Supporting Information for:

Ratiometric Fluorescent Probe for Imaging of Pantetheinase in Living Cells

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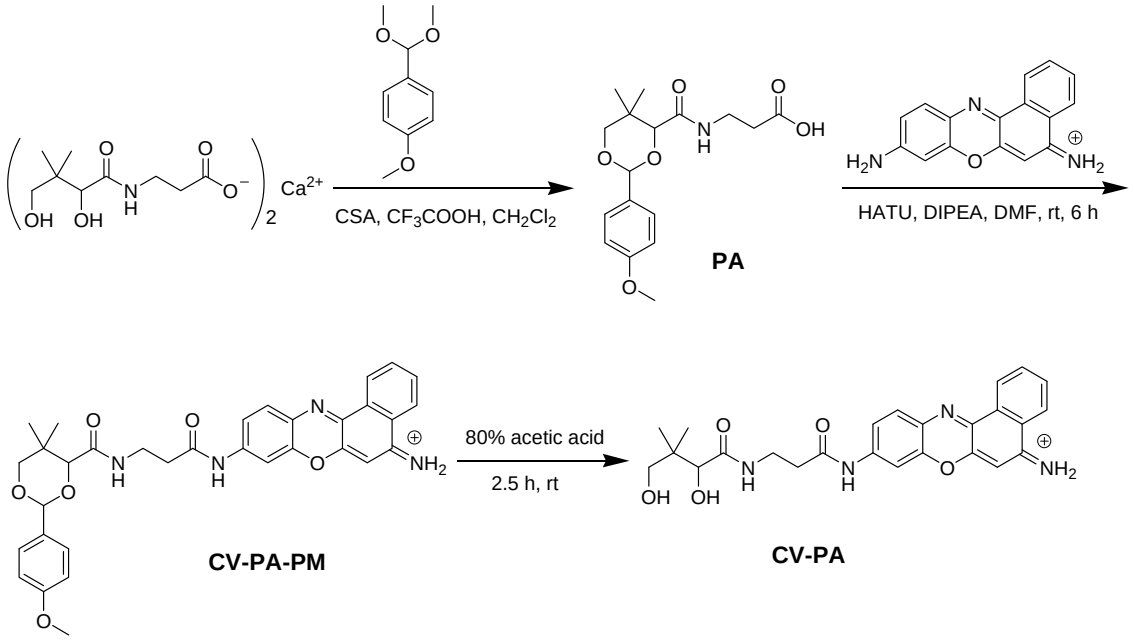
Table of Contents



Scheme S1. Synthesis of CV-PA.

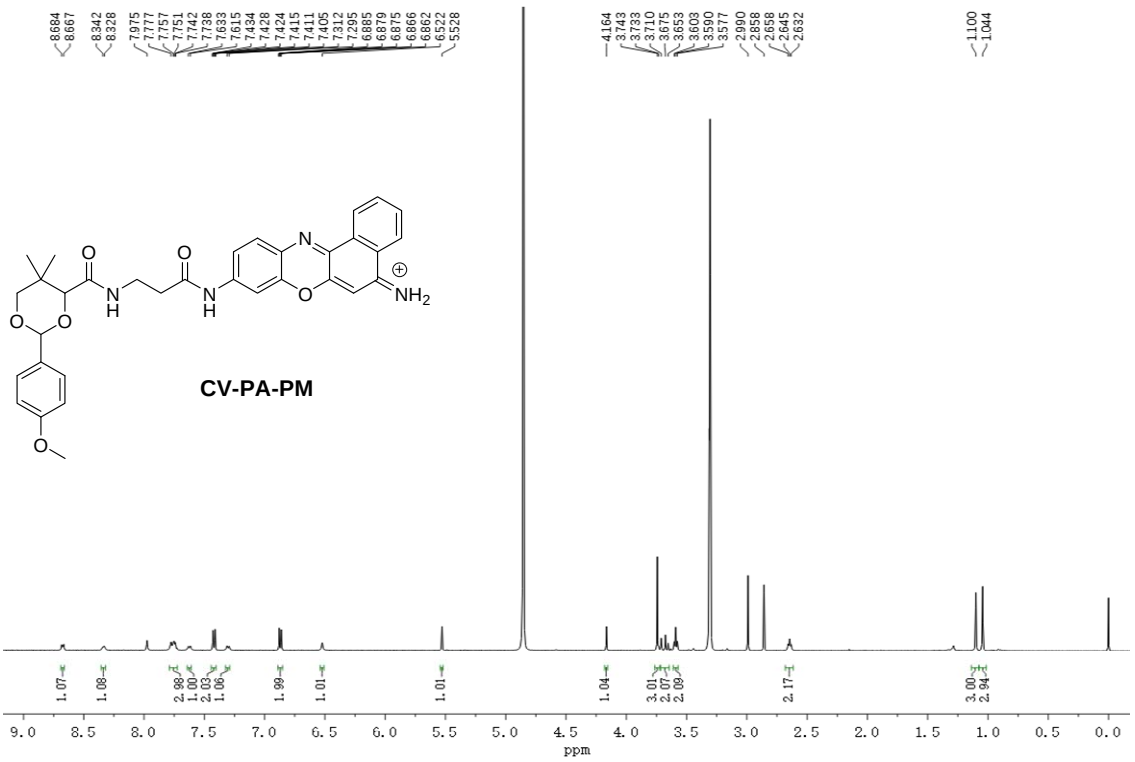


Figure S1. ^{1}H NMR spectrum of CV-PA-PM (500 MHz, CD_3OD, 298 K).

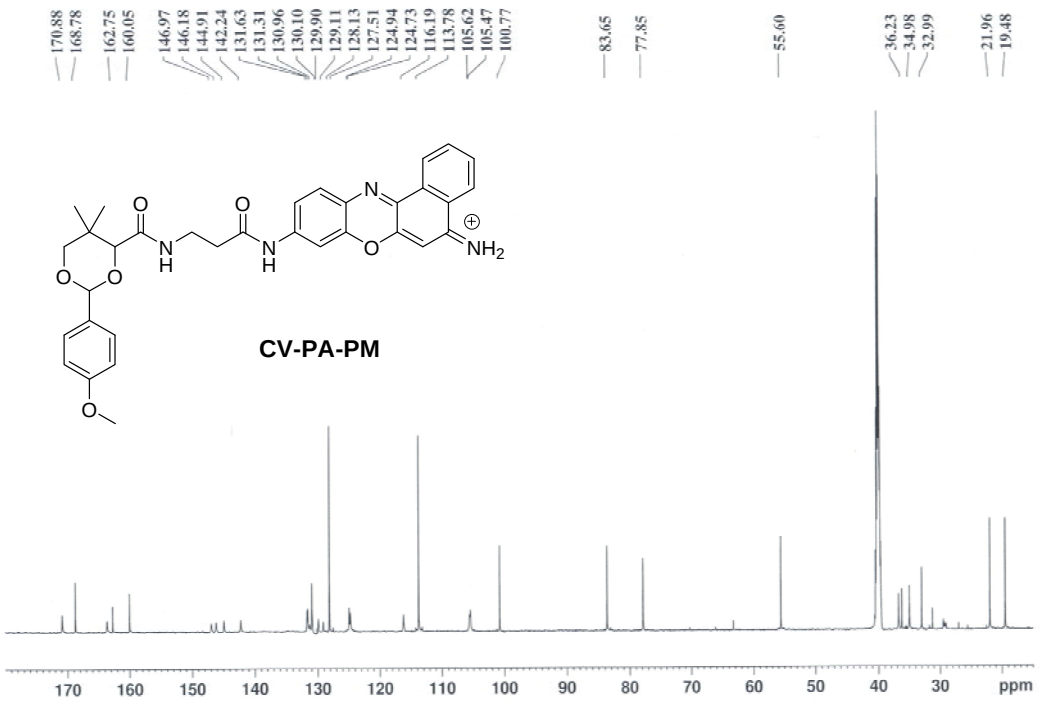


Figure S2. ^{13}C NMR spectrum of CV-PA-PM (150 MHz, DMSO-d_6, 298 K).

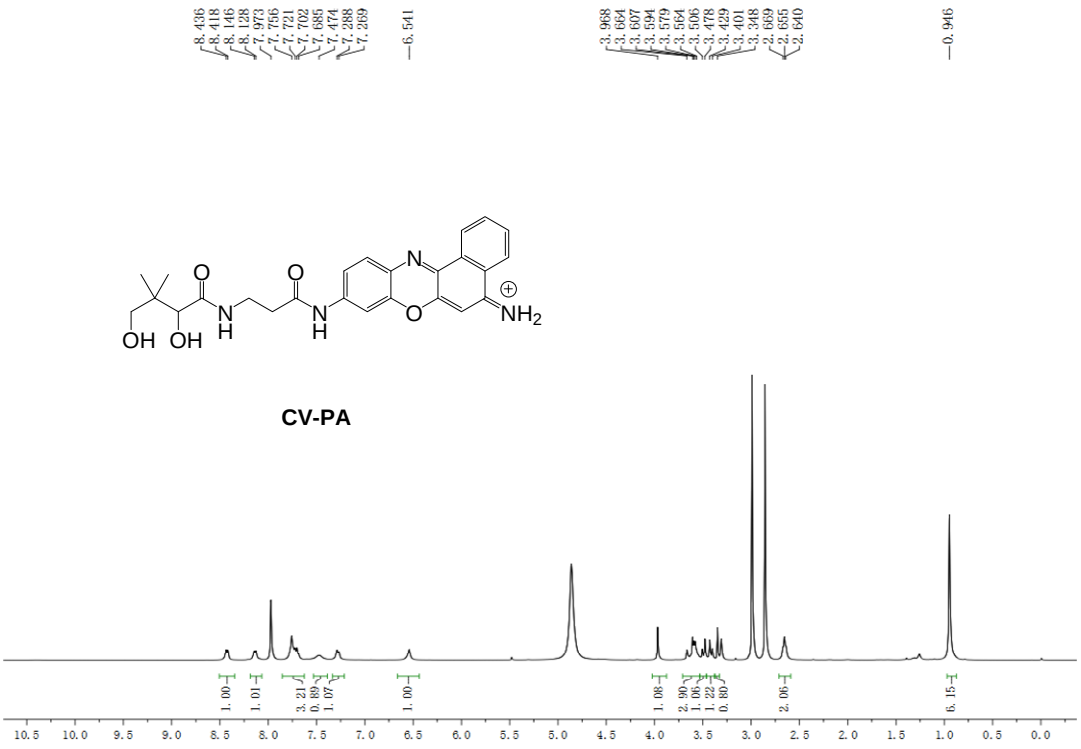


Figure S3. ^{1}H NMR spectrum of CV-PA (400 MHz, CD_3OD, 298 K).

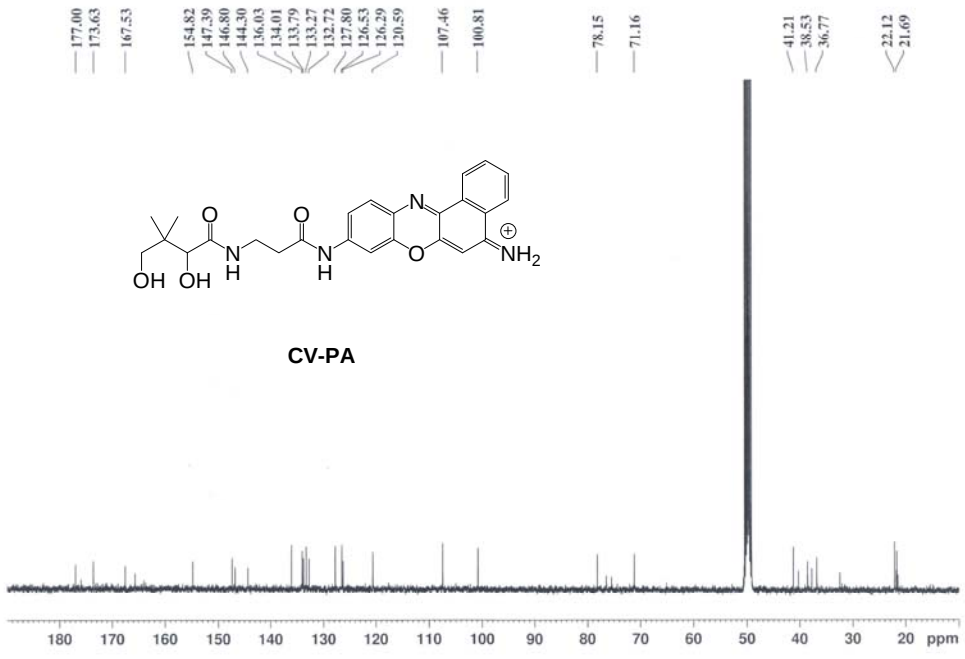


Figure S4. ^{13}C NMR spectrum of CV-PA (100 MHz, CD_3OD, 298 K).

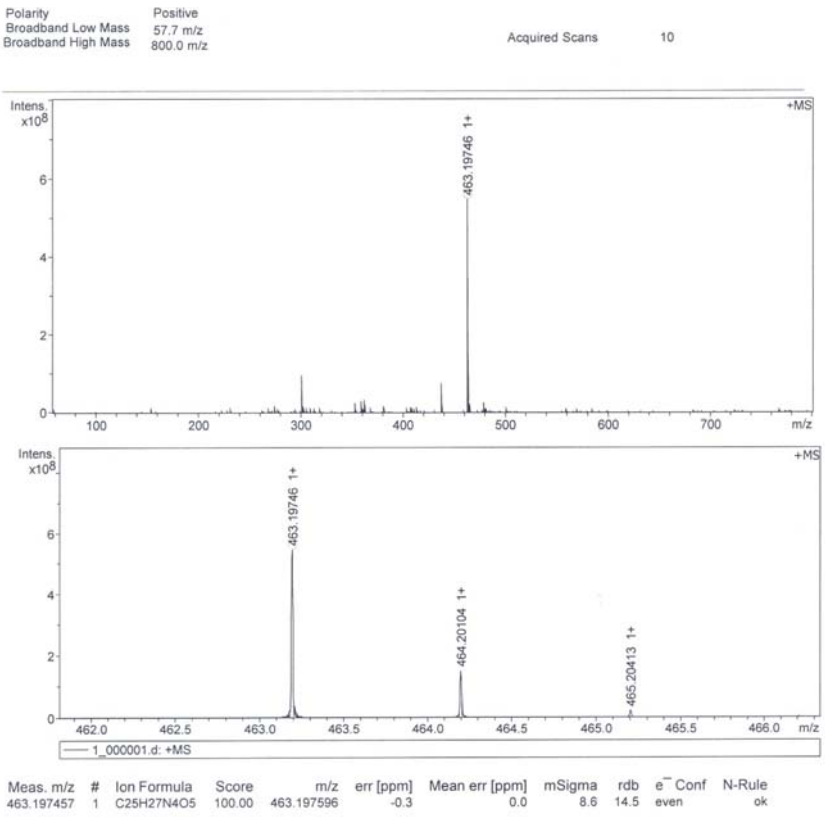


Figure S5. HR-ESI-MS of CV-PA.

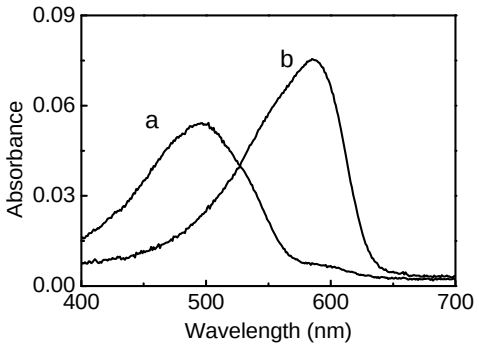


Figure S6. Absorption spectra of CV-PA (10 μM) reacting without (a) and with (b) pantetheinase (400 ng/mL) at 37 °C for 1 h in 20 mM phosphate buffer (pH 7.4).

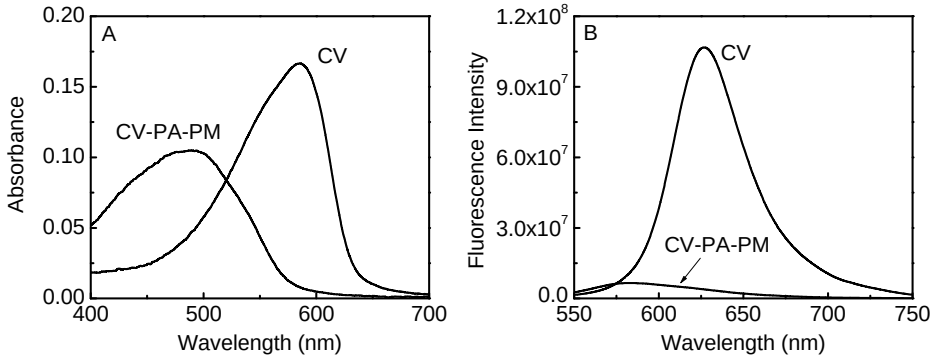


Figure S7. (A) Absorption and (B) fluorescence spectra of 10 μM CV and CV-PA-PM in pH 7.4 phosphate buffer (20 mM). λ_{ex} = 525 nm.

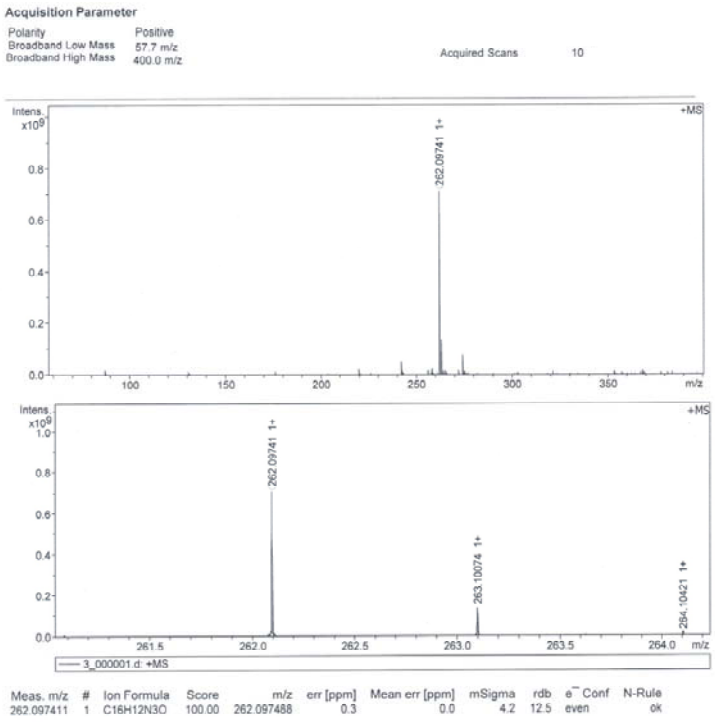


Figure S8. HR-ESI-MS of the reaction solution of CV-PA with pantetheinase.

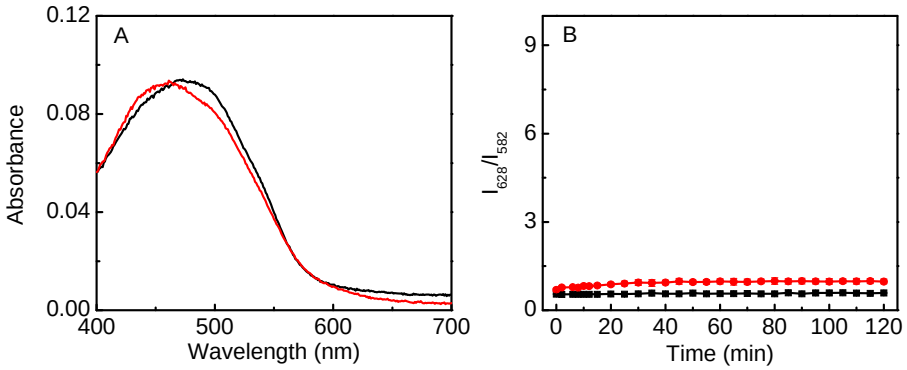


Figure S9. (A) Absorption spectra of CV-PA-PM (10 μM) before (black) and after (red) reaction with 400 ng/mL pantetheinase. (B) Ratiometric fluorescence responses of CV-PA-PM (10 μM) to 0 (black) and 400 ng/mL (red) pantetheinase with time.

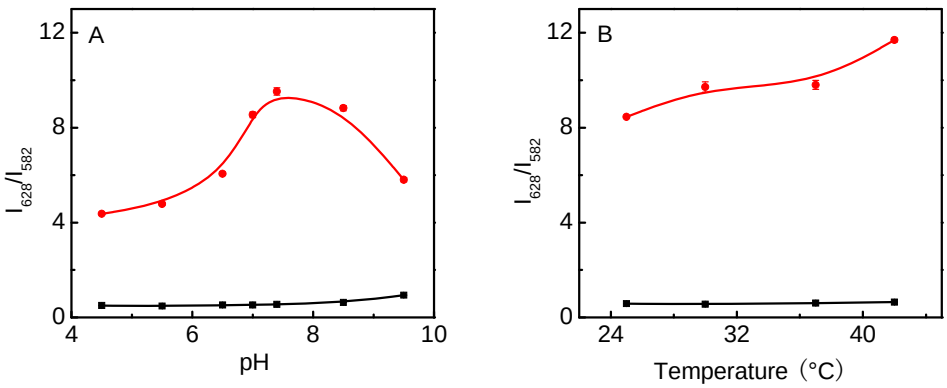


Figure S10. Effects of pH (A) and temperature (B) on the ratiometric signal of CV-PA (10 μM) reacting with (■) 0 and (●) 400 ng/mL pantetheinase for 2 h. λ_{ex} = 525 nm.

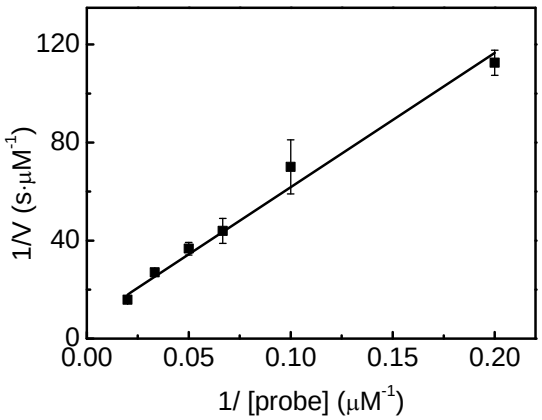


Figure S11. Lineweaver-Burk plot of CV-PA (5-50 μM) catalyzed by pantetheinase (50 ng/mL) in the phosphate buffer (20 mM, pH 7.4) at 37 °C. λ_{ex} = 525 nm.

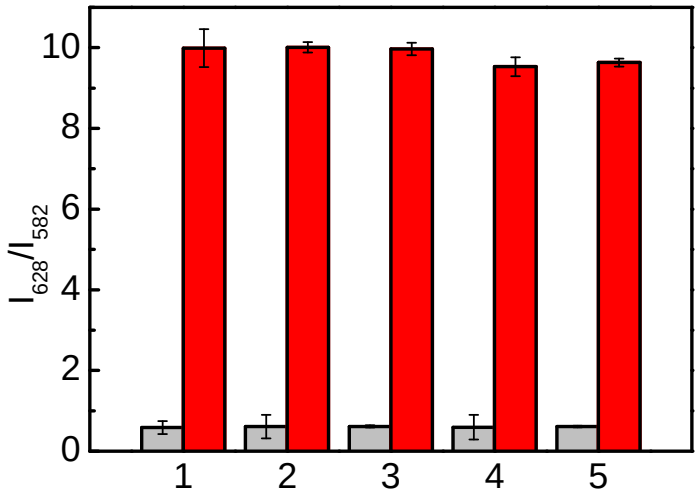
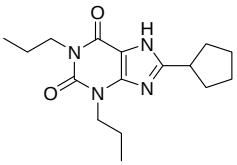
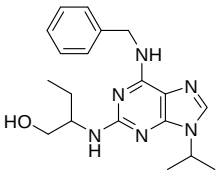
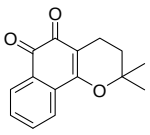
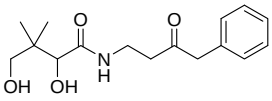


Figure S12. Effects of different inhibitors (400 μM) on the fluorescence of 10 μM of CV-PA (grey) and CV (red). (1) Without inhibitor (control); (2) 8-cyclopentyl-1,3-didropylxanthine (CPDX); (3) roscovitine; (4) β-lapachone; (5) RR6.

Table S1. IC_{50} values of inhibitors towards pantetheinase

Inhibitor	Structure	IC_{50} value (μM)[a]
CPDX		2.12 ± 0.21
roscovitine		10.10 ± 1.25
β-lapachone		0.45 ± 0.05
RR6		0.63 ± 0.03

[a] Mean of three determinations ± standard deviation.

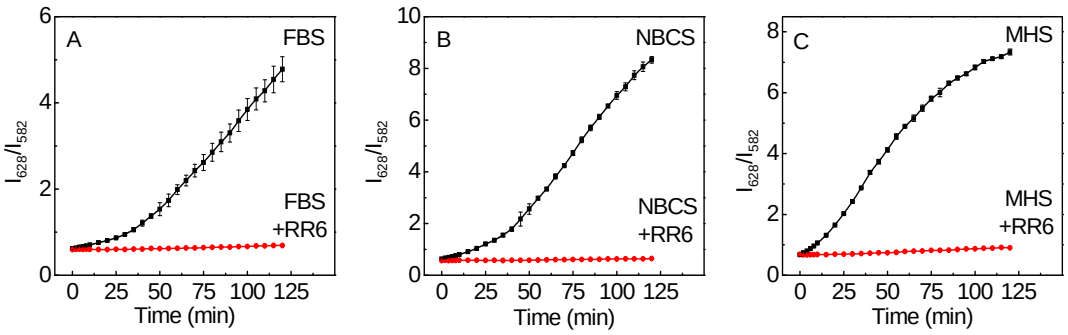


Figure S13. Time-dependent fluorescence signal of CV-PA (10 μM) in serum samples. (A) FBS; (B) NBCS and (C) MHS without (■) and with (●) 10 μM RR6.

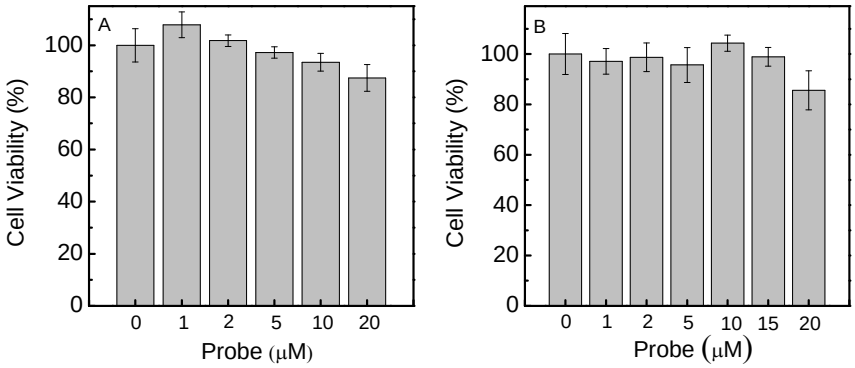


Figure S14. Cell viability of (A) HK-2 and (B) LO2 treated with CV-PA at varied concentrations. The results are the mean ± standard deviation of five separate measurements

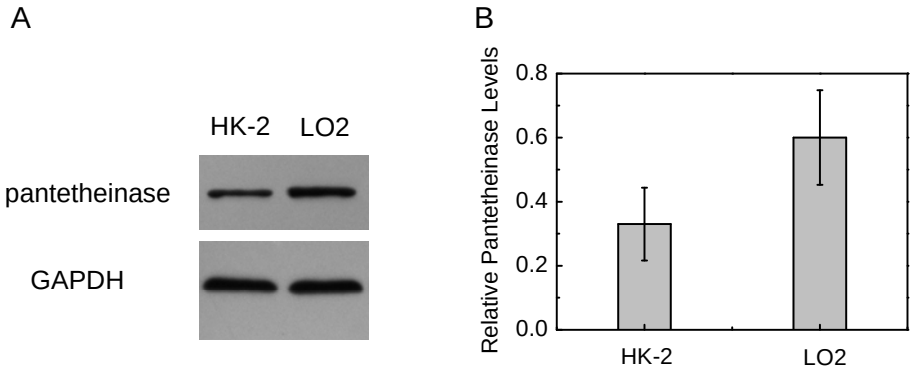


Figure S15. (A) Western blot analysis of HK-2 and LO2 cell lines. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a protein standard. (B) Relative pantetheinase level in panel A.

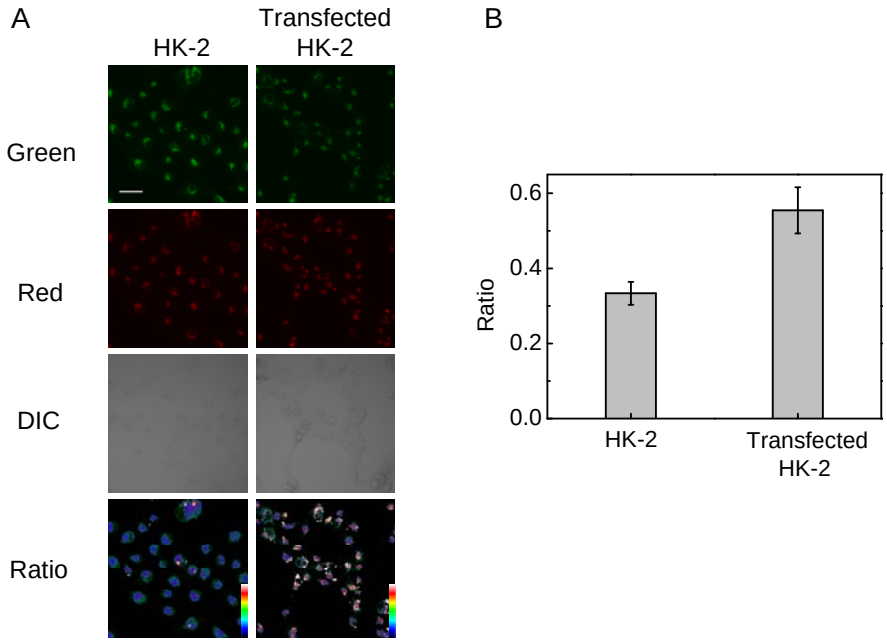


Figure S16. (A) Confocal fluorescence images of HK-2 and pantetheinase transfected HK-2 cells. Scale bar: 50 μm. (B) The relative ratio value of the corresponding ratio images in panel A.