Supporting information for

A novel two-photon fluorescent probe for detecting FA based on the coumarin derivative and its applications in living cells, zebrafish and tissues

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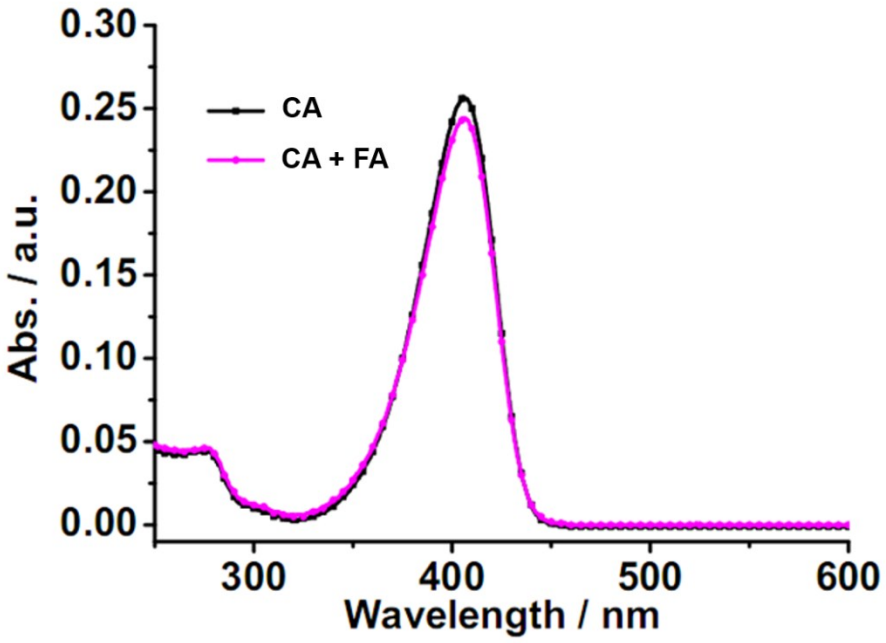

Fig. S1. The absorption spectra of fluorescent probe **CA** (10 μM) in the presence or absence of FA (200 μM).

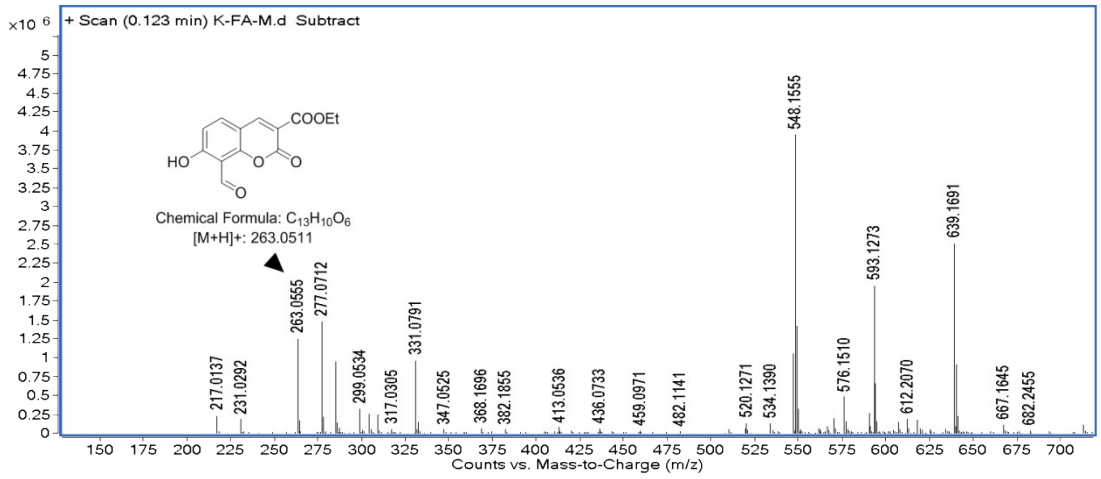

Fig. S2. The HRMS spectrum for reaction mixture of **CA** (10 μM) and FA (200 μM).

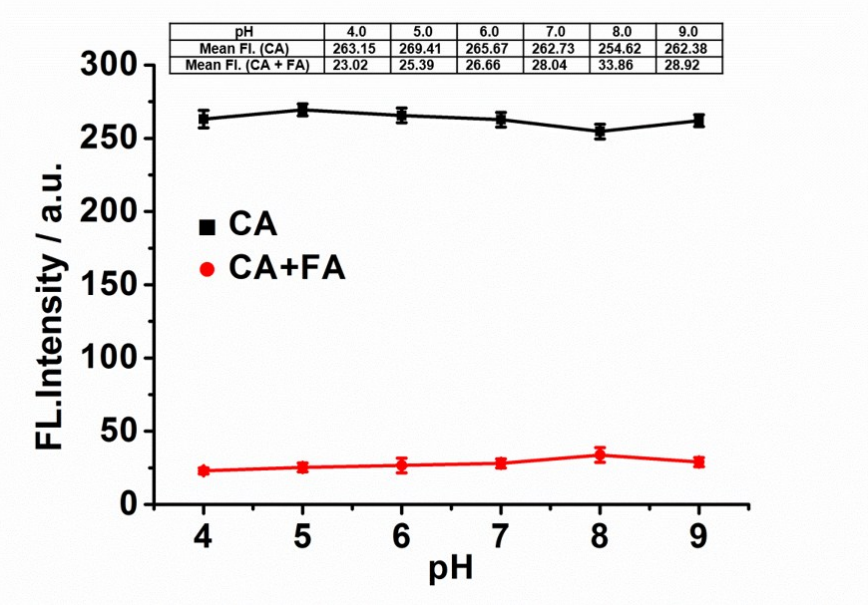

Fig. S3. The fluorescent intensities (I_{451}) of **CA** (10 μM) in the presence or absence of FA (200 μM) under various pH (pH = 4.0 - 9.0) conditions.

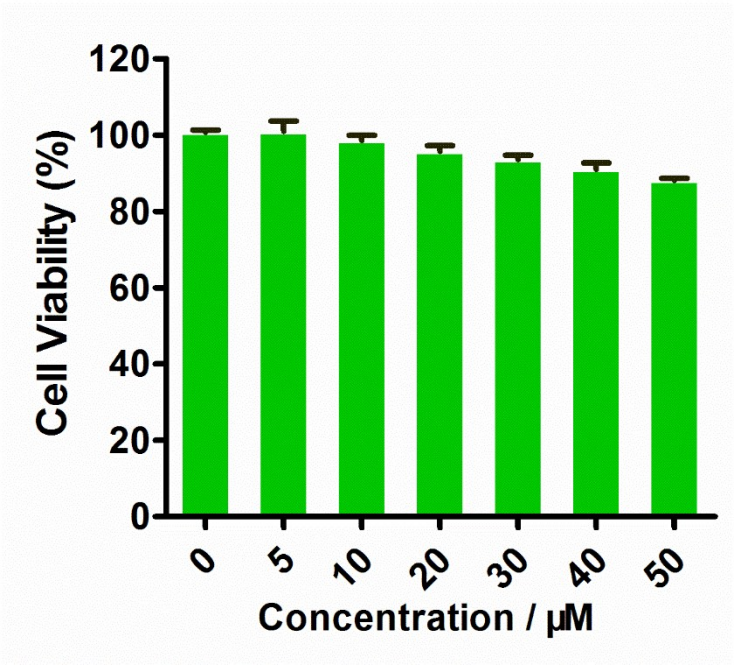

Fig. S4. Cell viability of HeLa cells treated with different concentrations (0 - 50 μM) of **CA** for 24 h.

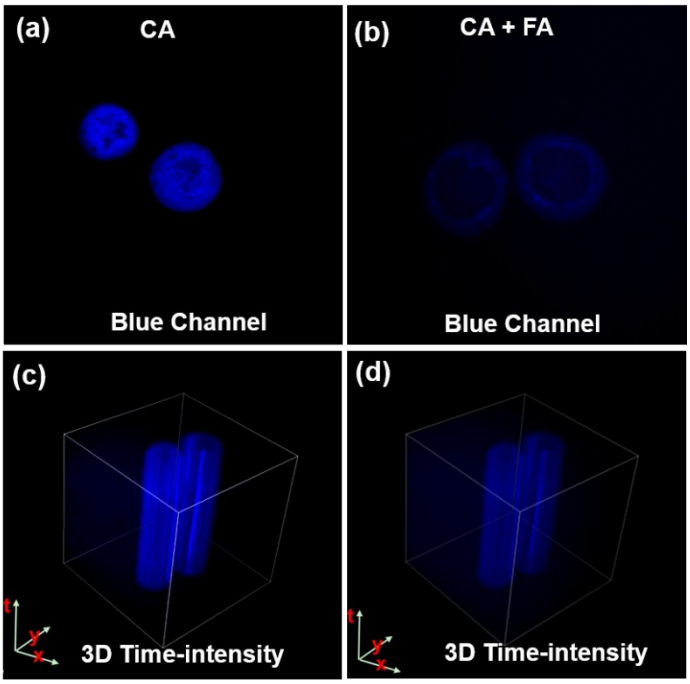

Fig. S5. Fluorescence images for detecting exogenous FA in HeLa cells with **CA** after continuous irradiation for 30 min. (a) HeLa cells incubated with 10 μM only **CA** for 30 min, and then irradiation for 30 min; (b) cells were firstly incubated with 10 μM **CA** for 30 min, and then treated with FA (50 μM) for another 30 min, followed by irradiation for 30 min. Upper row of a and b: the representative images in blue channels at 15 min with irradiation; bottom row of c and d: 3D time-dependent fluorescence images after 30 min irradiation in blue channels of a and b respectively. Blue Channel: λ_{ex} = 404 nm, λ_{em} = 425 - 475 nm.

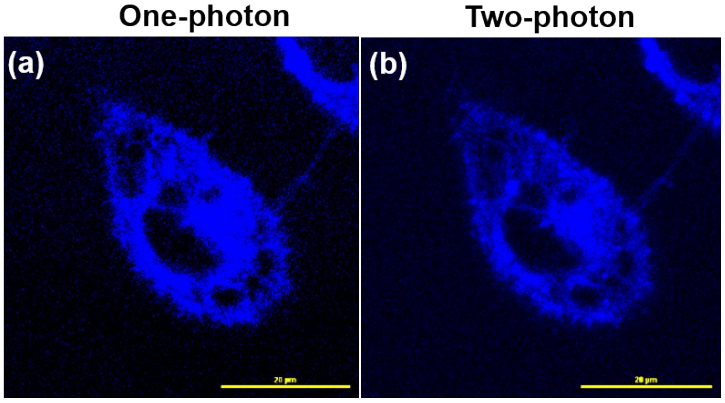

Fig. S6. One-photon (a) and two-photon (b) fluorescence images of HeLa cells treated with **CA** for 30 min. One-photon mode: λ_{ex} = 404 nm, λ_{em} = 425-475 nm; two-photon mode: λ_{ex} = 800 nm, λ_{em} = 425-475 nm.

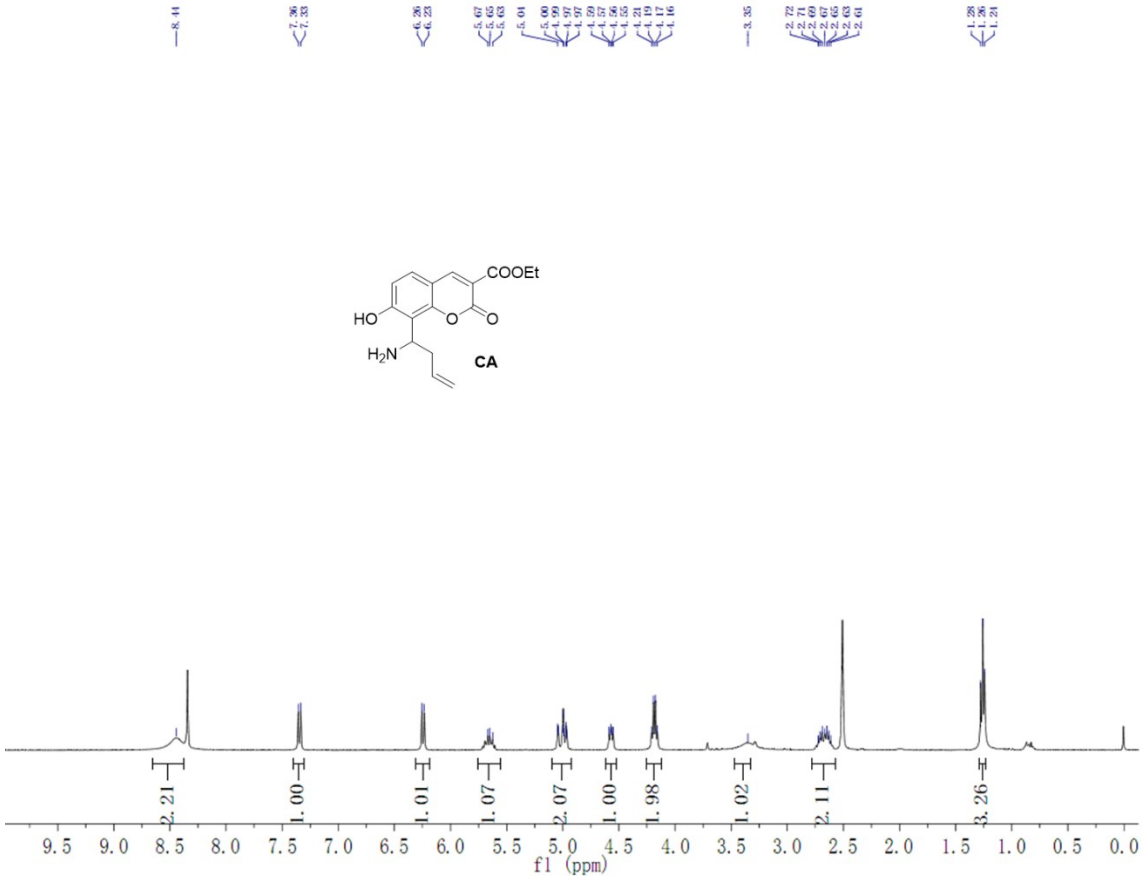

Fig. S7. ^{1}HNMR (100 MHz, DMSO-d_6) spectrum of **CA**.

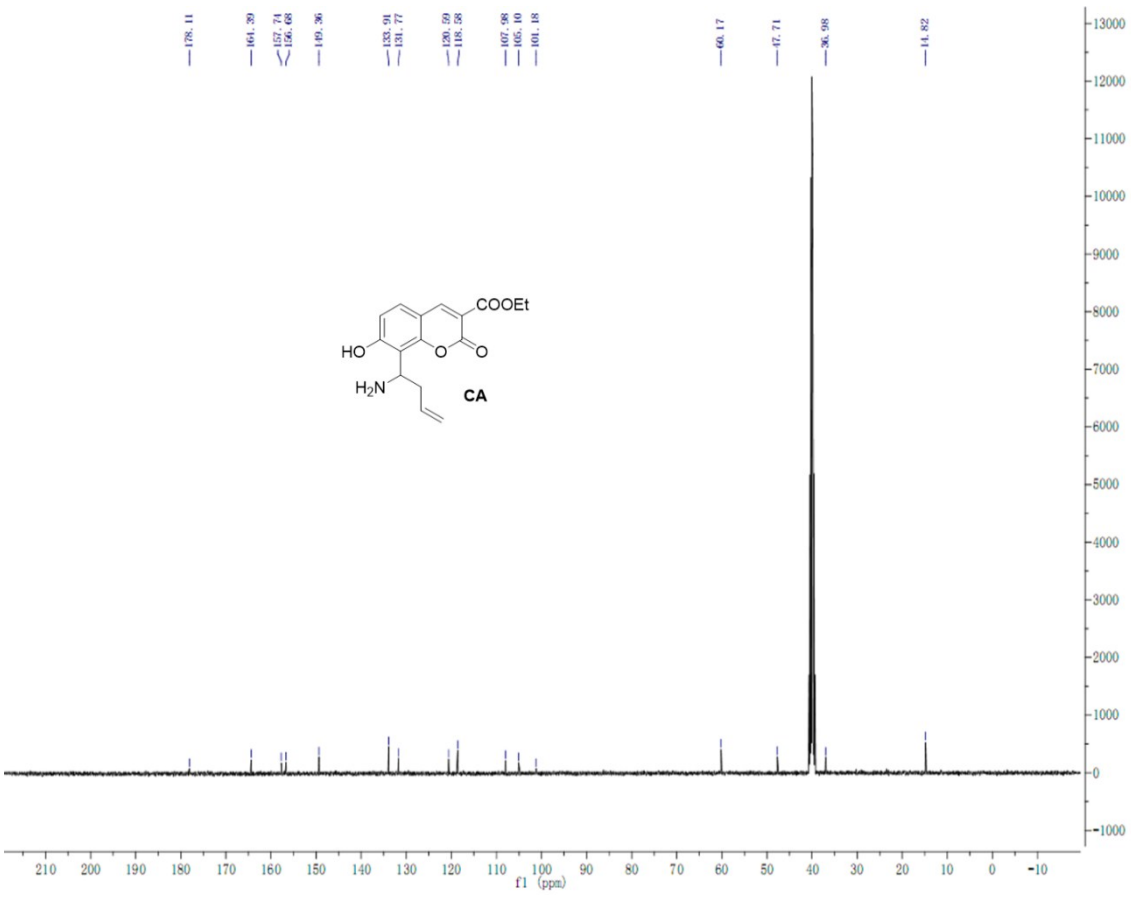

Fig. S8. ^{13}CNMR (100 MHz, DMSO-d_6) spectrum of **CA**.

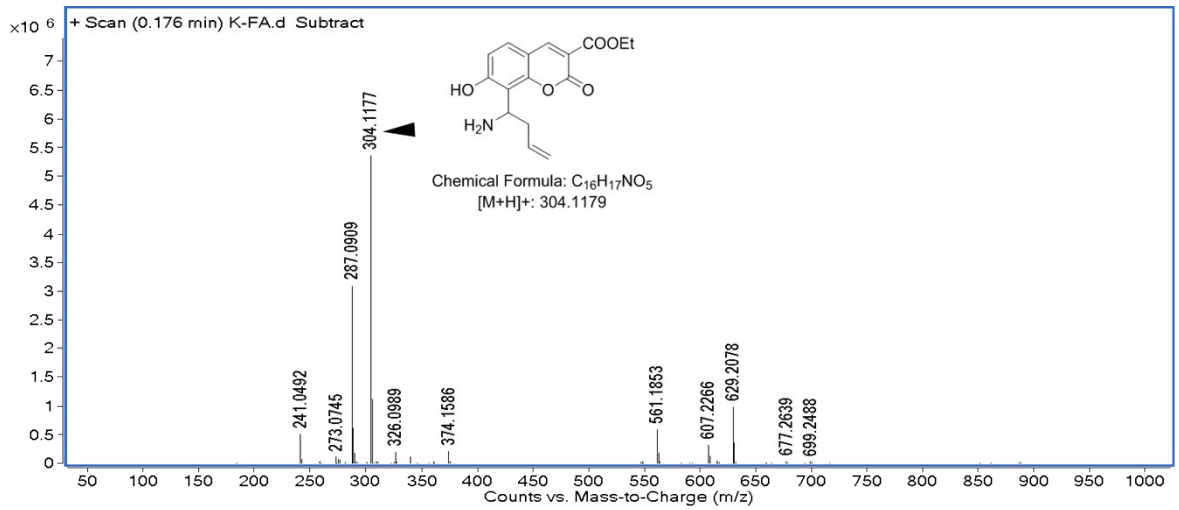

Fig. S9. HRMS spectrum of **CA**.