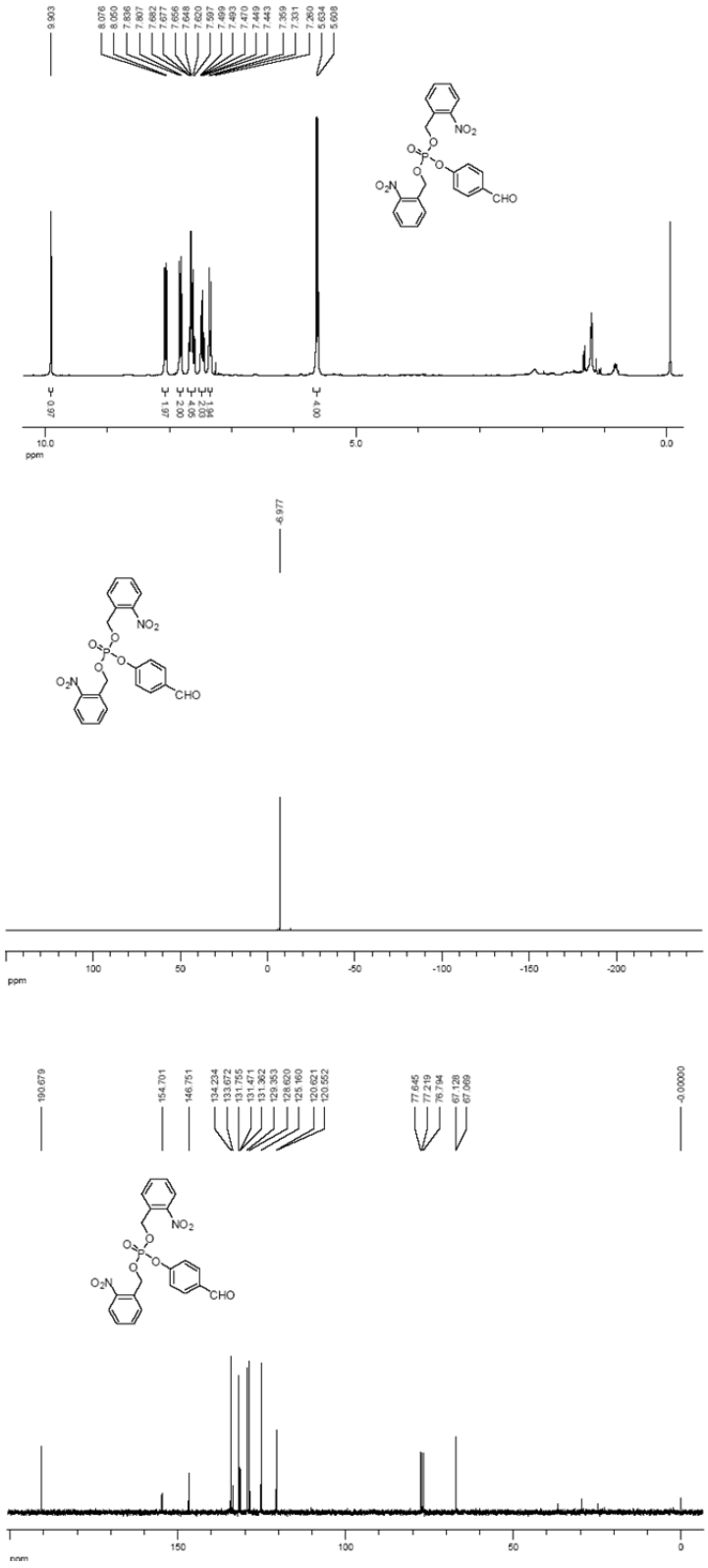
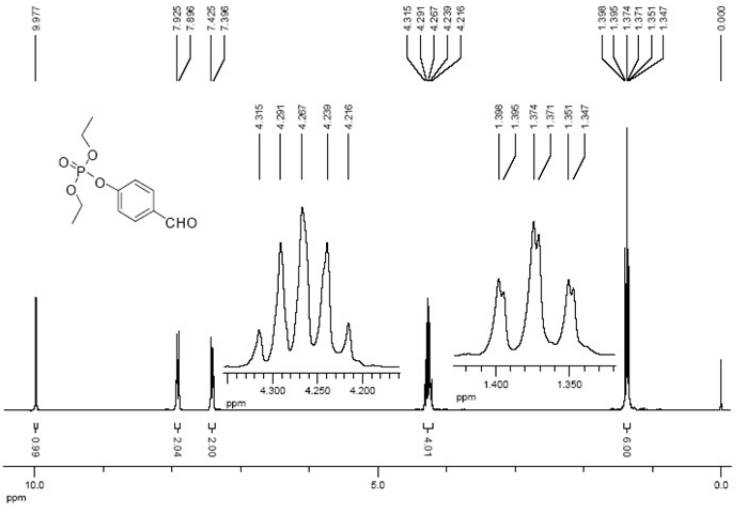


Figure S10. One-ohoton excited fluorescence image of ELF 97™ detection of phosphatase activities in fresh brains of one day-old live female Drosophila. Images were taken at a depth of ~ 35 μm and ~110 μm with 40 × magnification. Drosophila brains were incubated with ELF 97™ phosphatase substrate (100-fold dilution from commercial kit). Scale bar = 150 μm.

^{1}H, ^{31}P and ^{13}C NMR spectra.

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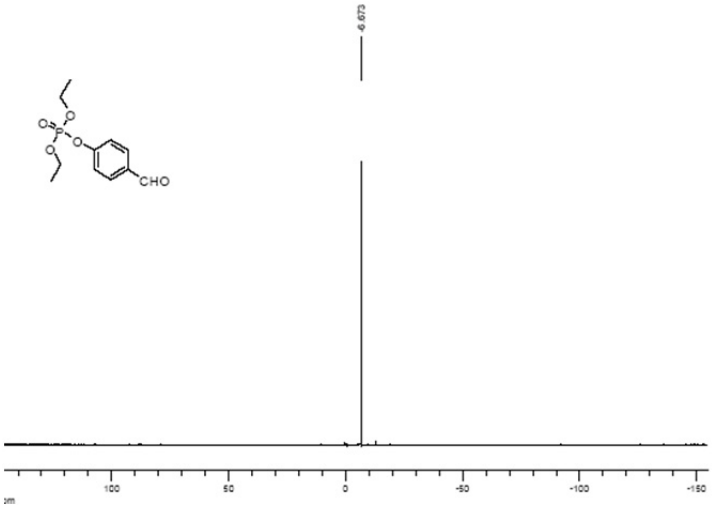
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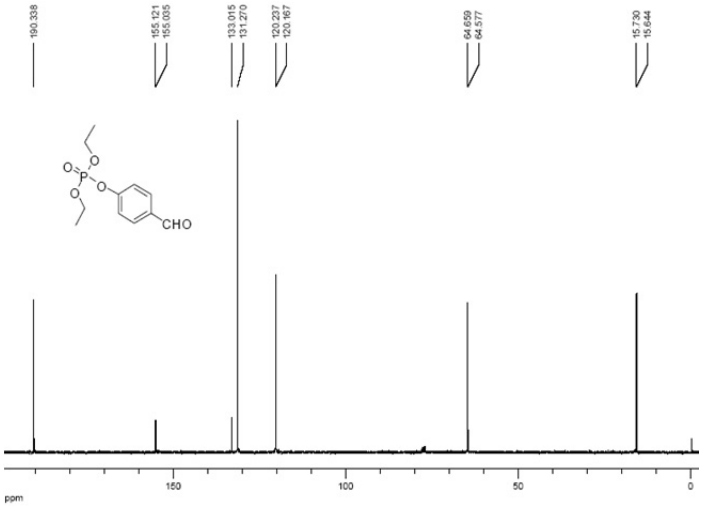
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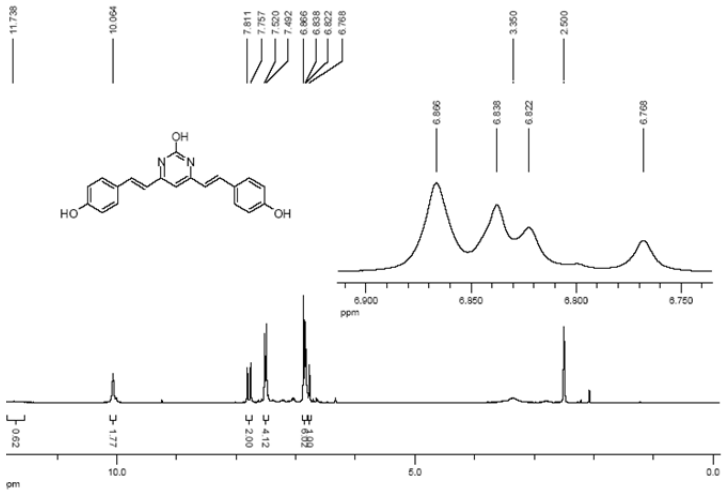
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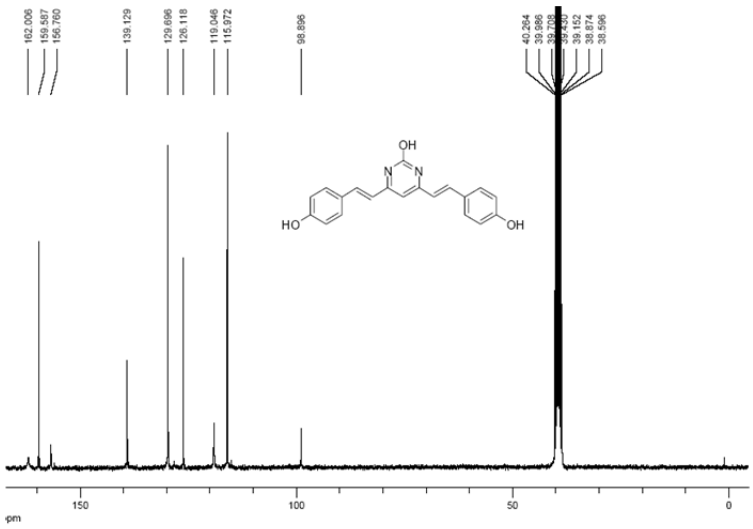
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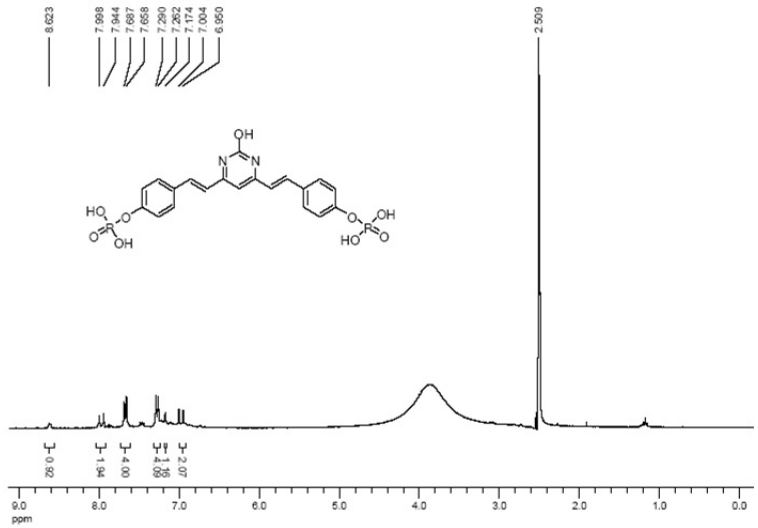
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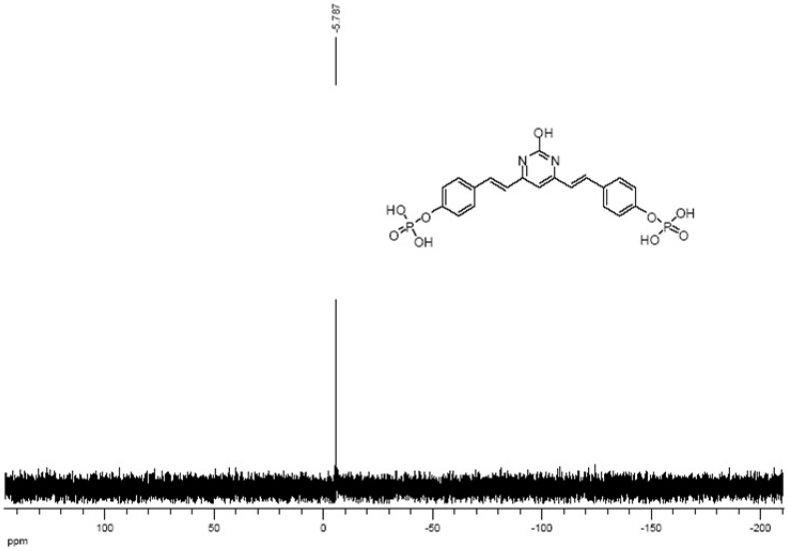
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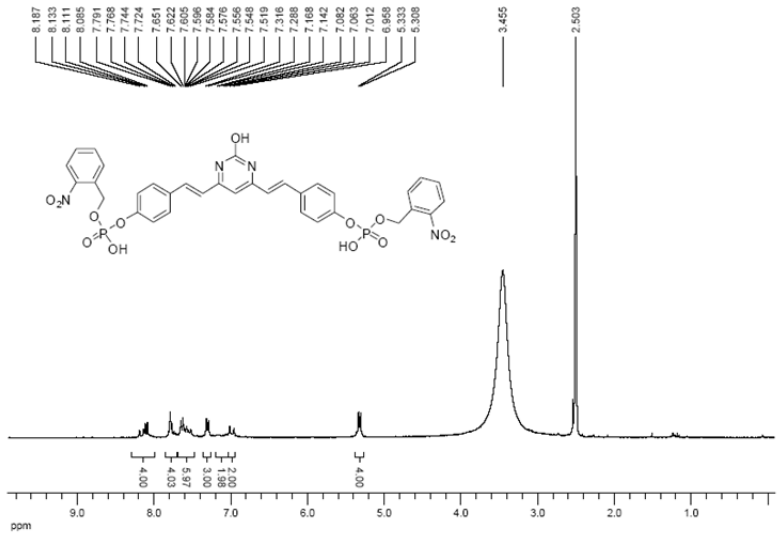
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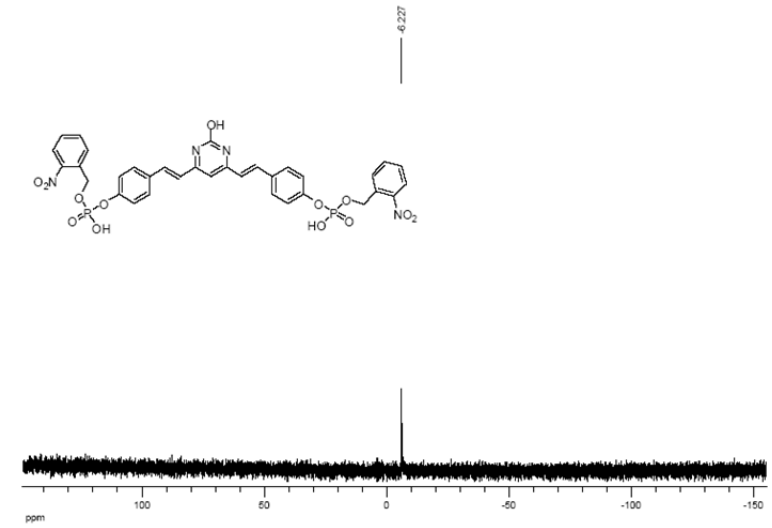
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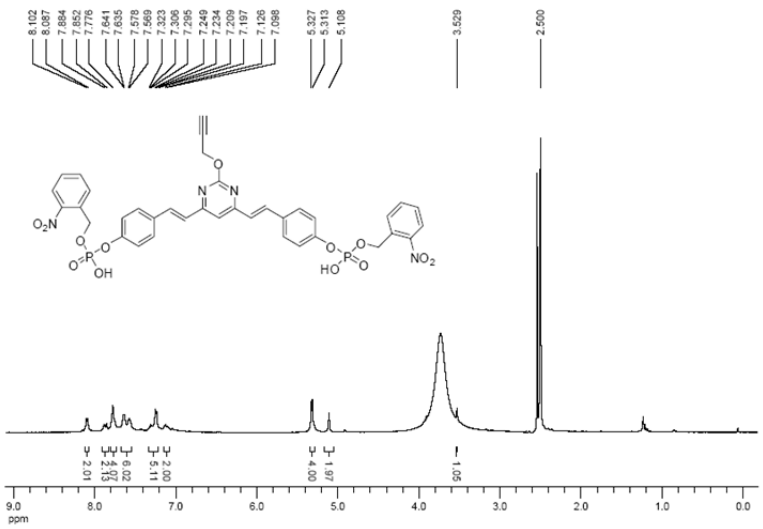
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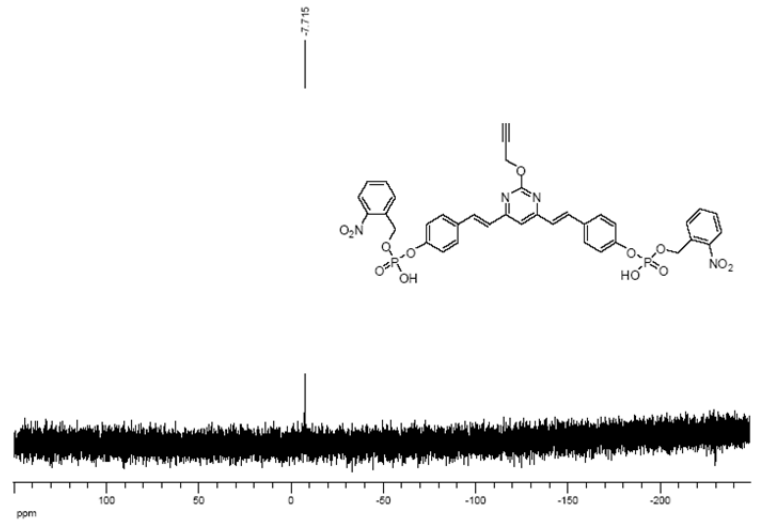
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