

Electronic Supplementary Information for:

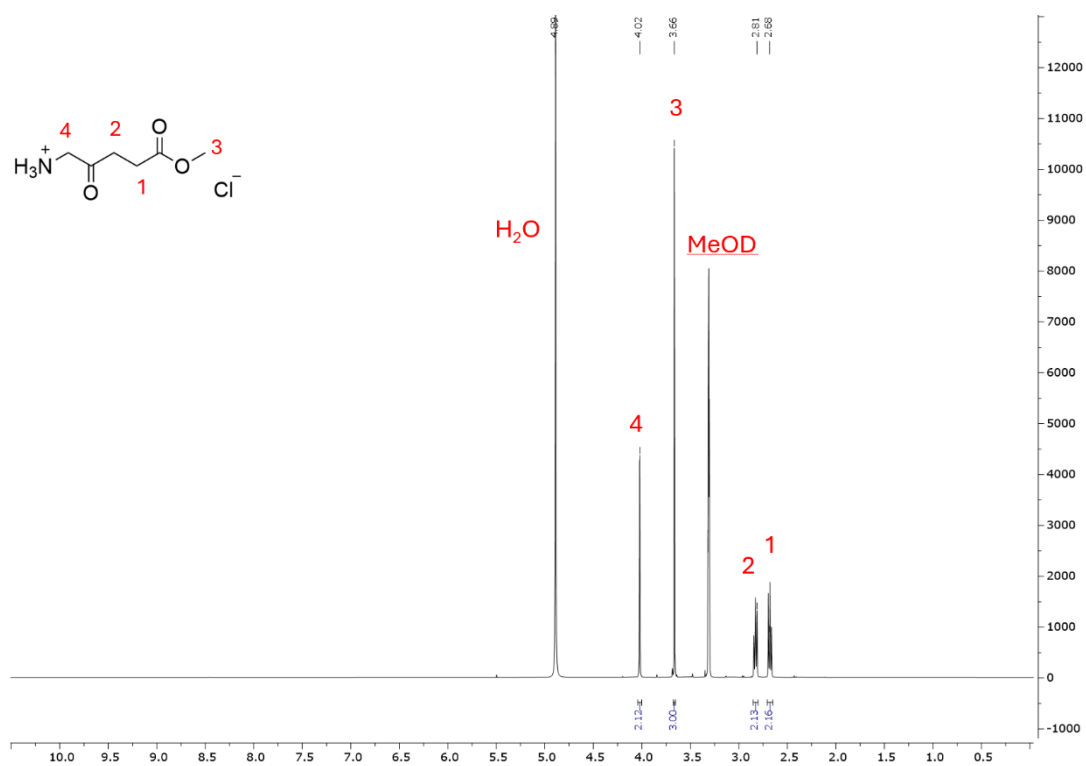
**Doubly protected ester prodrug of 5-aminolevulinic acid for enhanced cell uptake and
photoinactivation**

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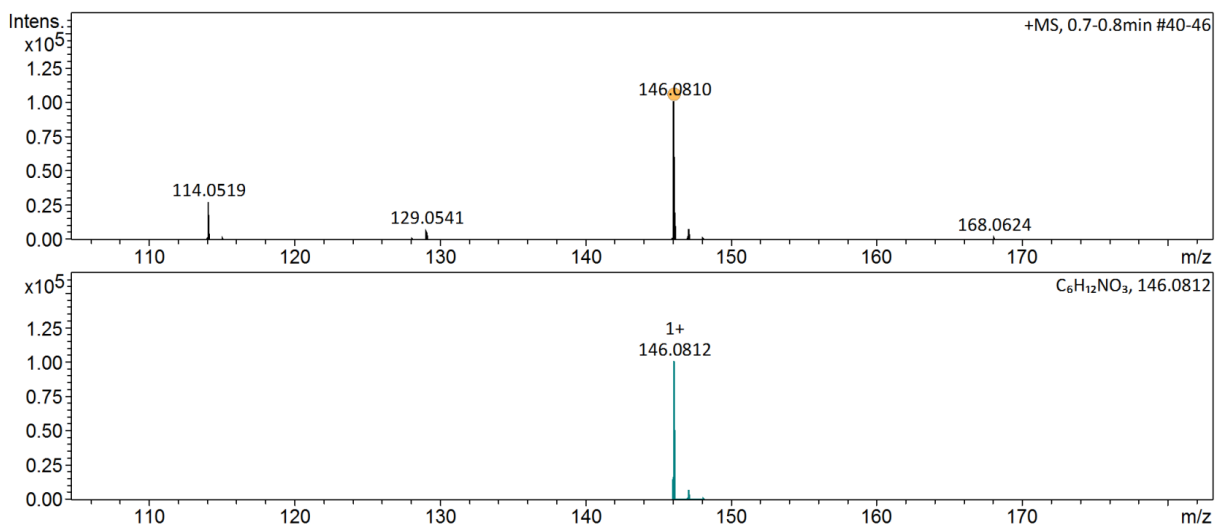
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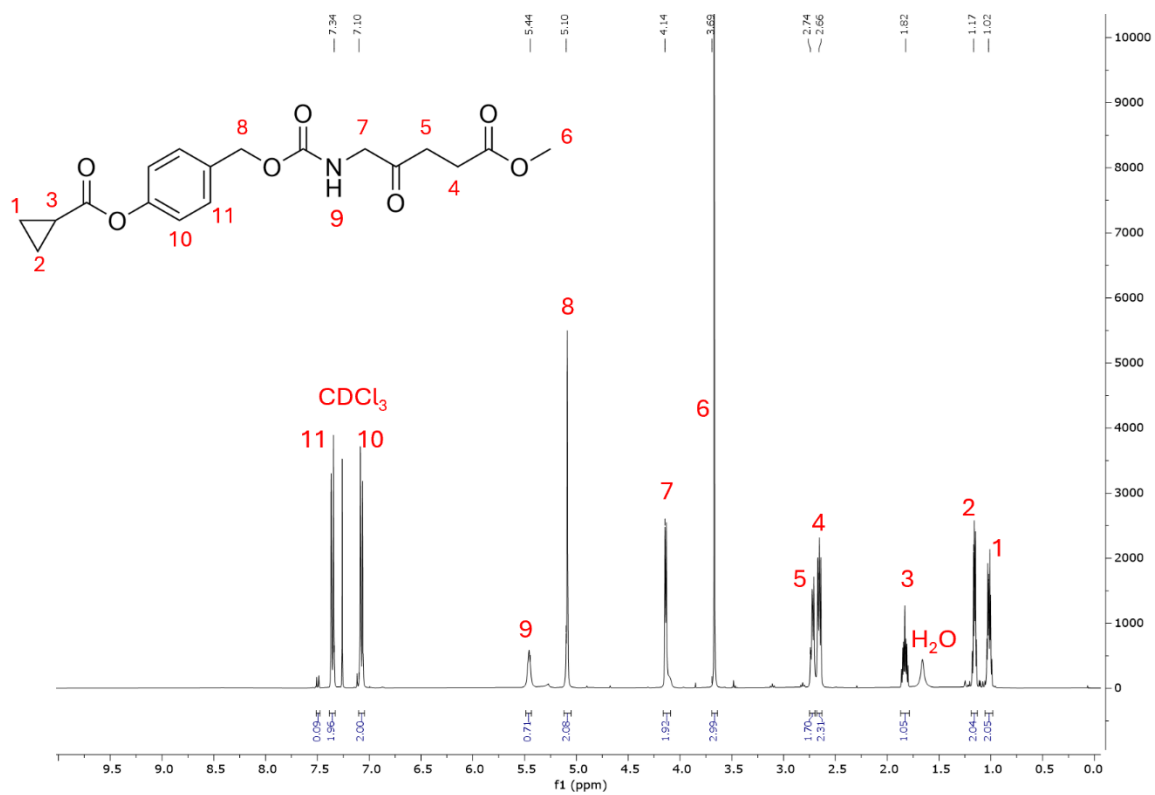
Compound Spectra



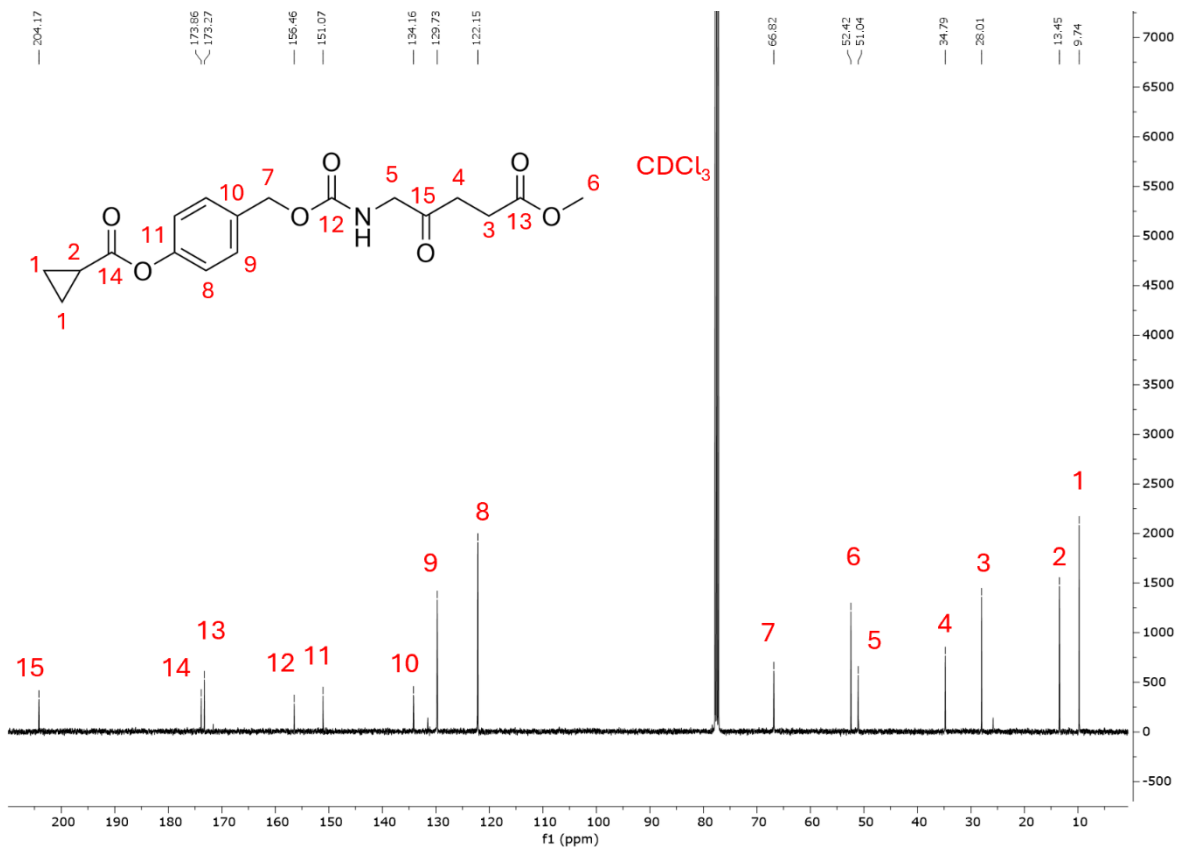
$^1\text{H NMR}$ (400 MHz, MeOD) of MAL



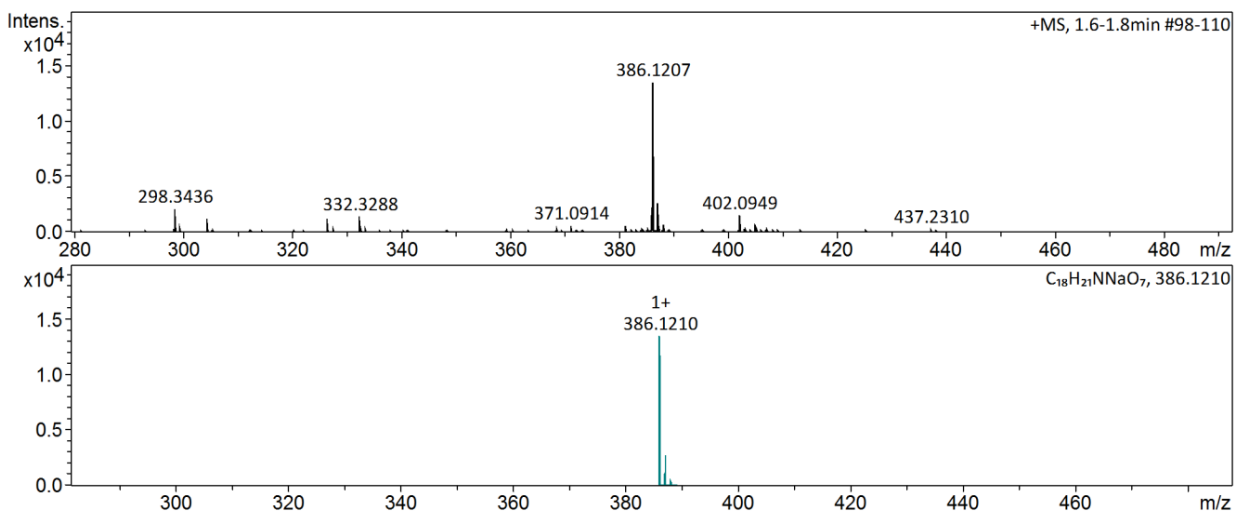
HRMS (SI-TOF) spectrum of MAL



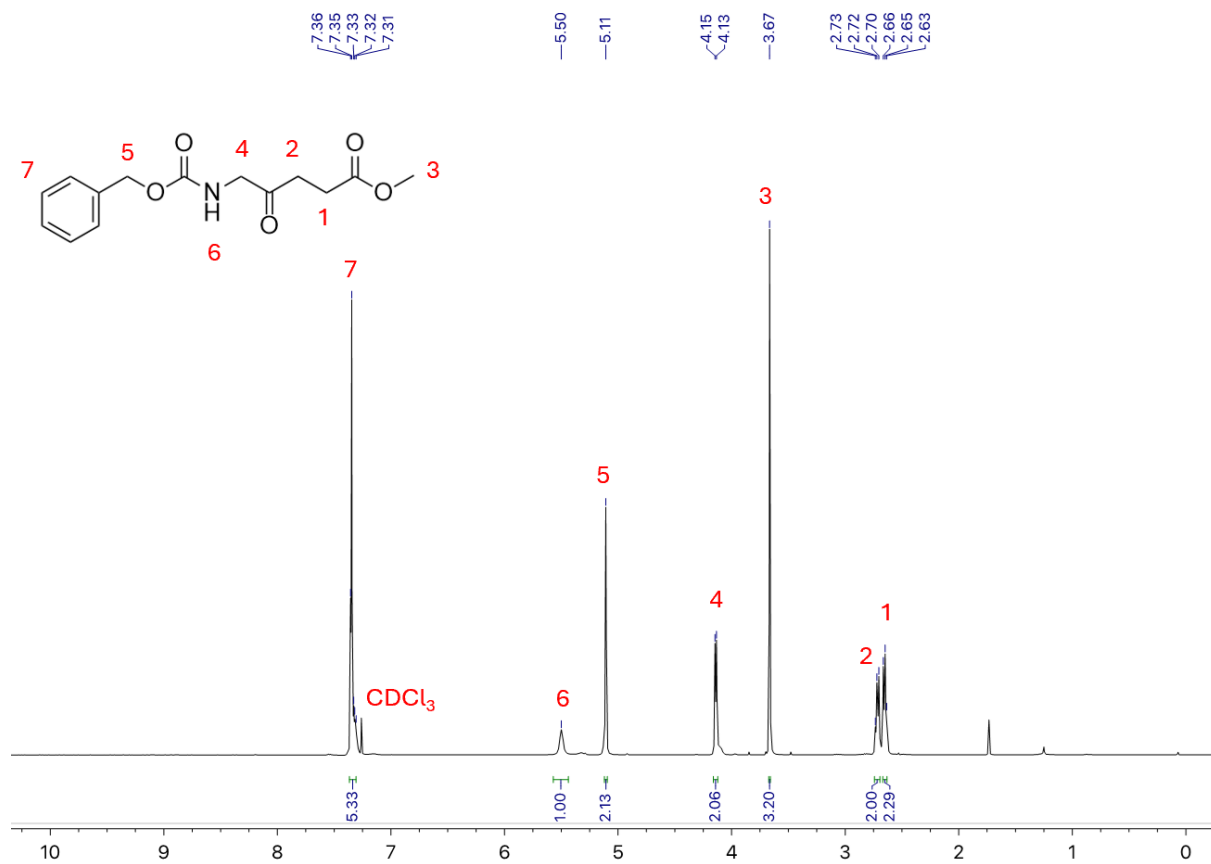
¹H NMR (400 MHz, CDCl₃) of 5-eMAL



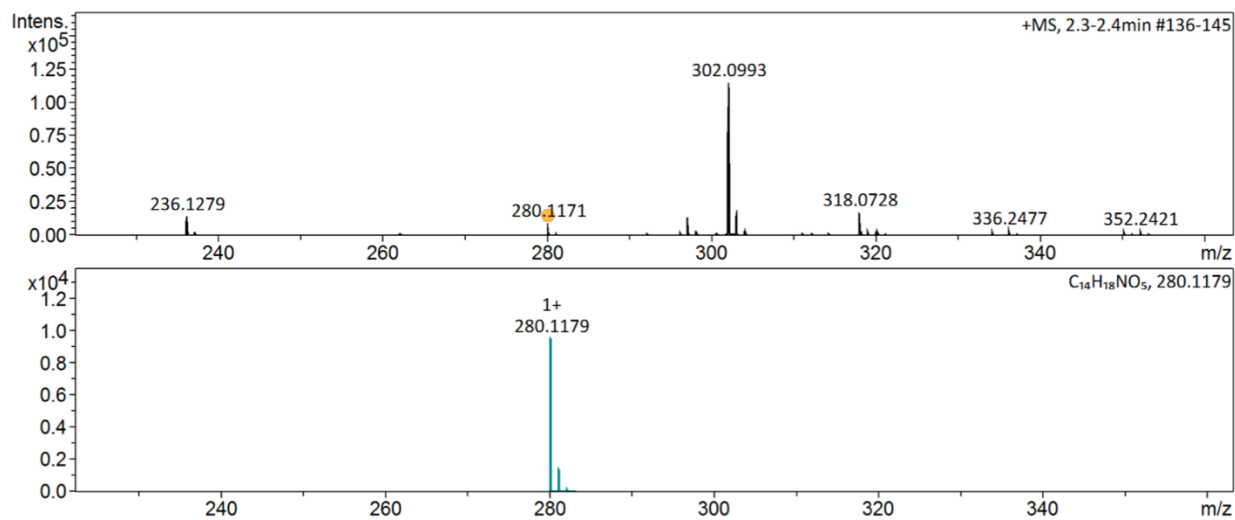
^{13}C NMR (400 MHz, CDCl_3) of 5-eMAL



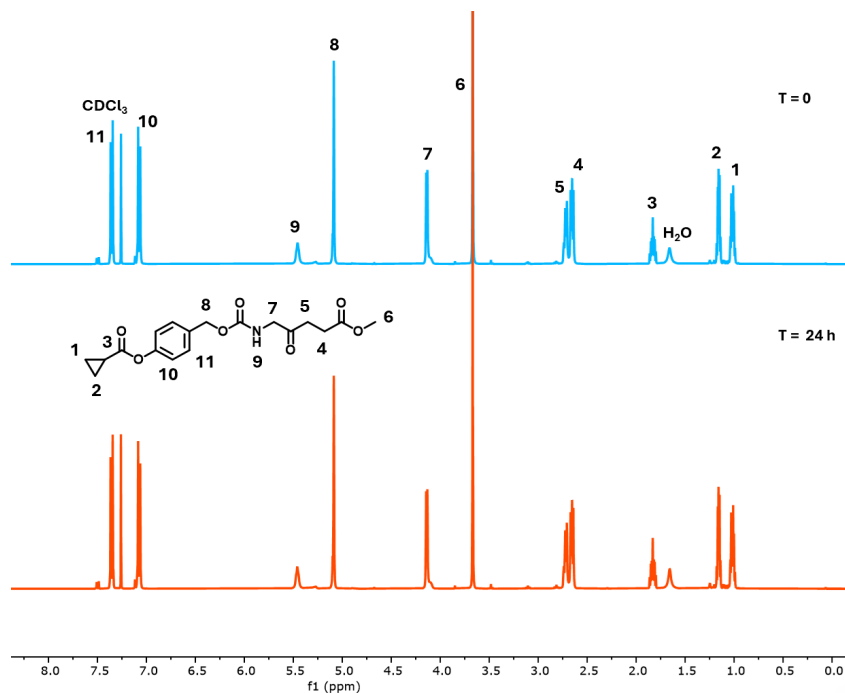
HRMS (ESI-TOF) spectrum of 5-eMAL



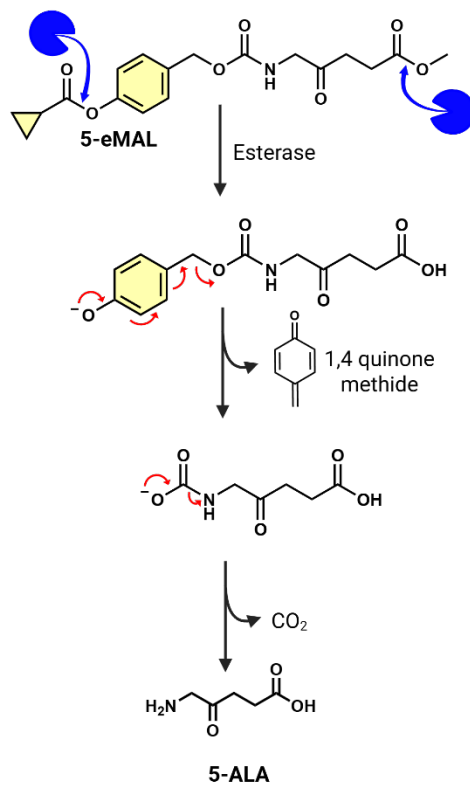
^1H NMR spectrum (400 MHz, CDCl_3) of **5-bMAL**



HRMS (ESI-TOF) spectrum of **5-bMAL**



Scheme S1: ¹H NMR spectrum (400 MHz, CDCl₃, 25°C) showing that a solution of 5-eMAL is stable over 24 hours at room temperature.



Scheme S2: Esterase triggered conversion of 5-eMAL into 5-ALA.

Biological Controls

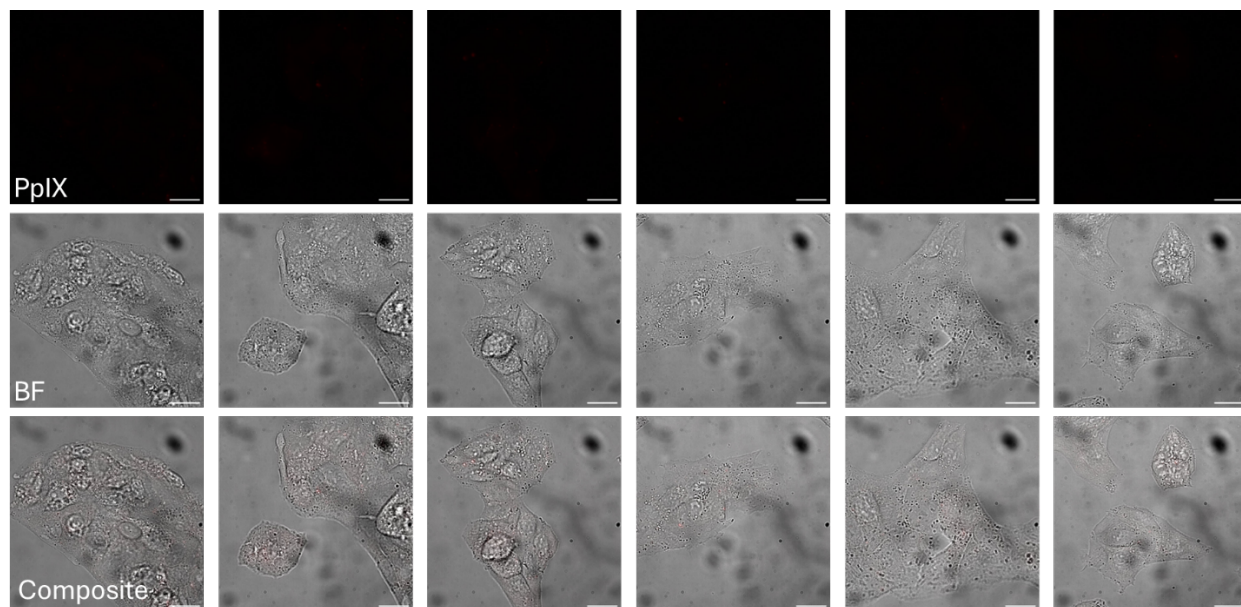


Figure S1: Micrographs of untreated HepG2 cells. Ex: 387/11 nm; Dichromatic mirror: 409 nm; Em: 655/40 nm. Scale Bar = 30 μ m.

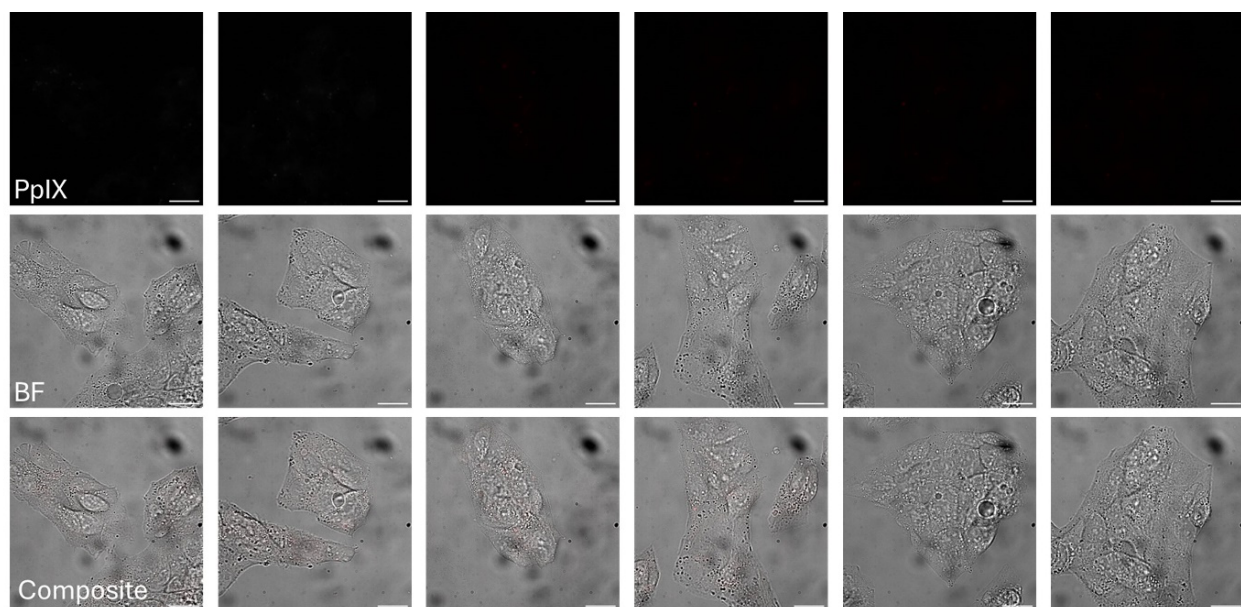


Figure S2: Micrographs of HepG2 cells incubated three hours with 5-ALA (5 μ M). Ex: 387/11 nm; Dichromatic mirror: 409 nm; Em: 655/40 nm. Scale Bar = 30 μ m.

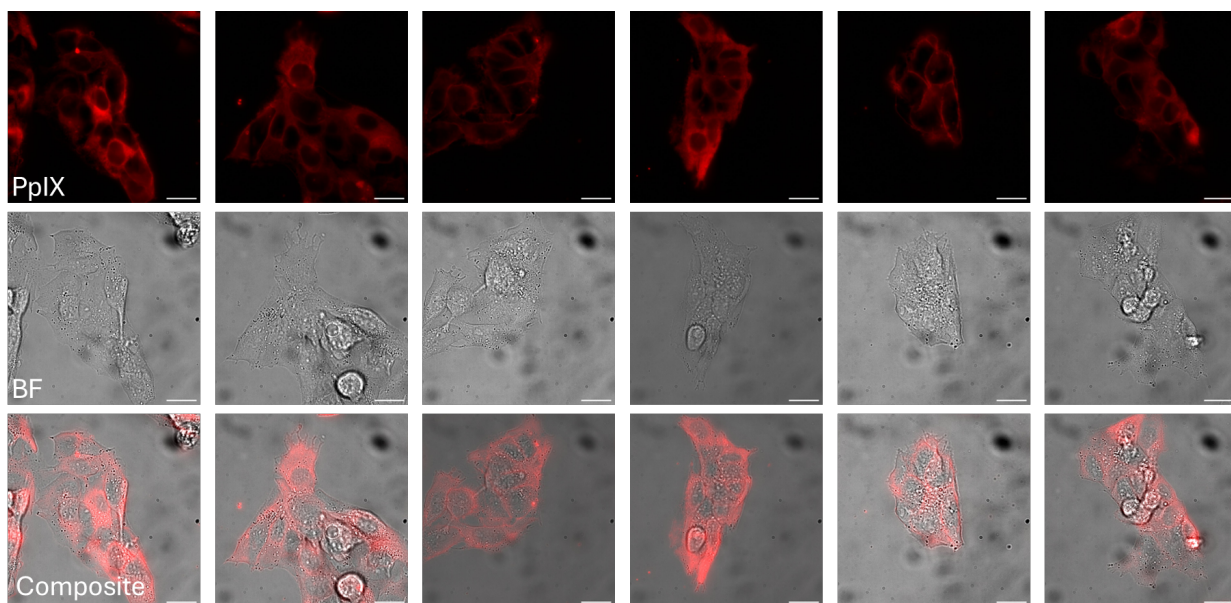


Figure S3: Micrographs of HepG2 cells incubated three hours with 5-eMAL (5 μ M). Ex: 387/11 nm; Dichromatic mirror: 409 nm; Em: 655/40 nm. Scale Bar = 30 μ m.

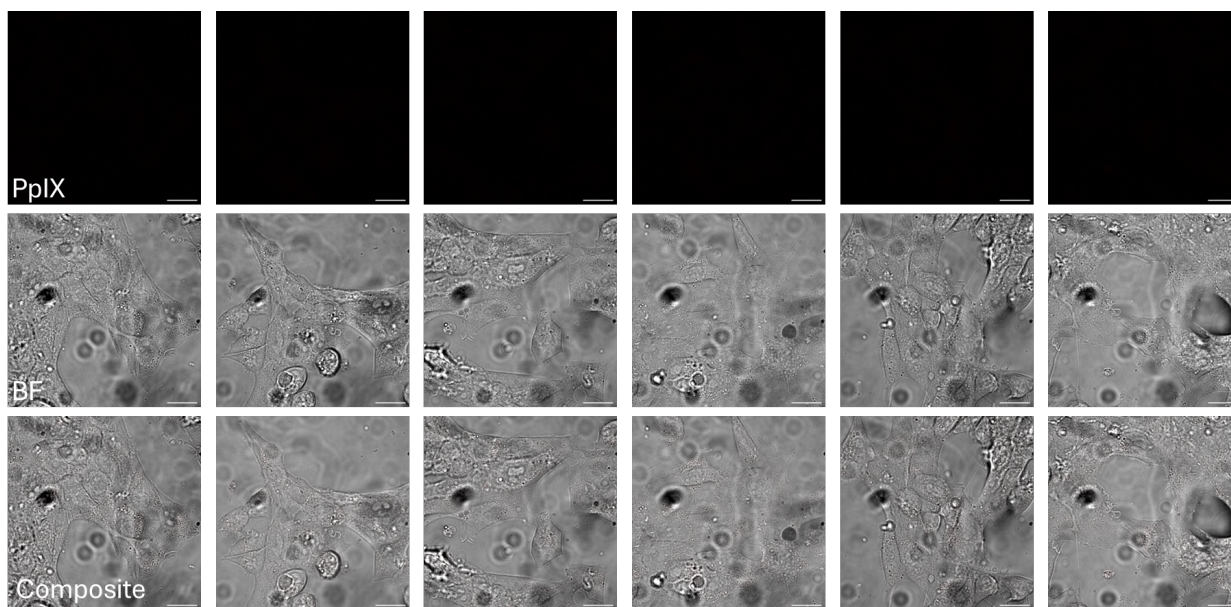


Figure S4: Micrographs of untreated 4T1 cells. Ex: 387/11 nm; Dichromatic mirror: 409 nm; Em: 655/40 nm. Scale Bar = 30 μ m.

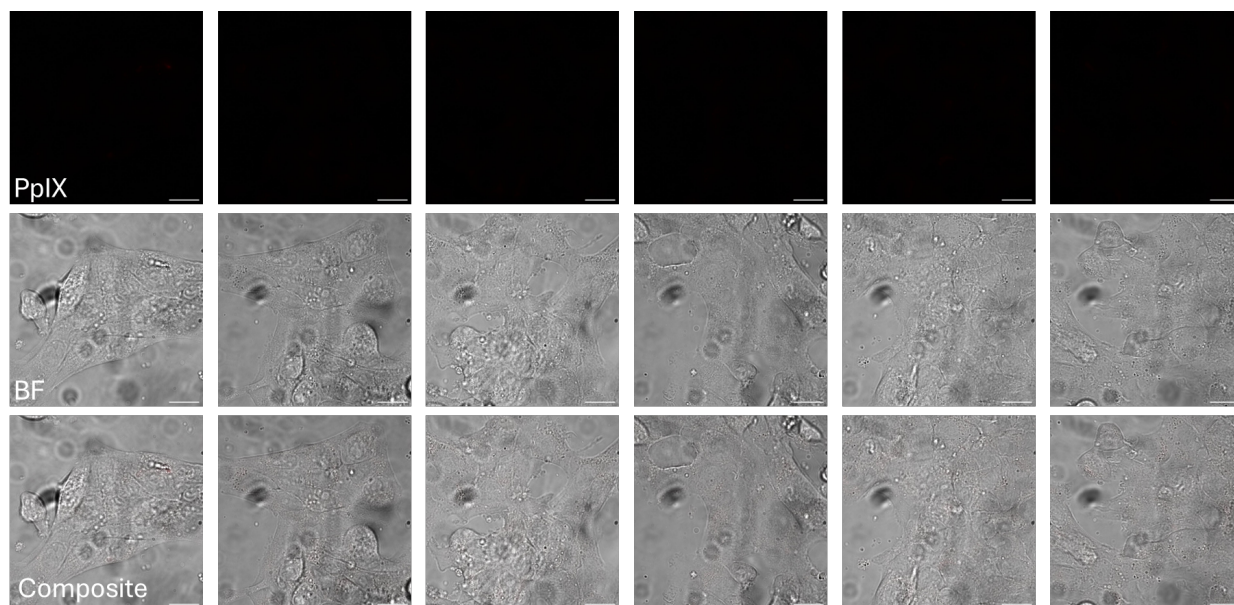


Figure S5: Micrographs of 4T1 cells incubated three hours with 5-ALA (5 μ M). Ex: 387/11 nm; Dichromatic mirror: 409 nm; Em: 655/40 nm. Scale Bar = 30 μ m.

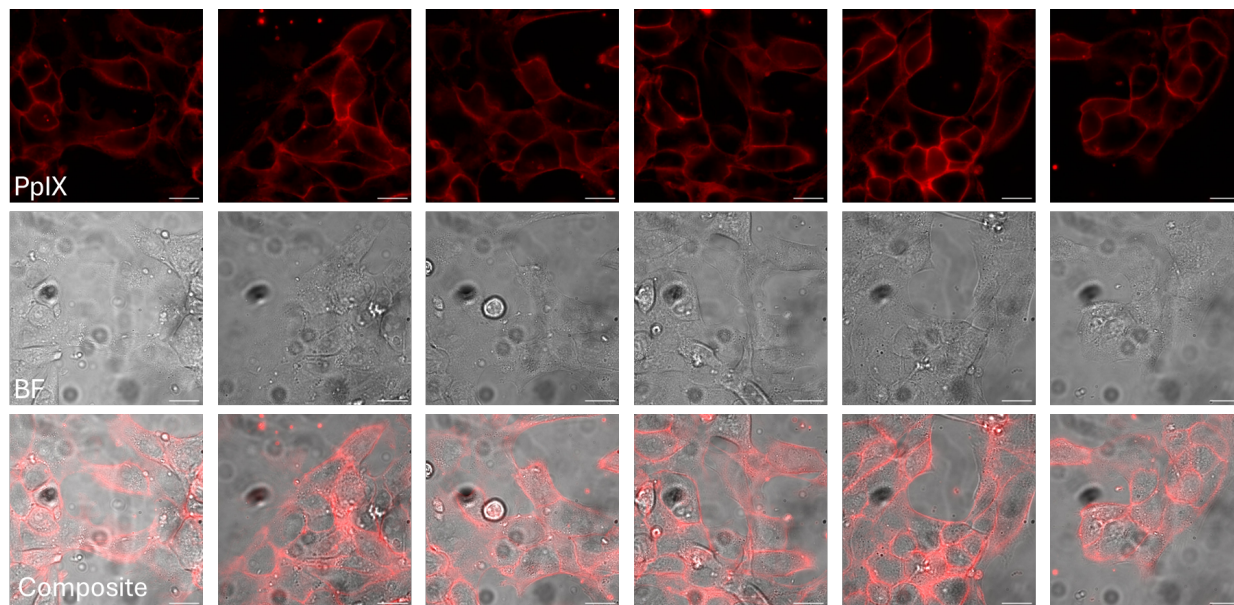


Figure S6: Micrographs of 4T1 cells incubated three hours with 5-eMAL (5 μ M). Ex: 387/11 nm; Dichromatic mirror: 409 nm; Em: 655/40 nm. Scale Bar = 30 μ m.

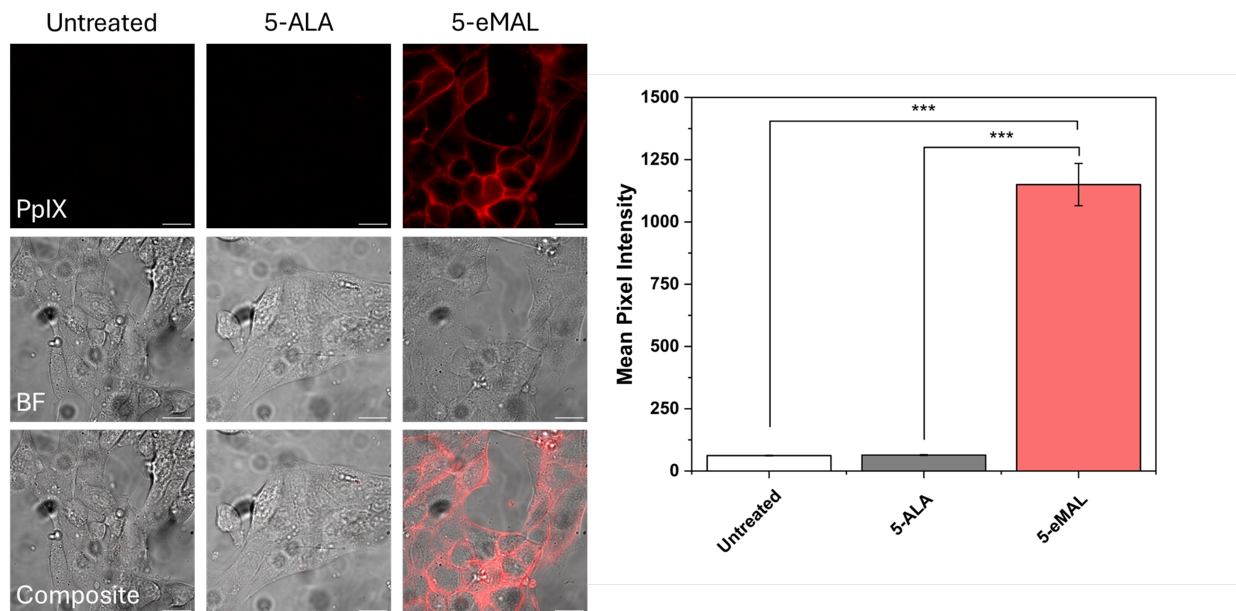


Figure S7: (a) Representative micrographs of 4T1 cells left untreated, incubated three hours with 5-ALA (5 μ M), or incubated three hours with 5-eMAL (5 μ M). (b) Average mean pixel intensity of 4T1 cells. Bars represent standard error (N = 6). *** $p < 0.001$. Ex: 387/11 nm; Dichromatic mirror: 409 nm; Em: 655/40 nm. Scale bar = 30 μ m.

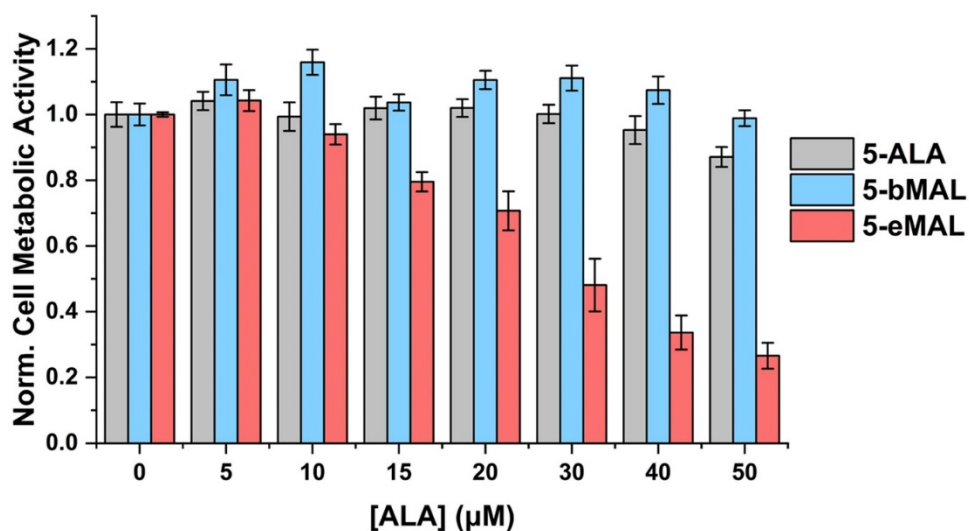


Figure S8: Normalized metabolic activity of HepG2 cells incubated 24 hours with either 5-ALA, 5-eMAL, or 5-bMAL (0 – 50 μ M) in dark. Bars represent standard error of one experiment where N = 3 replicates of the same concentration.

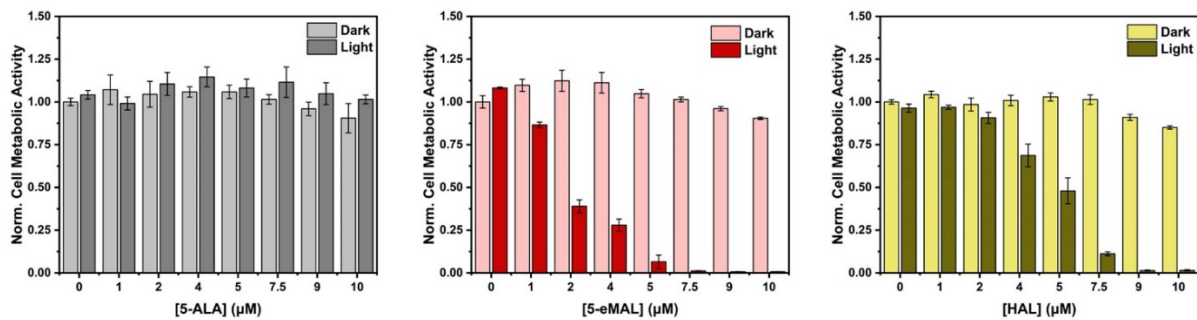


Figure S9: Normalized metabolic activity of HepG2 cells incubated 3 hours with 5-ALA, 5-eMAL, or HAL (0 – 10 μM) and kept in dark or cells incubated 3 hours with 5-ALA, 5-eMAL, or HAL (0 – 10 μM) and then irradiated with 405 nm light (6 mW) for 10 minutes. Bars represent error where N = 3.

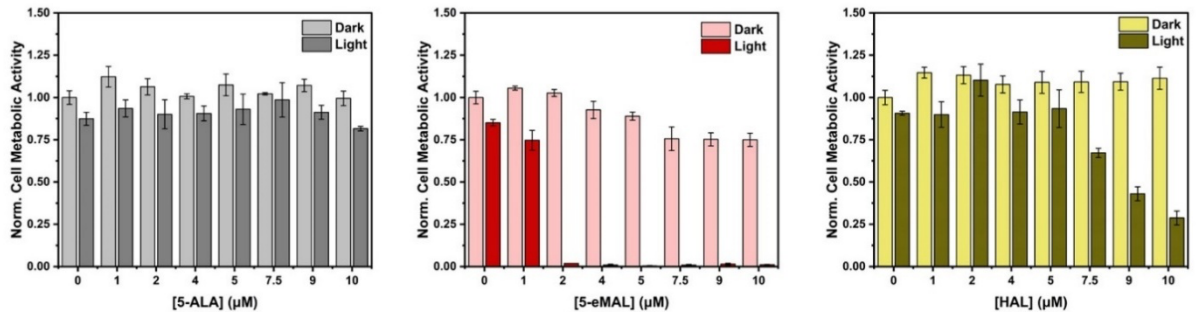


Figure S10: Normalized metabolic activity of 4T1 cells incubated 3 hours with 5-ALA, 5-eMAL, or HAL (0 – 10 μM) and kept in dark or cells incubated 3 hours with 5-ALA, 5-eMAL, or HAL (0 – 10 μM) and then irradiated with 405 nm light (6 mW) for 10 minutes. Bars represent error where N = 3.