A Fluorogenic Probe for Ultrafast and Reversible Detection of Formaldehyde in Neurovascular Tissues

Supplementary Figures

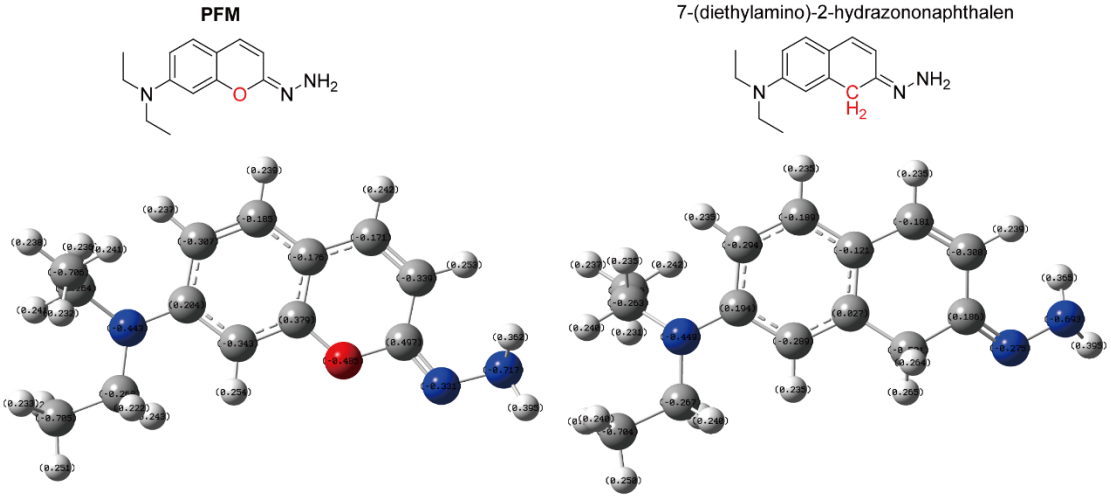


Figure S1. Electron density map of **PFM** and the control compound calculated by Gaussian using B3LYP method.

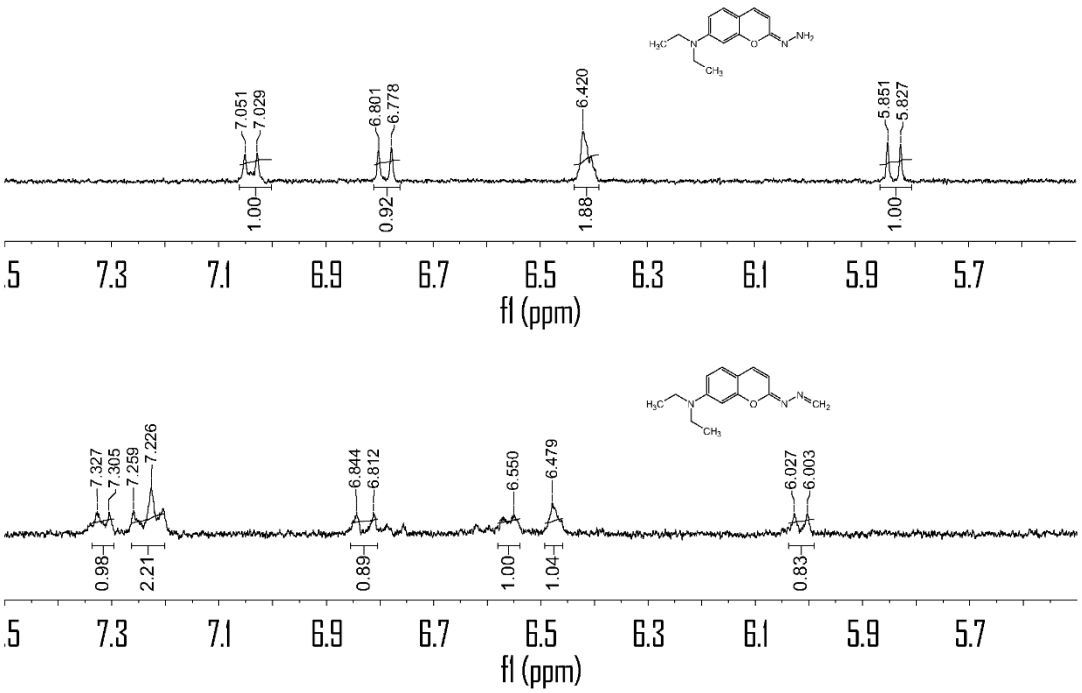


Figure S2. ^{1}H NMR of **PFM** in DMSO-d_6/D_2O (1:1) before (top) and after (bottom) the addition of 10 eq. formaldehyde.

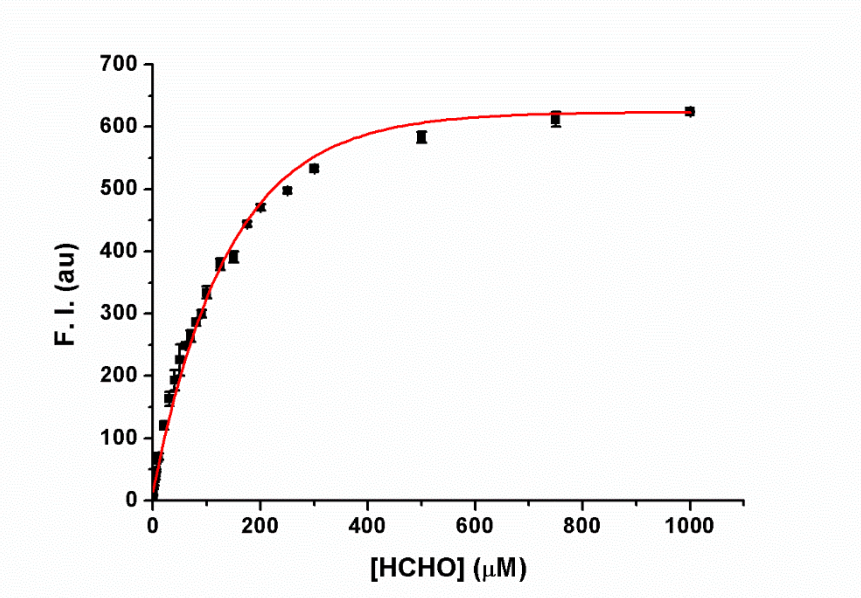


Figure S3. Plot of fluorescent intensity at 500 nm of **PFM** (10 μM) against the concentrations of FA gave an exponential dependence. Data were acquired in PBS (10 mmol, pH 7.4) at ambient temperature after a reaction time of 5 min (λ_{ex} 451 nm).

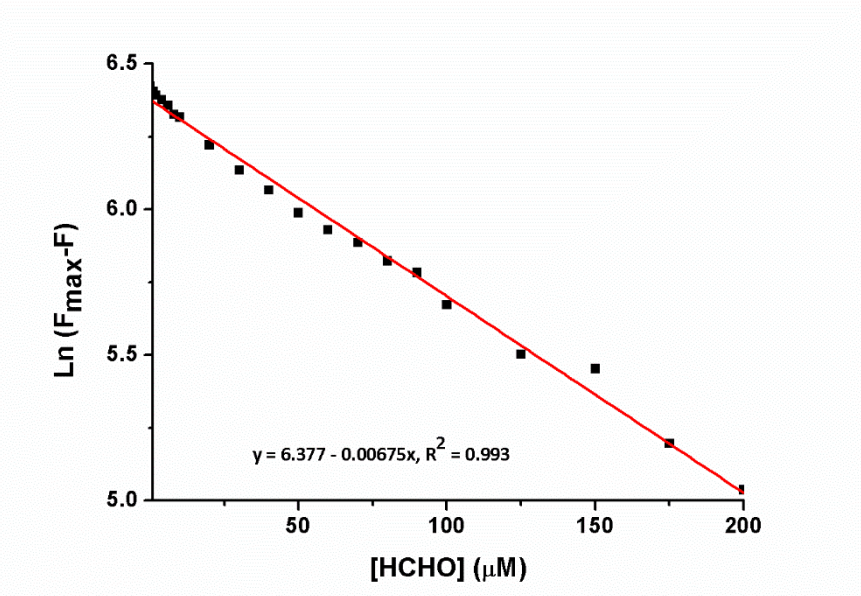


Figure S4. The Napierian logarithm of F_{max} minus F correlated linearly with FA concentrations (0 to 200 μM), and wherein F_{max} is the maxium fluorescent intensity at 500 nm of **PFM** after the treatment of a large enough amount of FA, and F is the fluorescence after the treatment of corresponding amount of FA. Data were acquired in the same way as those in **Figure S3**.

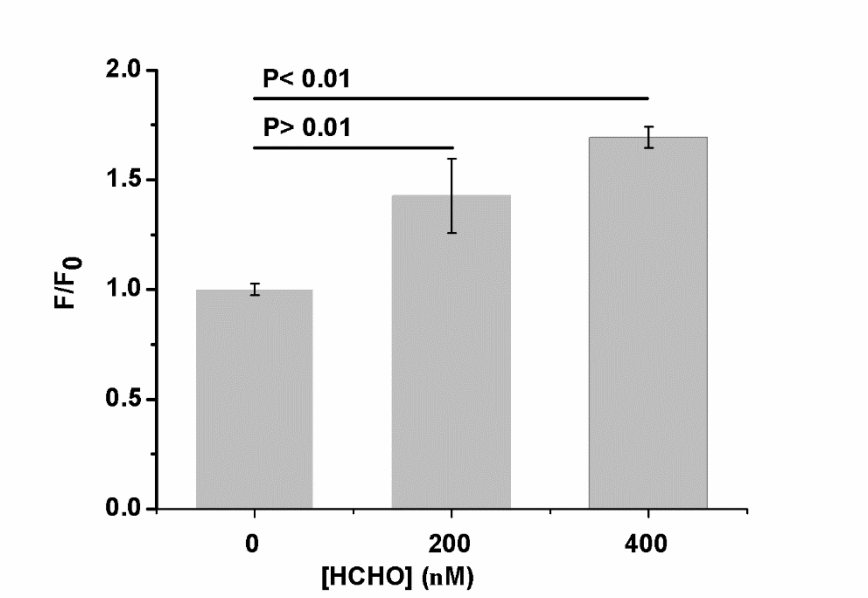


Figure S5. The detection limit determination of **PFM.** Results were obtained as the concentration of FA that induced a statistically significant increase in fluorescence intensity at 500 nm compared with a blank control after 5 min with a P-value < 0.01. Experiments were carried out by incubating **PFM** (10 μM) with FA (0, 200, 400 nM) in PBS (10 mM, pH 7.4) at ambient temperature for 5 min and then collecting the emission at 500 nm by excitation at 451 nm. F: fluorescence intensity at 500 nm after treating **PFM** with various concentrations of FA; F_0: fluorescence intensity at 500 nm of probe blank control. Statistical analyses were performed with a two-tailed Student's t-test (n = 3). Error bars are standard deviation.

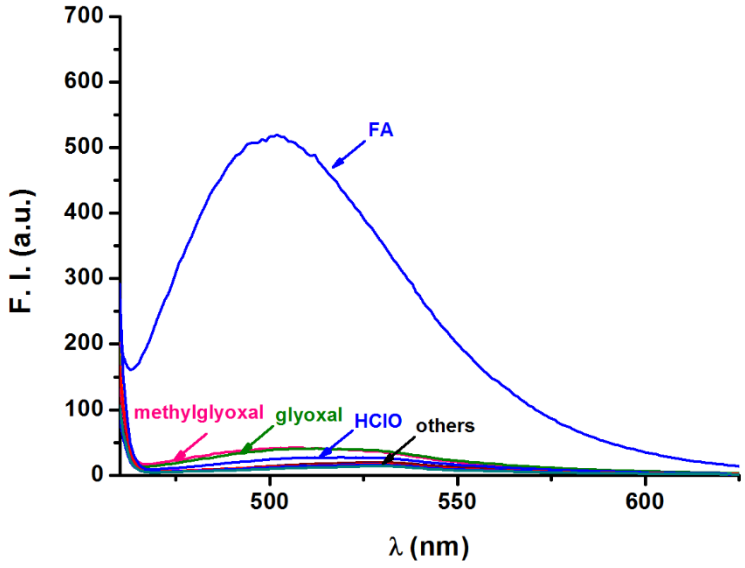


Figure S6. Fluorescent spectra of **PFM** (10 μM) in the presence of various bio-relevant carbonyl species (300 μM) or biologically oxidizing or reducing conditions. Spectra were taken in PBS (10 mM, pH 7.4) at ambient temperature with excitation 451 nm.

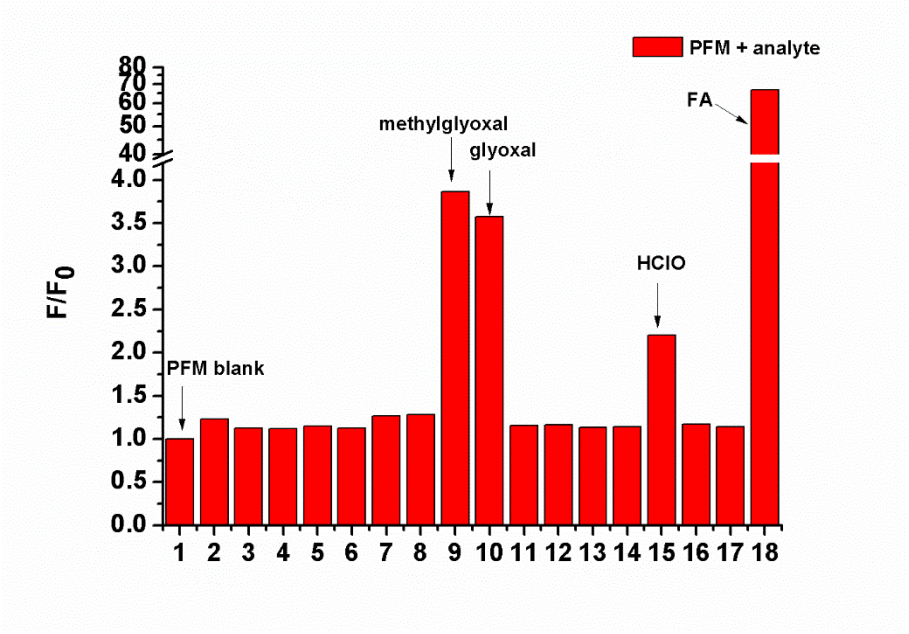


Figure S7. Enlarged picture of Figure 2D (with the selectivity results only).

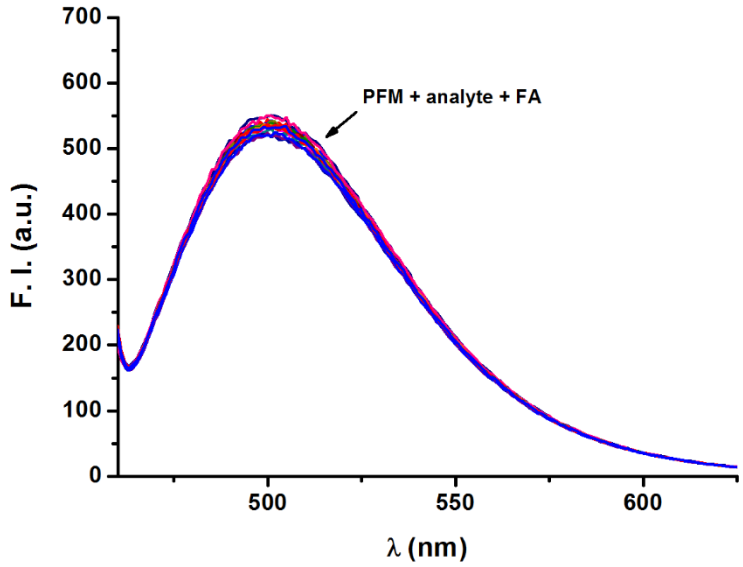


Figure S8. Fluorescent spectra of **PFM** (10 μM) in the presence of FA (300 μM) together with one of the various bio-relevant carbonyl species (300 μM). Spectra were taken in PBS (10 mM, pH 7.4) at ambient temperature with excitation 451 nm.

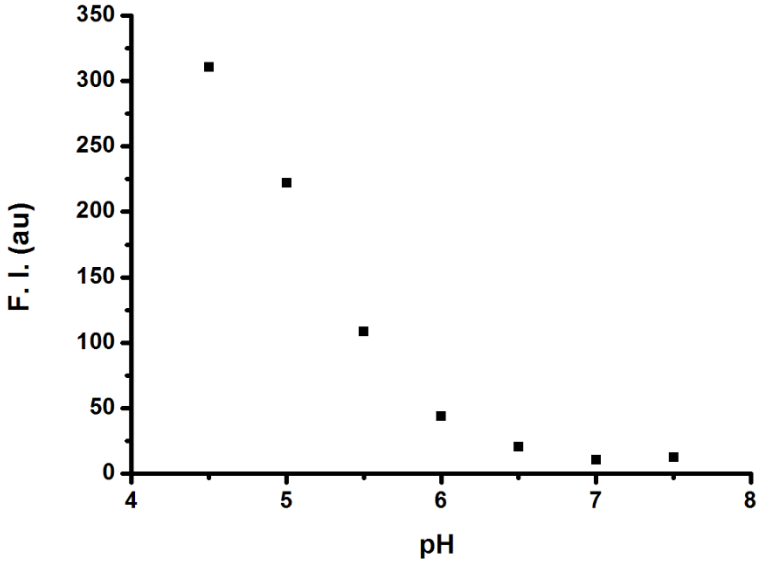


Figure S9. Fluorescent intensity of **PFM** (10 μM) at 513 nm in PBS of various pH values. Data were obtained at ambient temperature (λ_{ex} 451 nm).

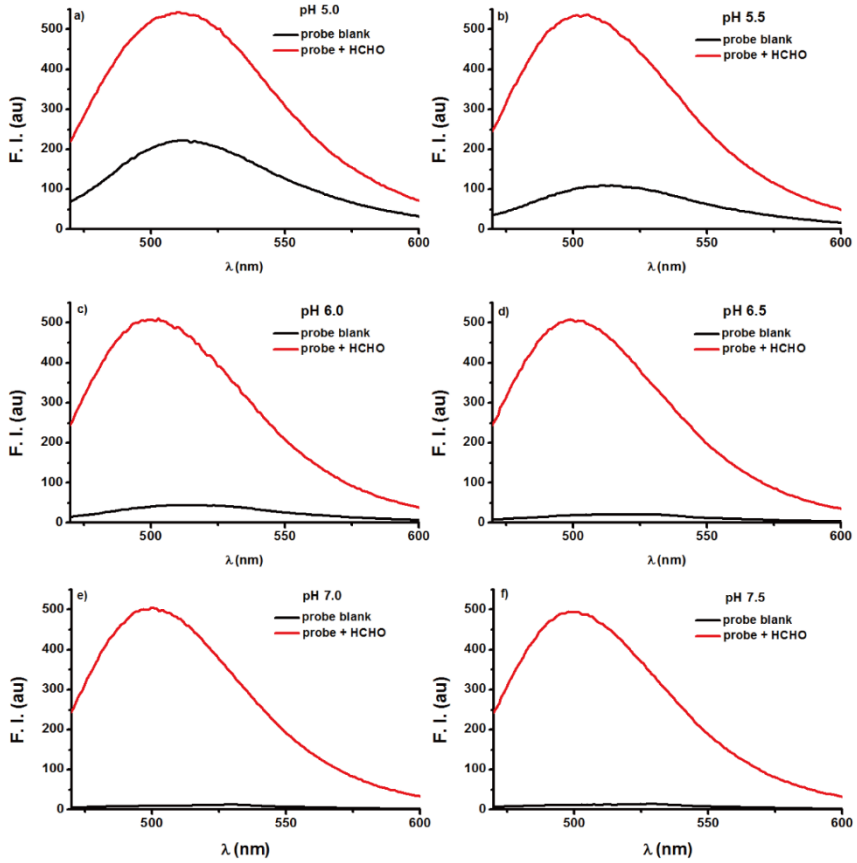


Figure S10. Spectra of **PFM** (10 μM) before and after the treatment of FA (200 μM, 5 min) in PBS (10 mM) of various pH values at ambient temperature (λ_{ex} 451 nm).

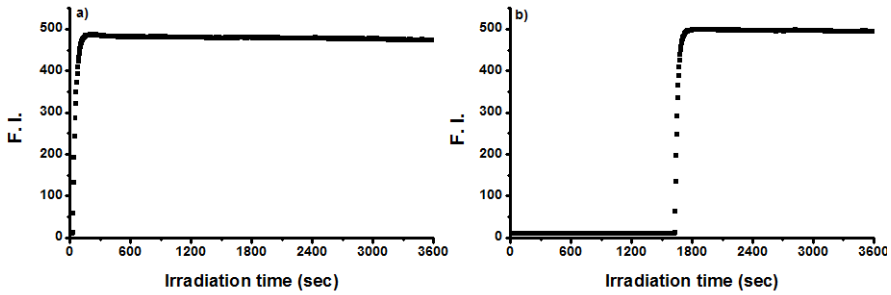


Figure S11. Photo-stability of **PFM** (10 μM) and the **PFM**-FA product. (a) **PFM** (10 μM) was treated with FA (200 μM) and the intensity at 500 nm was collected under continuous excitation. (b) **PFM** (10 μM) was continuously excited and then treated with FA (200 μM). Data were collected at ambient temperature with excitation 451 nm.

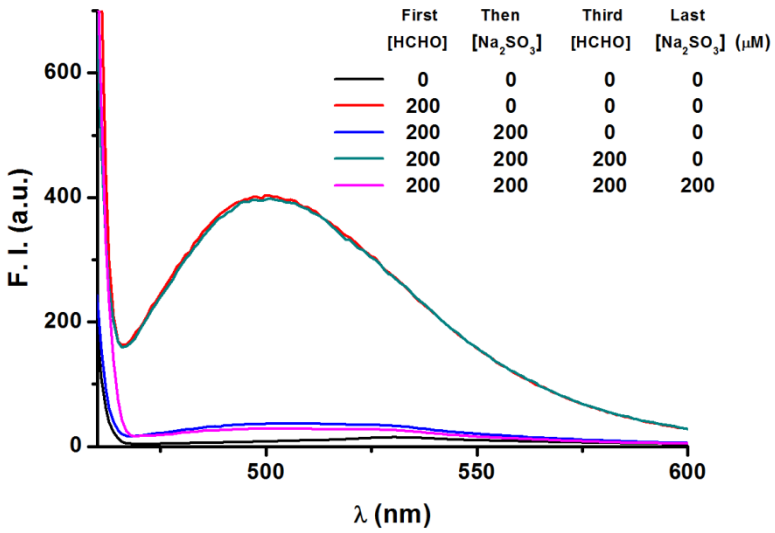


Figure S12. Spectra of **PFM** (10 μM) after the sequential treatment of FA (200 μM, 5 min), $NaHSO_3$ (200 μM, 15 min). Spectra were recorded in PBS (10 mM, pH 7.4) at ambient temperature (λ_{ex} 451 nm).

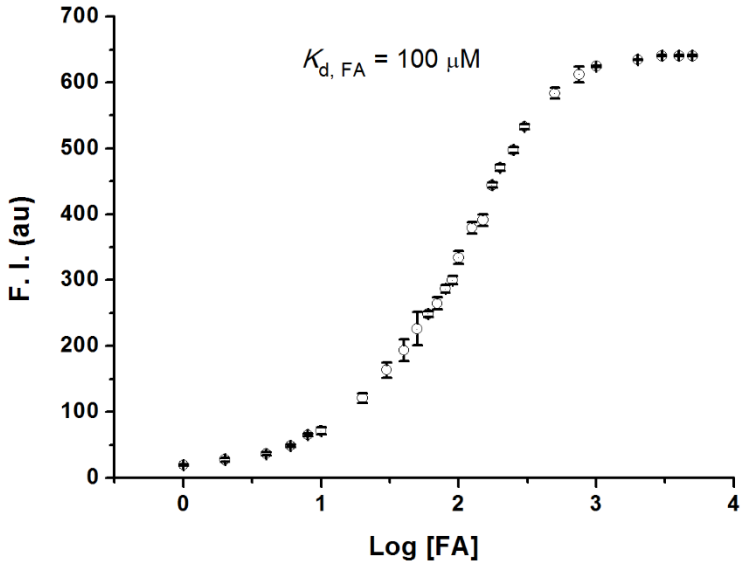


Figure S13. Plot of fluorescent intensity at 500 nm of **PFM** (10 μM) against the concentrations of FA. Data were acquired in the same way as those in **Figure S3**. Employing a literature method [41], the $K_{d, FA}$ of **PFM** was calculated to be around 100 μM.

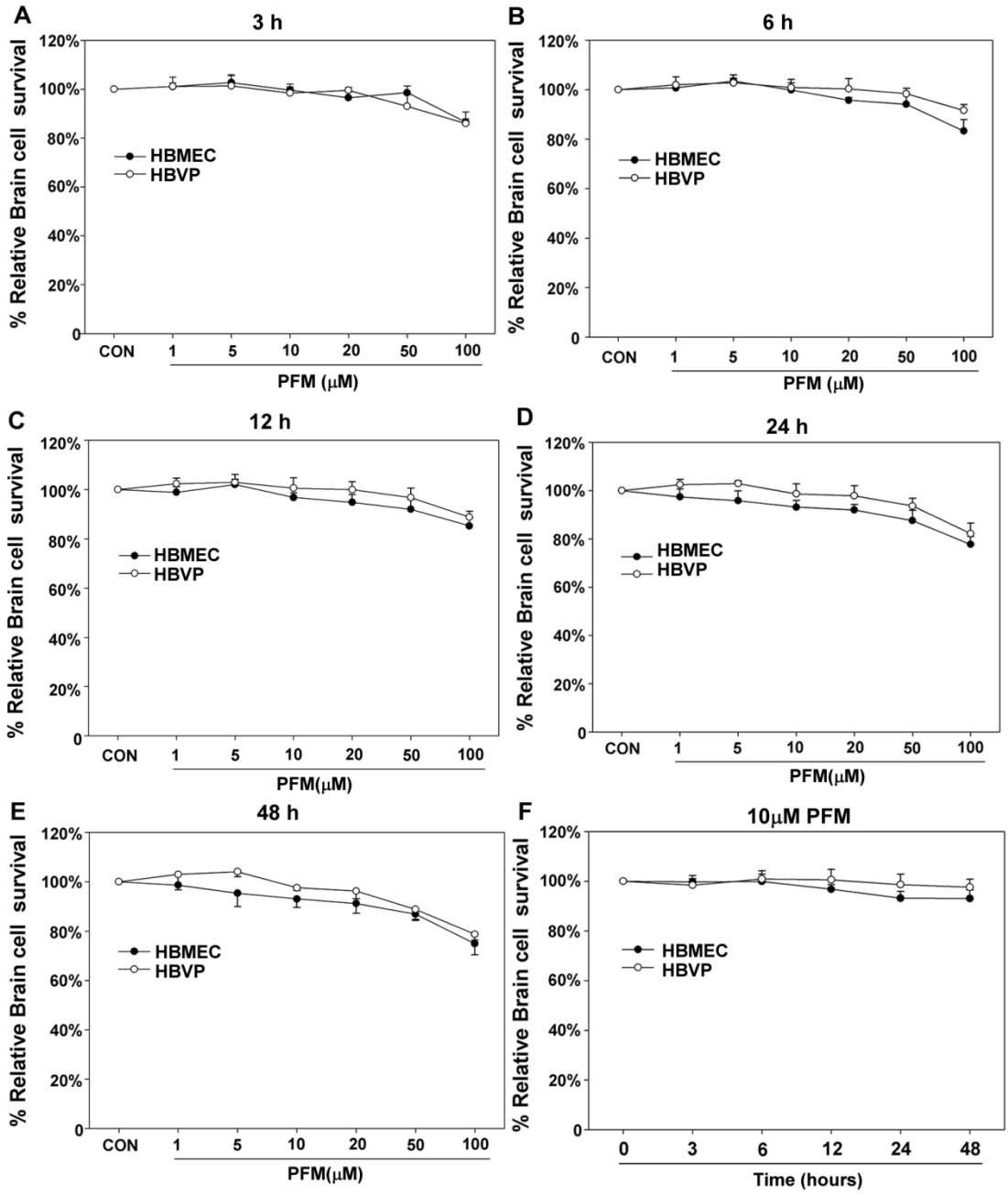


Figure S14. Cytotoxicity of **PFM** was assessed in HBEMCs and HBVPs by CCK8 assays. **PFM** of 1 μM, 5 μM, 10 μM, 25 μM, 50 μM, and 100 μM were added to cells, and they were allowed to incubate for of 3 h (A), 6 h (B), 12 h (C), 24 h (D), 48 h (E). After introducing CCK8 (10 μL) solution at 37 ºC in a 95% humidified atmosphere with 5% CO_2 for 1 h, the absorption at 450 nm was measured by Microplate Spectrophotometer (MD I3X). (F) The cytotoxicity of **PFM** (10 μM) was assessed in HBEMCs and HBVPs from 0 h-48 h.

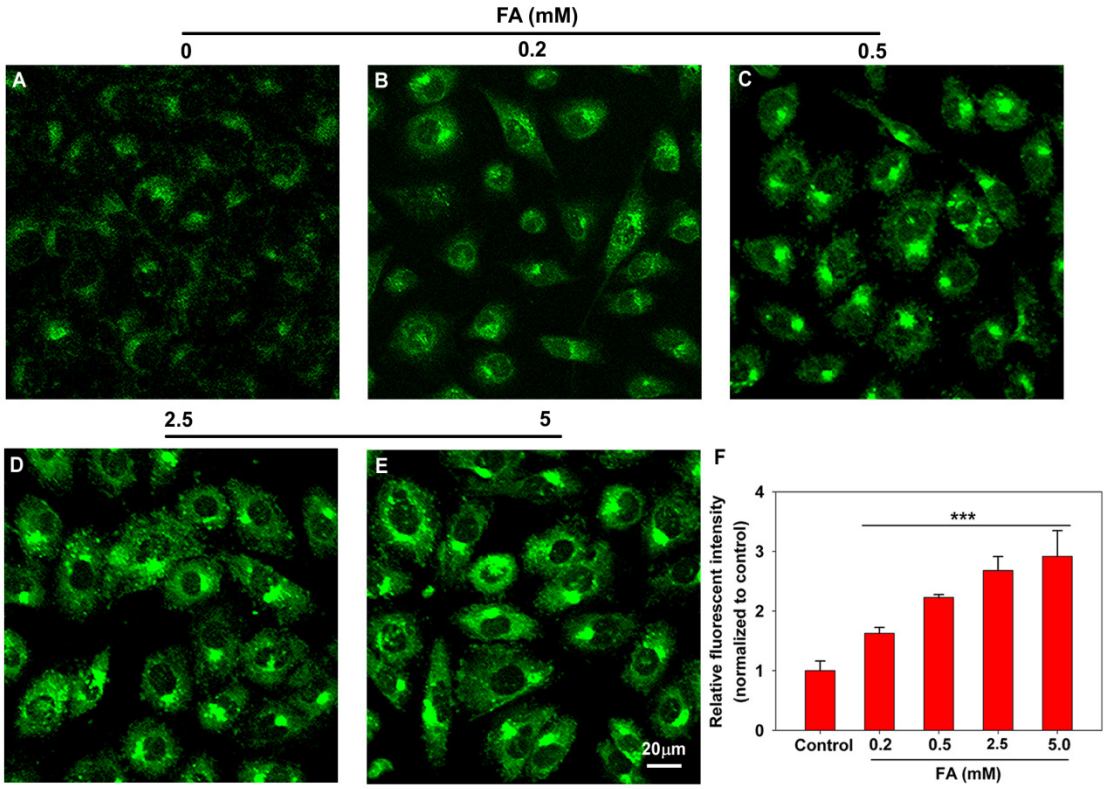


Figure S15. Confocal imaging of **PFM** for detecting exogenous FA in live HBVPs. Cells are treated with 10 μM **PFM** for 15 min at 37 °C, then washed with PBS (pH 7.4), followed by FA incubation for 15 min. control (A); 0.2 mM FA (B); 0.5 mM FA (C); 2.5 mM FA (D); 5 mM FA (E). (F) Quantification of image data. **PFM** fluorescence was monitored at 515-545 nm (λ_{ex}=488 nm). Scale bar=20 μm.

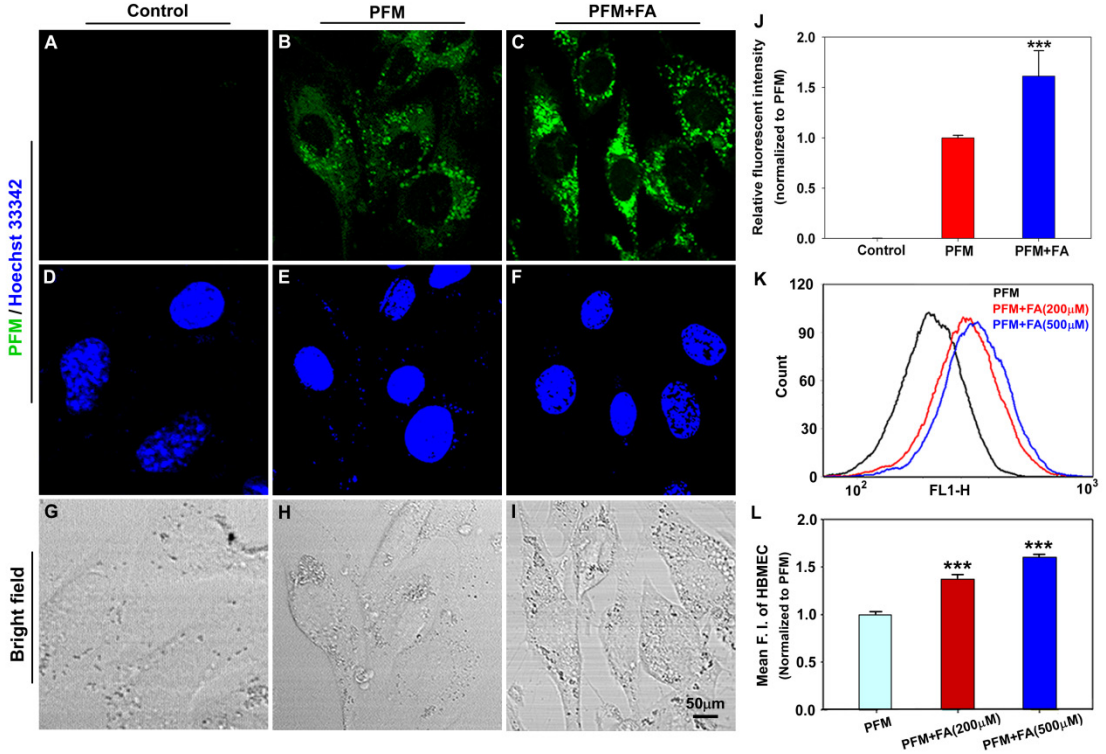


Figure S16. Imaging exogenous FA in living HBMECs. The fluorescence intensity of **PFM** was assessed by the confocal microscope (A-J) and flow cytometry analysis (K, L). HBMECs were treated with **PFM** (10 μM) for 15 min at 37 °C, then washed with PBS (pH 7.4) and followed by FA (200 μM for C, and 200 μM or 500 μM for K and L) incubation for 15 min. Nuclei were stained with Hoechst 33342. (J) Quantification of image data. Data are expressed as mean±S.E.M., n=10, ***P<0.01 versus **PFM** alone. For flow cytometry analysis, each plot represented 10,000 viable cells (non-viable cells were excluded from FACS analysis by appropriate gating). Data are expressed as mean±S.E.M., a minimum of 3 images for each condition were quantified and averaged, ***P<0.001 versus **PFM** alone. The **PFM** fluorescence was monitored at 515-545 nm (λ_{ex}=488 nm).

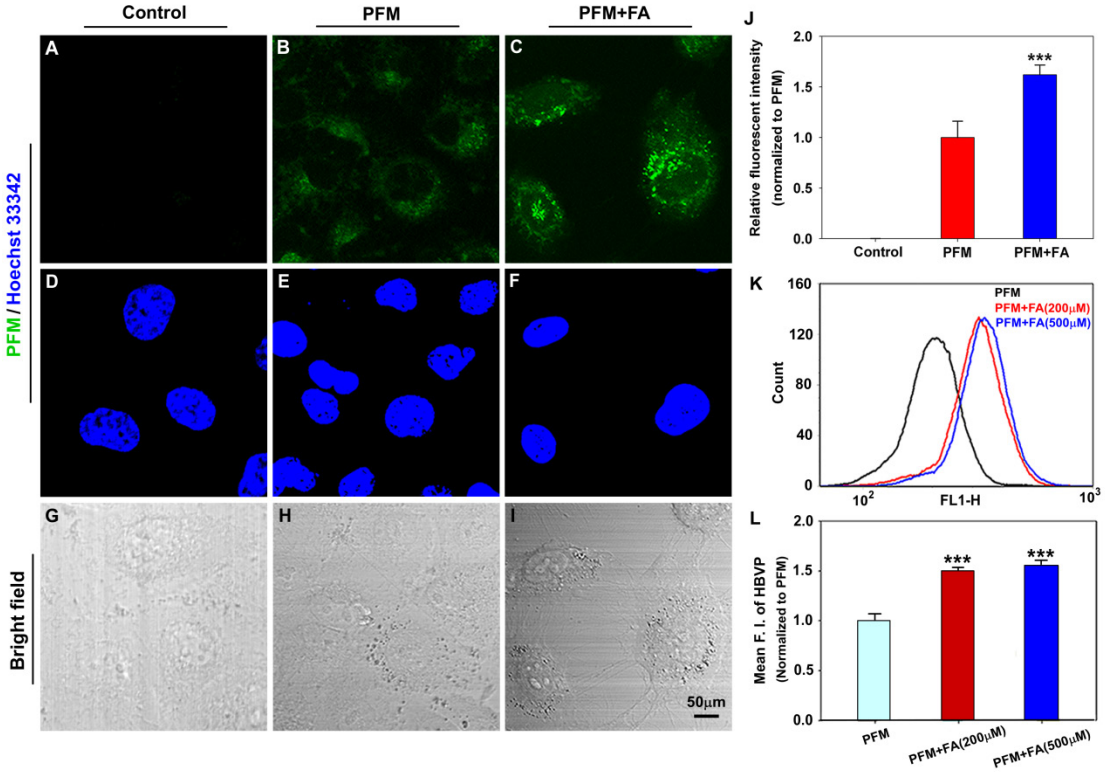


Figure S17. Imaging exogenous FA in living HBVPs. The fluorescence intensity of **PFM** was assessed by the confocal microscope (A-J) and flow cytometry analysis (K, L). HBVPs were treated with **PFM** (10 μM) for 15 min at 37 °C, then washed with PBS (pH 7.4) and followed by FA (200 μM for C, and 200 μM or 500 μM for K and L) incubation for 15 min. Nuclei were stained with Hoechst 33342. (J) Quantification of image data. For flow cytometry analysis, each plot represented 10,000 viable cells (non-viable cells were excluded from FACS analysis by appropriate gating). Data are expressed as mean±S.E.M., a minimum of 3 images for each condition were quantified and averaged, ***P<0.001 versus **PFM** alone. The **PFM** fluorescence was monitored at 515-545 nm (λ_{ex}=488 nm).

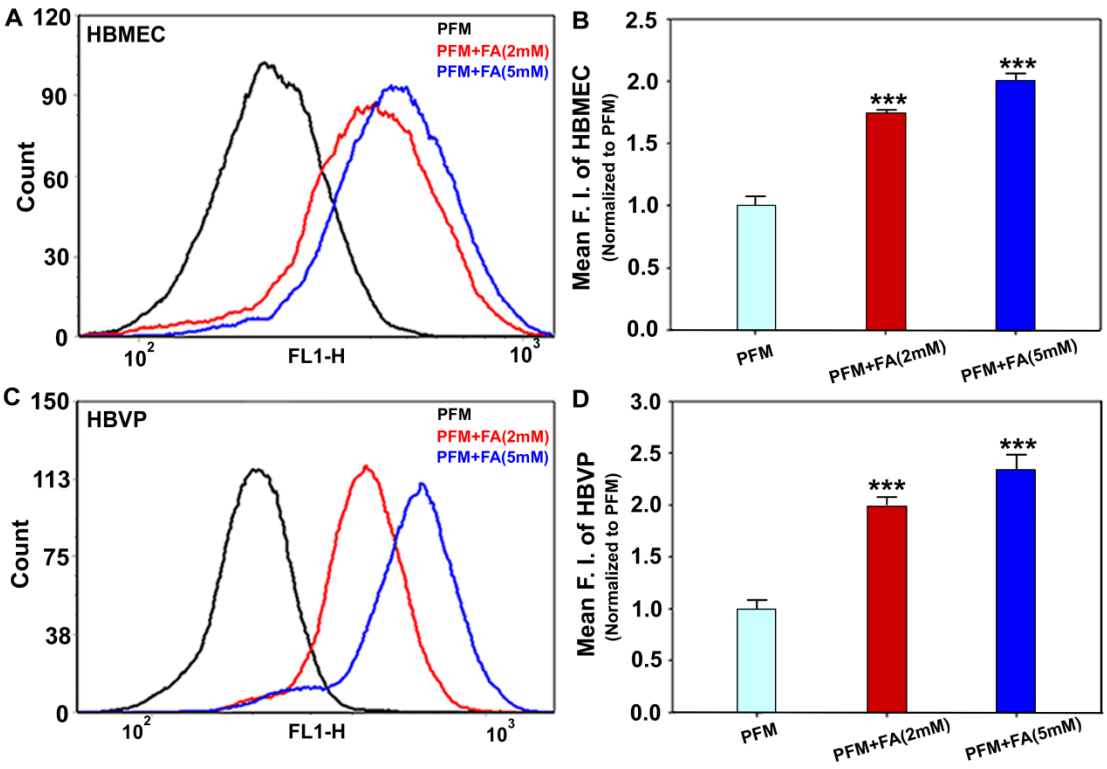


Figure S18. Flow cytometry analysis of exogenous FA of higher concentrations in living HBMEC (A, B) and HBVP (C, D) stained with **PFM** (10 μM) for 15 min at 37 °C, and then incubated with FA (2 mM and 5 mM) at 37 °C for 15 min. The **PFM** fluorescence was monitored at 515-545 nm (λ_{ex}=488 nm). Quantification of fluorescence intensity by flow cytometry analysis of HBMECs or HBVPs (B, D). Each plot represented 10,000 viable cells (non-viable cells were excluded from FACS analysis by appropriate gating). Data are expressed as mean ± S.E.M., ***$P < 0.001$ versus **PFM**.

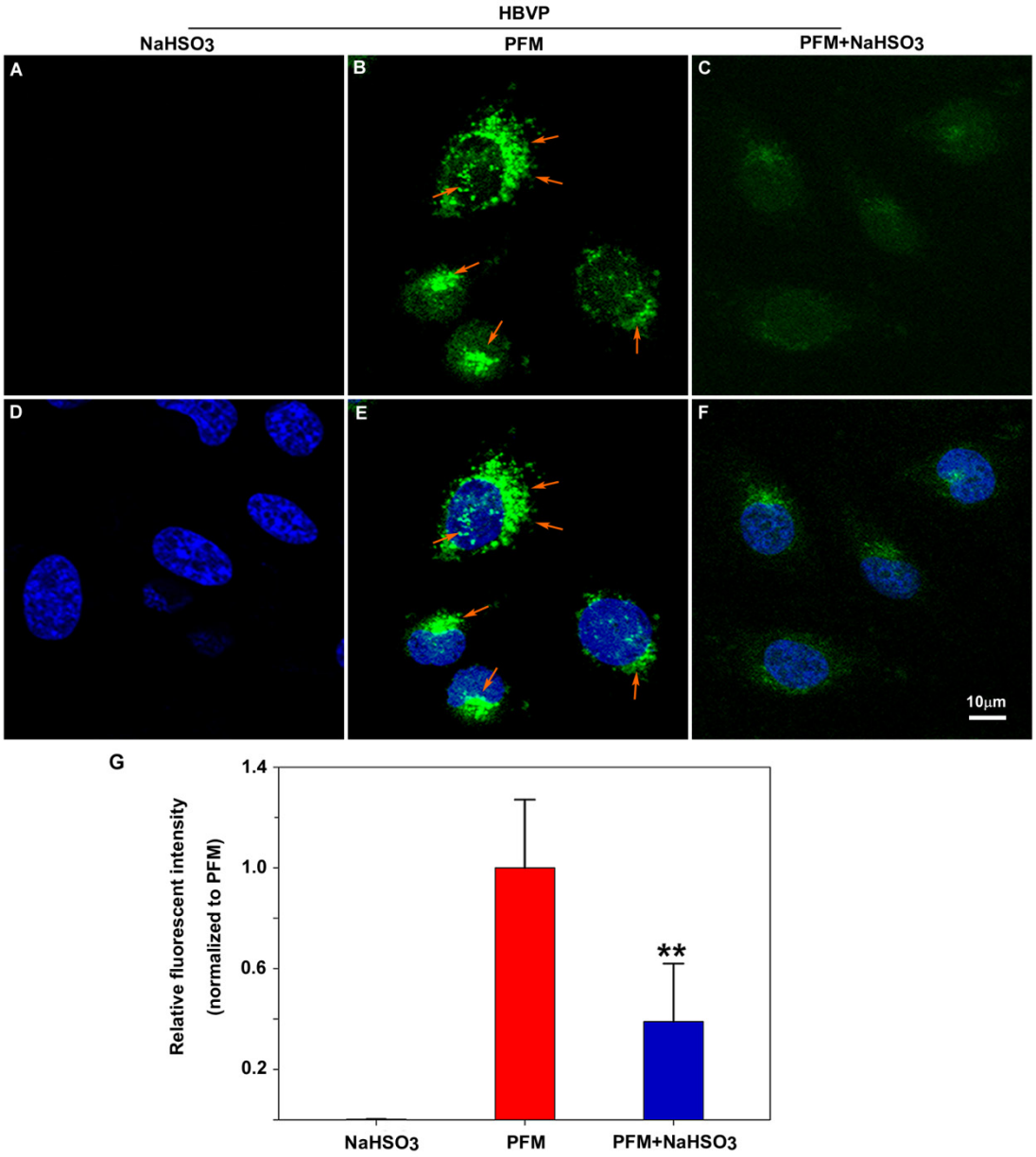


Figure S19. Imaging endogenous FA in live neurovascular cell HBVPs. The fluorescence of **PFM** was detected by confocal microscope (A-F), HBVPs are pre-treatment with $NaHSO_3$ for 30 min, then washed with PBS (pH 7.4) and followed by **PFM** (10 μM) incubation for 15 min at 37 °C. Nuclei were stained with Hoechst 33342. (G) Quantification of image data. Data are expressed as mean±S.E.M., a minimum of 3 images for each condition were quantified and averaged, $^{**}P<0.01$ versus **PFM** alone. The **PFM** fluorescence was monitored at 515-545 nm (λ_{ex}=488 nm).

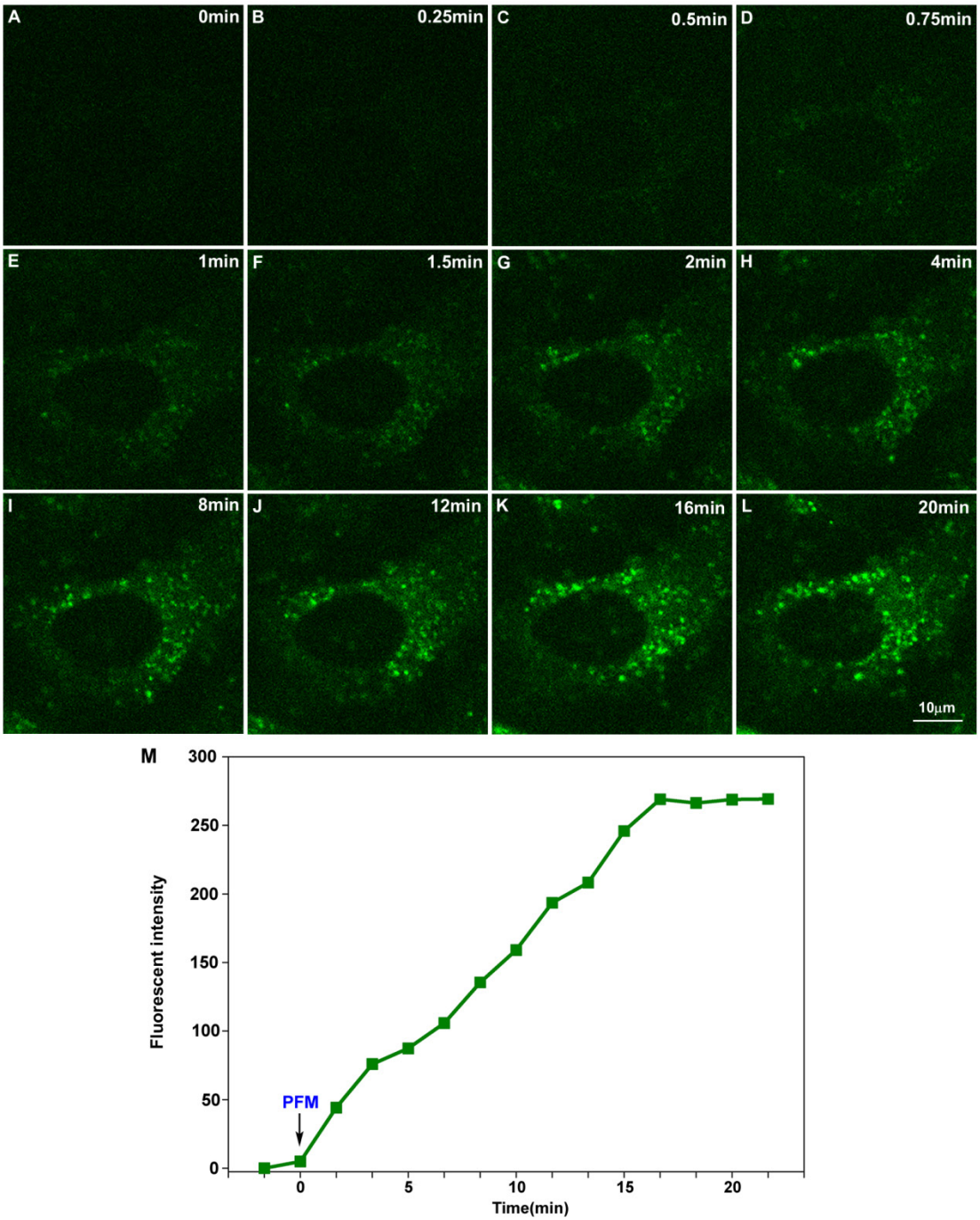


Figure S20. Real-time visualization of endogenous FA in living HBMECs. (A-L) The time-series images are individual frames from a continuous time-lapse movie and show dynamic fluorescence elevation with **PFM** (10 μM) treatment. (M) Mean values of fluorescence intensity from time-lapse movie (video S1) were measured to quantify the progressive of endogenous FA. The **PFM** fluorescence was monitored at 515-545 nm (λ_{ex}=488 nm).

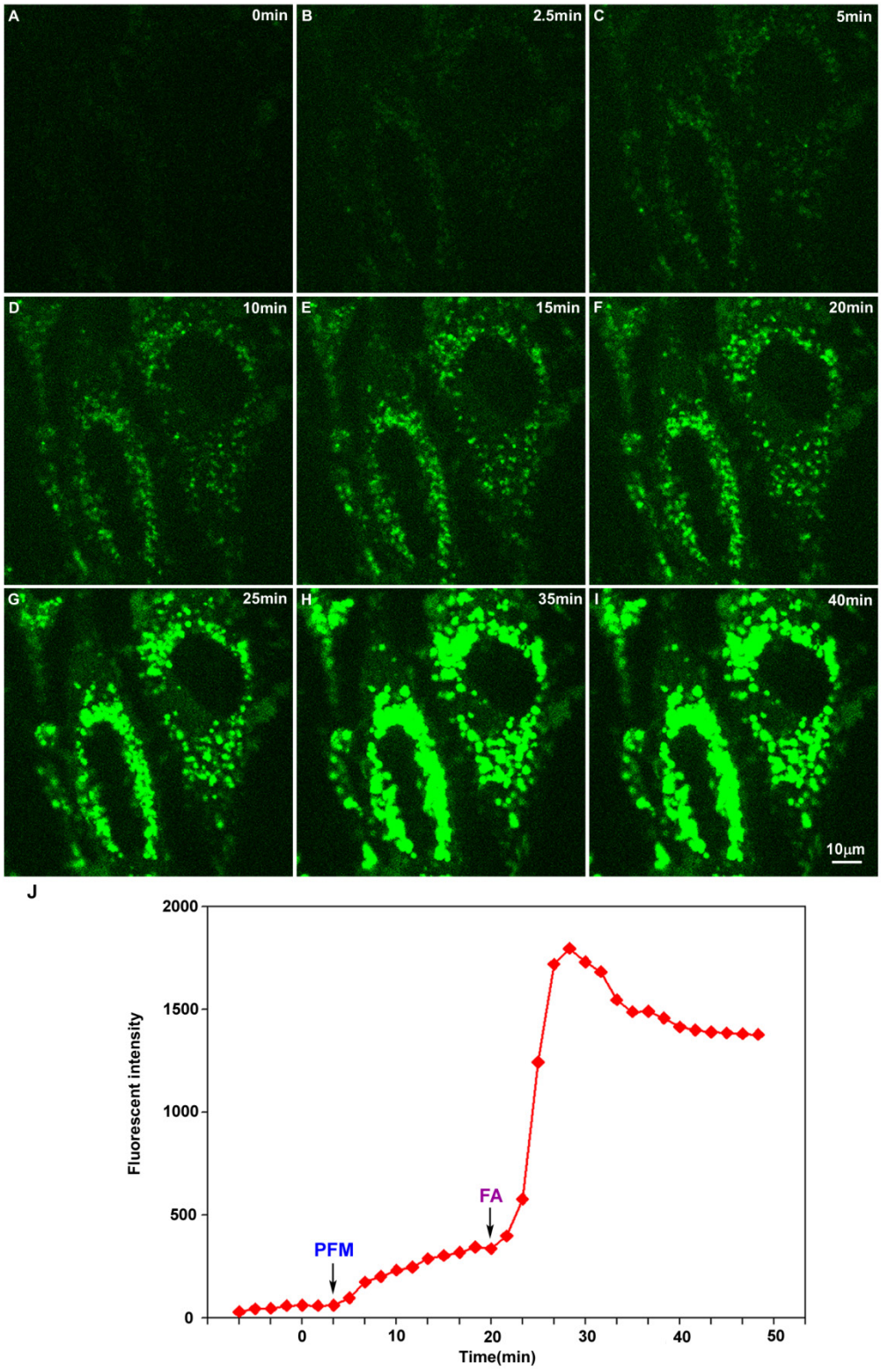


Figure S21. Real-time visualization of exogenous FA in living HBMECs. The time-series images are individual frames from a continuous time-lapse movie and show dynamic fluorescence elevation with **PFM** (10 μM) (A-F) treatment and exogenous FA (500 μM) (G-I). (J) Mean values of fluorescence intensity from time-lapse movie (video S2) were measured to quantify the progressive of endogenous and exogenous FA. The **PFM** fluorescence was monitored at 515-545 nm (λ_{ex}=488 nm).

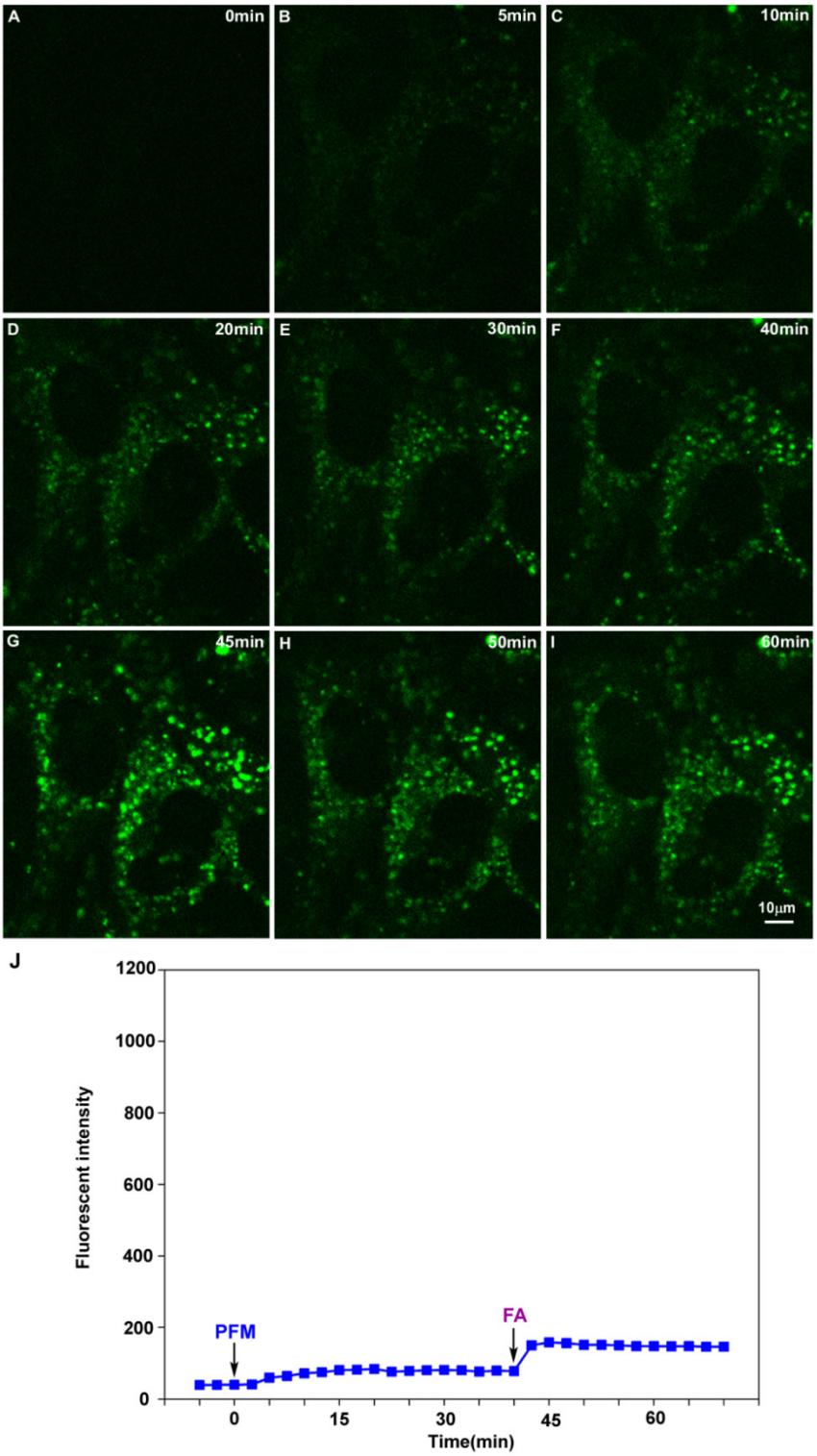


Figure S22. Real-time visualization of endogenous and exogenous FA in living HBMECs with $NaHSO_3$ (200 μM) pre-treatment for 30 min. The time-series images are individual frames from a continuous time-lapse movie and show dynamic fluorescence elevation with **PFM** (10 μM) (A-F) treatment and exogenous FA (500 μM) (G-I). (J) Mean values of fluorescence intensity from time-lapse imaging (video S3) were measured to quantify the progressive of endogenous and exogenous FA upon $NaHSO_3$ pre-treatment. The **PFM** fluorescence was monitored at 515-545 nm (λ_{ex}=488 nm).

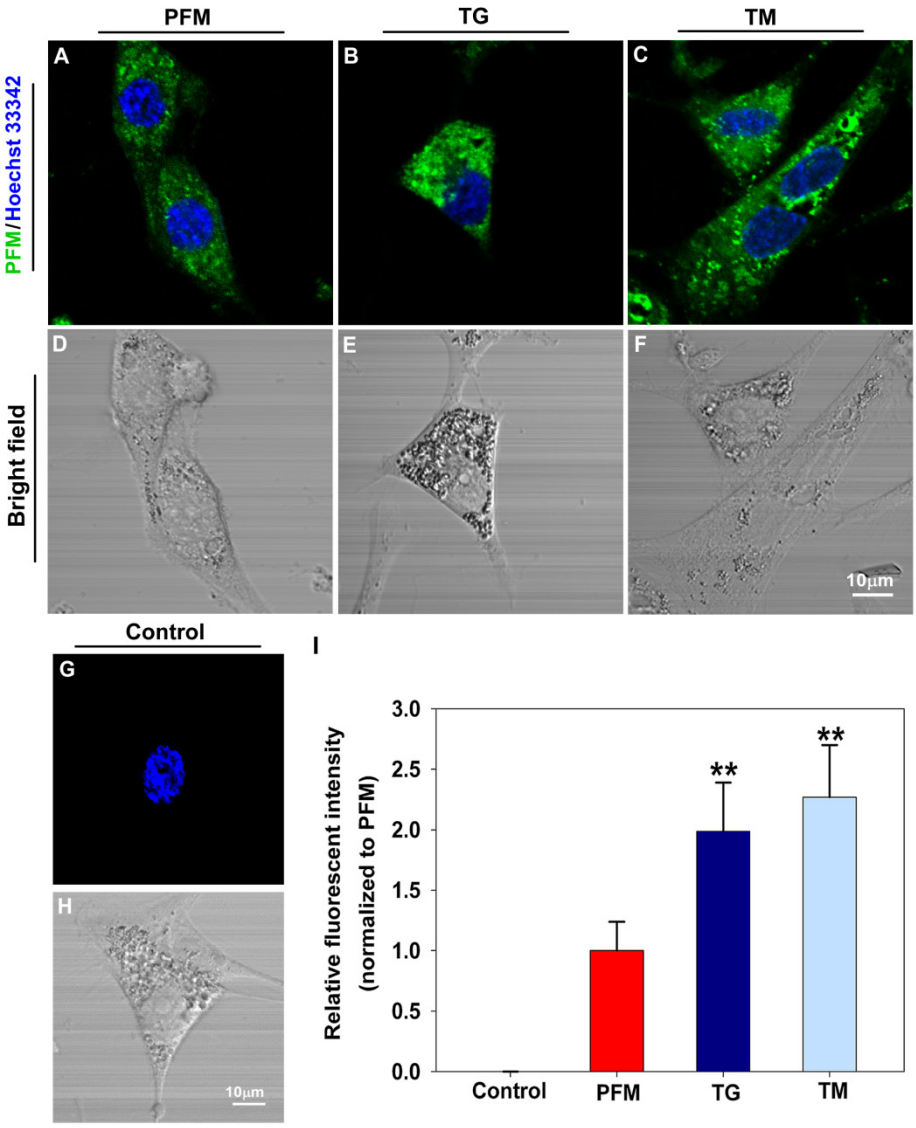


Figure S23. Imaging endogenous FA in living HBMECs upon ER stress. HBMECs were pre-treatment with (B, E) thapsigargin (TG, 5 μM) and (C, F) tunicamycin (TM, 5 μM) for 30 min, then washed with PBS (pH 7.4) and followed by **PFM** (10 μM) incubation for 15 min at 37 °C. Fluorescence were obtained after TG/TM incubation for 60 min. Nuclei were stained with Hoechst 33342. (A, D) HBMECs treated with **PFM** only. (G, H) HBMECs treated without **PFM**. (I) Quantification of image data. Data are expressed as mean±S.E.M., a minimum of 3 images for each condition were quantified and averaged, $^{**}P<0.01$ versus **PFM** alone. The **PFM** fluorescence was monitored at 515-545 nm (λ_{ex}=488 nm).

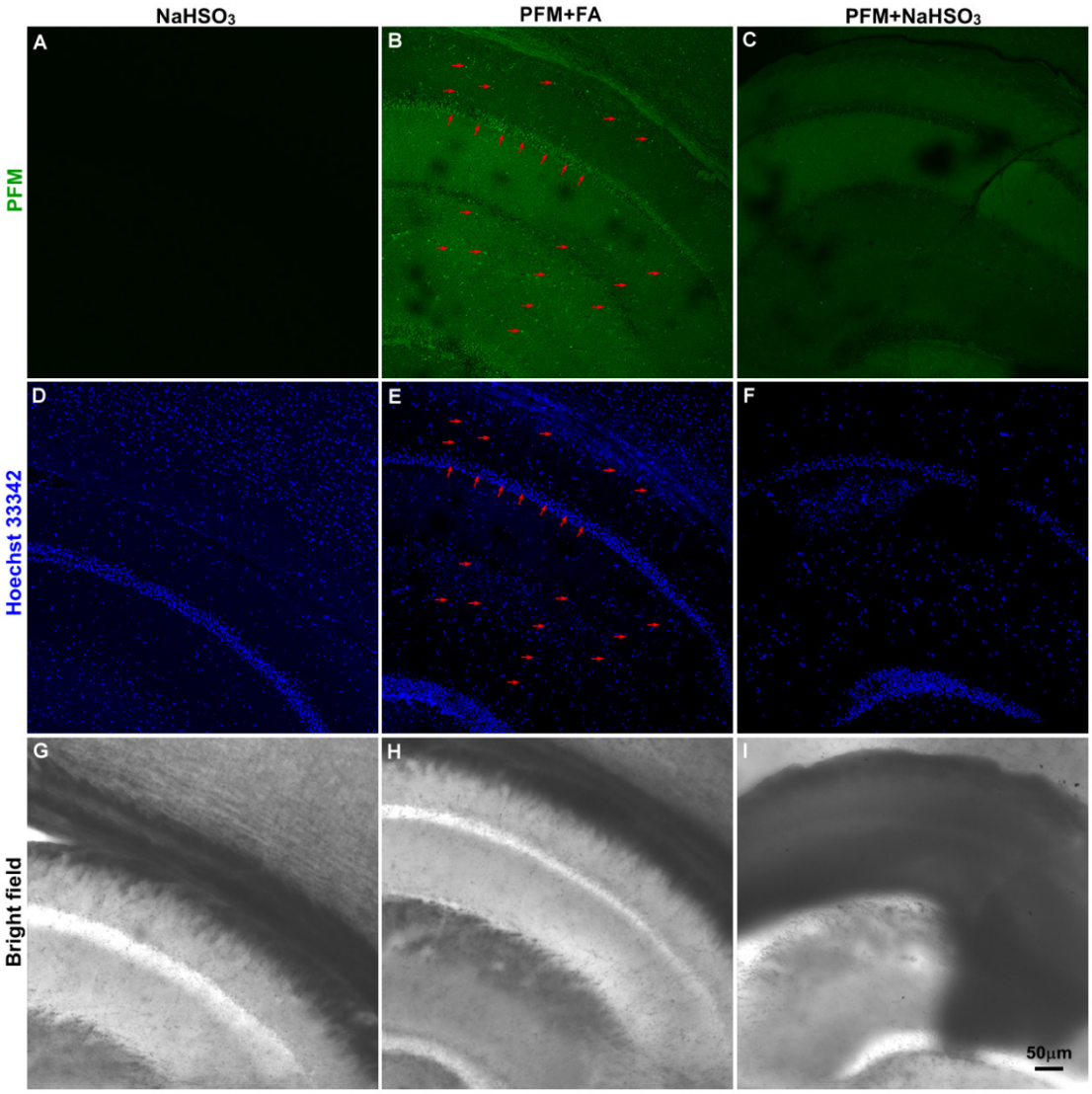


Figure S24. Application of **PFM** for monitoring FA in living brain tissue of mice. (A, D, G) Confocal fluorescence images of cerebral cortex incubated with $NaHSO_3$ (200 μM) for 30 min. (B, E, H) Fluorescence images of cerebral cortex incubated with exogenous FA (500 μM) for 30 min, followed by incubation with **PFM** (20 μM) for another 30 min. (C, F, I) Fluorescence images of cerebral cortex incubated with $NaHSO_3$ (200 μM) for 30 min, followed by incubation with **PFM** (20 μM) for another 30 min. The **PFM** fluorescence was monitored at 515-545 nm (λ_{ex}=488 nm). Nuclei were stained with Hoechst 33342 (A2, B2, C2). Bright field image (A3, B3, C3). Scale bar=50 μm.

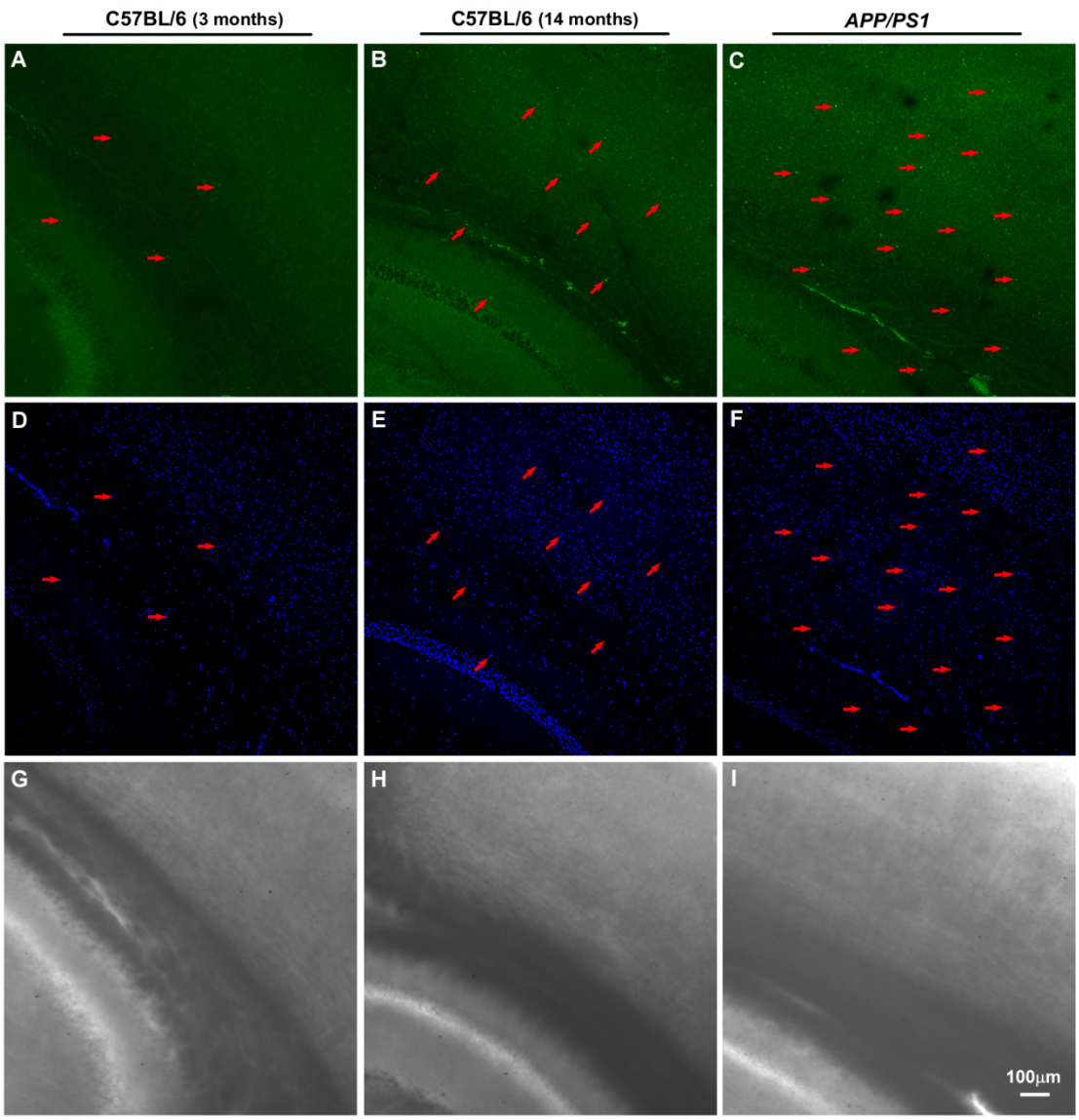


Figure S25. Application of **PFM** for monitoring endogenous FA in living brain tissue slides of C57BL/6 and *APP/PS1* transgenic mice. Fluorescence images of cerebral cortex of C57BL/6 and *APP/PS1* transgenic mice incubated with **PFM** (20 μM) for 30 min, (A, D, G) 3 month old C57BL/6 mice, (B, E, H) 14 month old C57BL/6 mice, (C, F, I) *APP/PS1* transgenic mice. The arrow indicated the cells with high fluorescence. Scale bar=100 μm. The **PFM** fluorescence was monitored at 515-545 nm (λ_{ex}=488 nm). Nuclei were stained with Hoechst 33342 (blue).

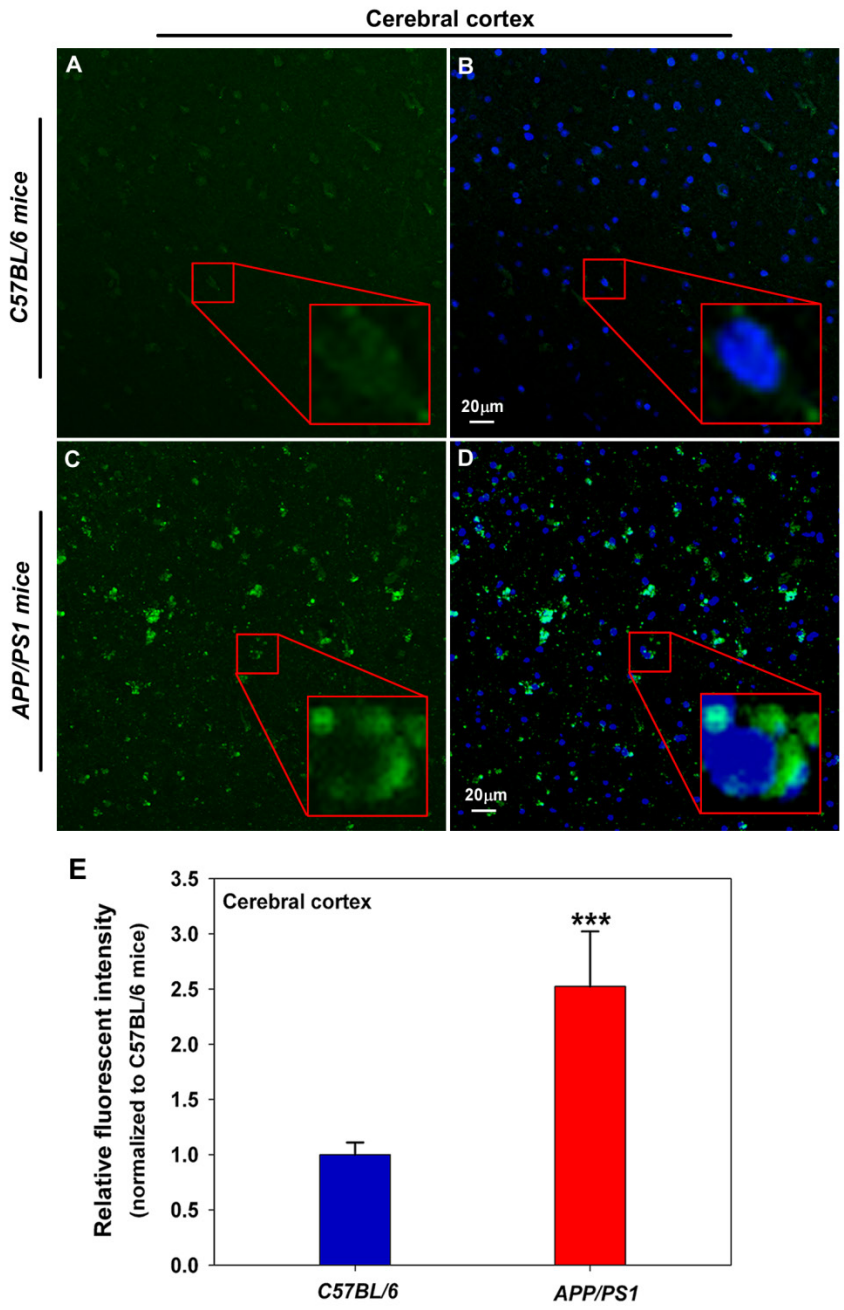
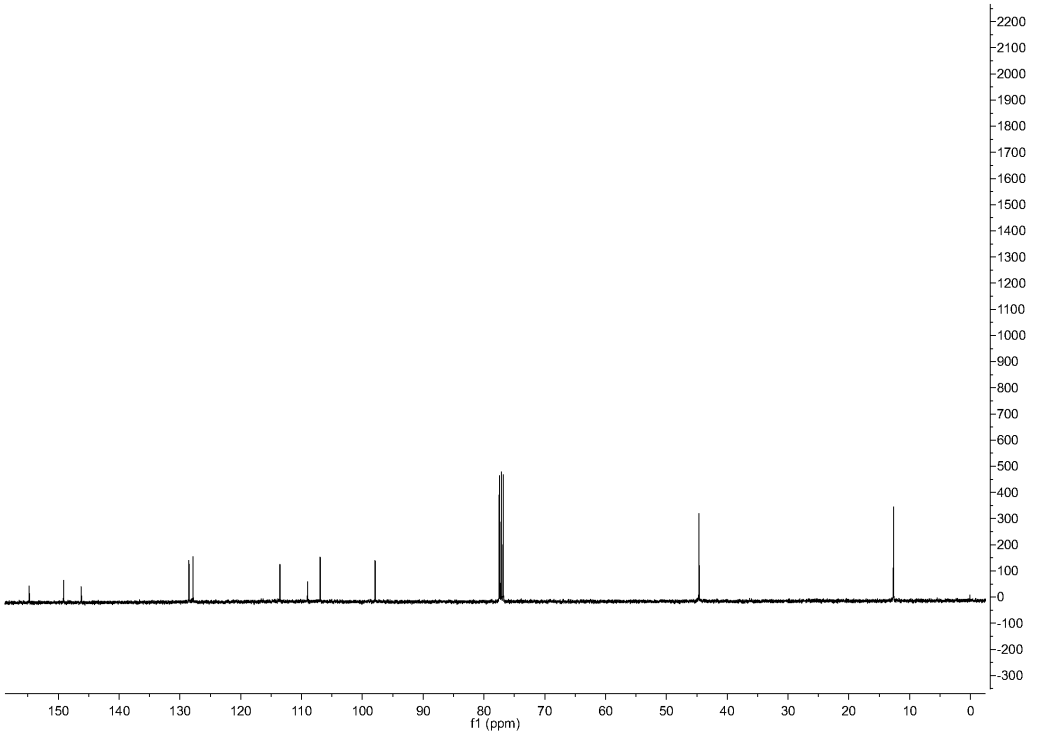
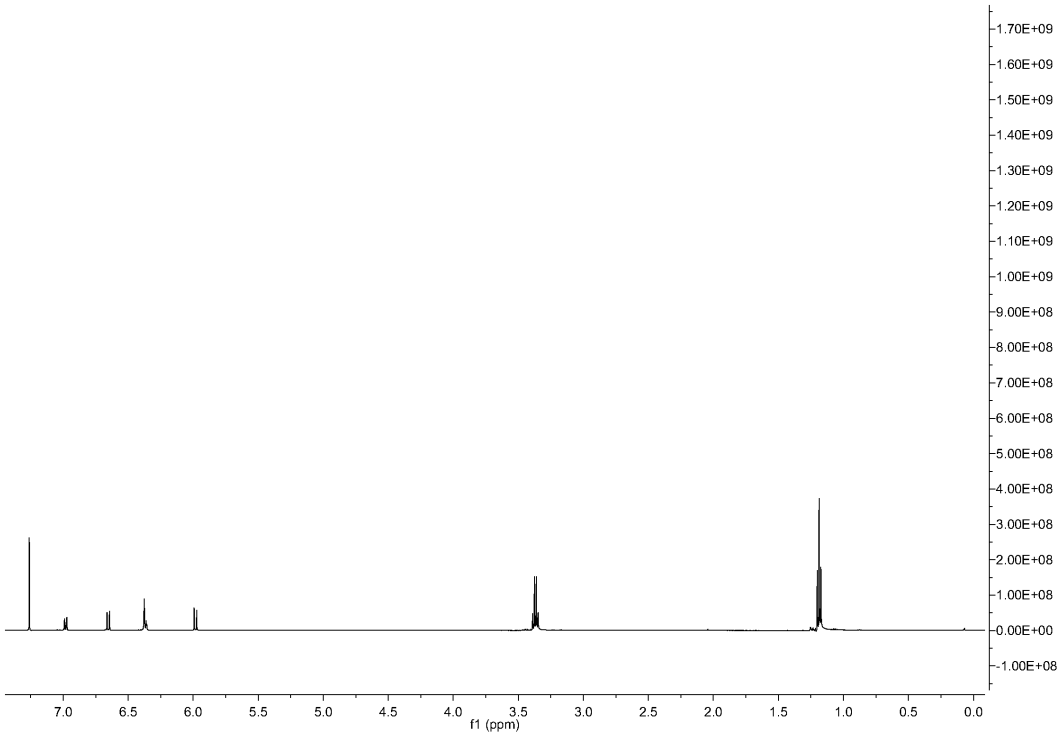


Figure S26. Application of **PFM** for monitoring endogenous FA in living brain tissues of *APP/PS1* transgenic mice. Fluorescence images of cerebral cortex of C57BL/6 (A, B) and *APP/PS1* transgenic mice (C, D) were detected following **PFM** (20 μM) incubation for 30 min. The **PFM** fluorescence was monitored at 515-545 nm (green, λ_{ex}=488 nm). Nuclei were stained with Hoechst 33342 (blue). Scale bar=20 μm. (E) Quantification of image data. Data are expressed as mean±S.E.M., ****P*<0.001 versus C57BL/6 mice.





NMR traces of **PFM**