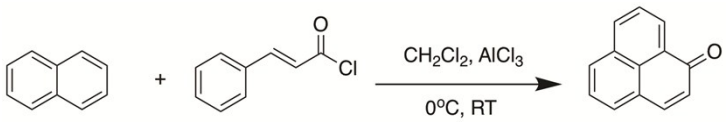
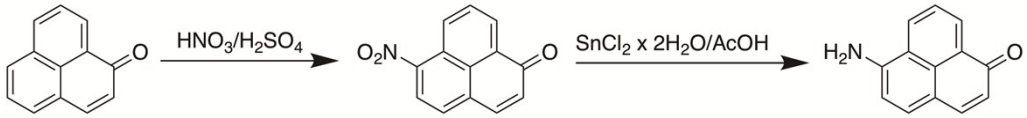
Electronic Supplementary Material (ESI) for Chemical Science.

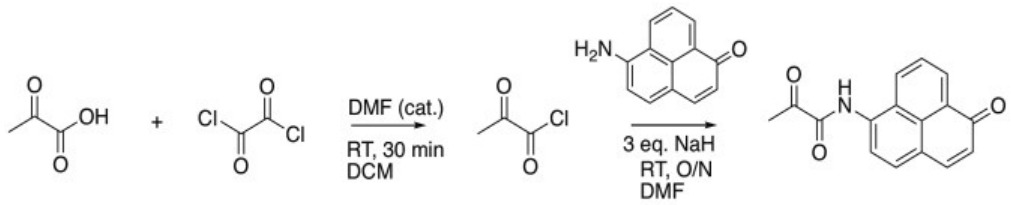
Supporting Information



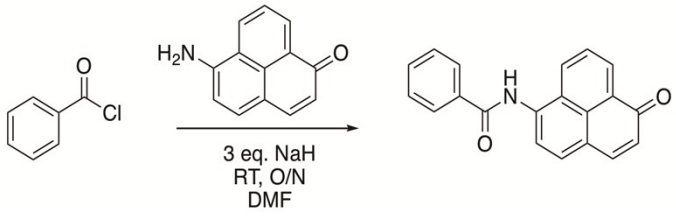
Scheme 1. Synthesis of Phenalenone.



Scheme 2. Synthesis of 6-amino-1-phenalenone.



Scheme 3. Synthesis of Probe **1**.



Scheme 4. Synthesis of Probe **2**.

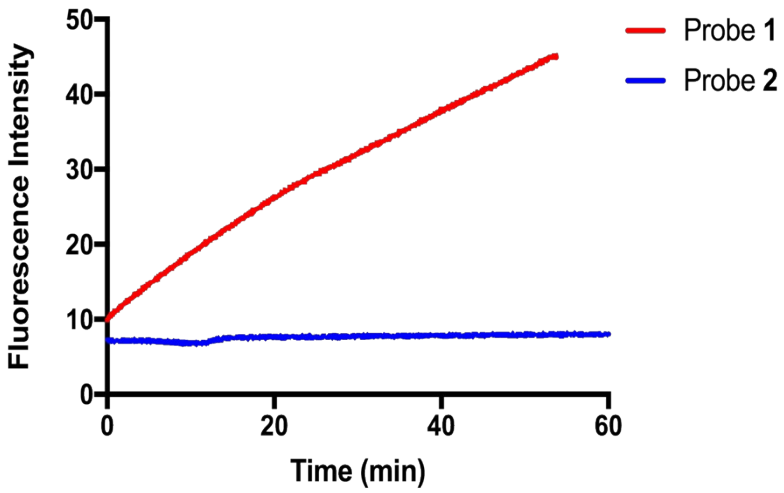


Figure S1. Aqueous stability of probe **1** and probe **2**. Time-dependent fluorescence intensity at 605 nm of 11 µM of probe **1** and probe **2** were measured in DPBS at 37°C with an excitation wavelength of 548 nm.

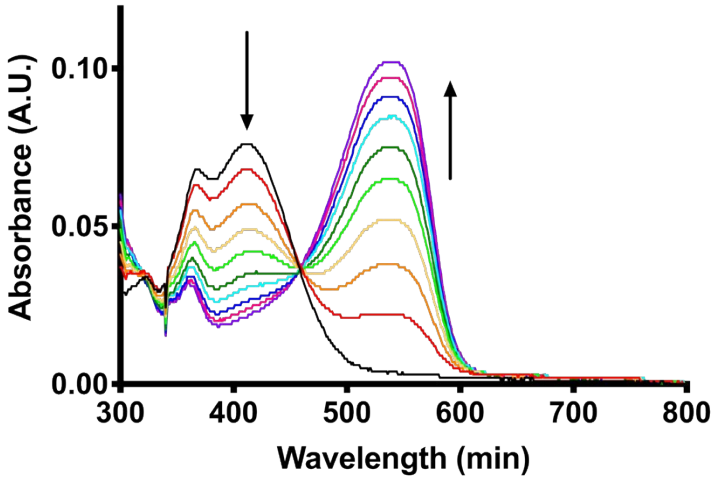


Figure S2. UV-Vis spectra changes of probe **2** (11 µM) upon addition of 110 nM hCES2. Spectra were recorded in 20 min intervals in DPBS at 37°C.

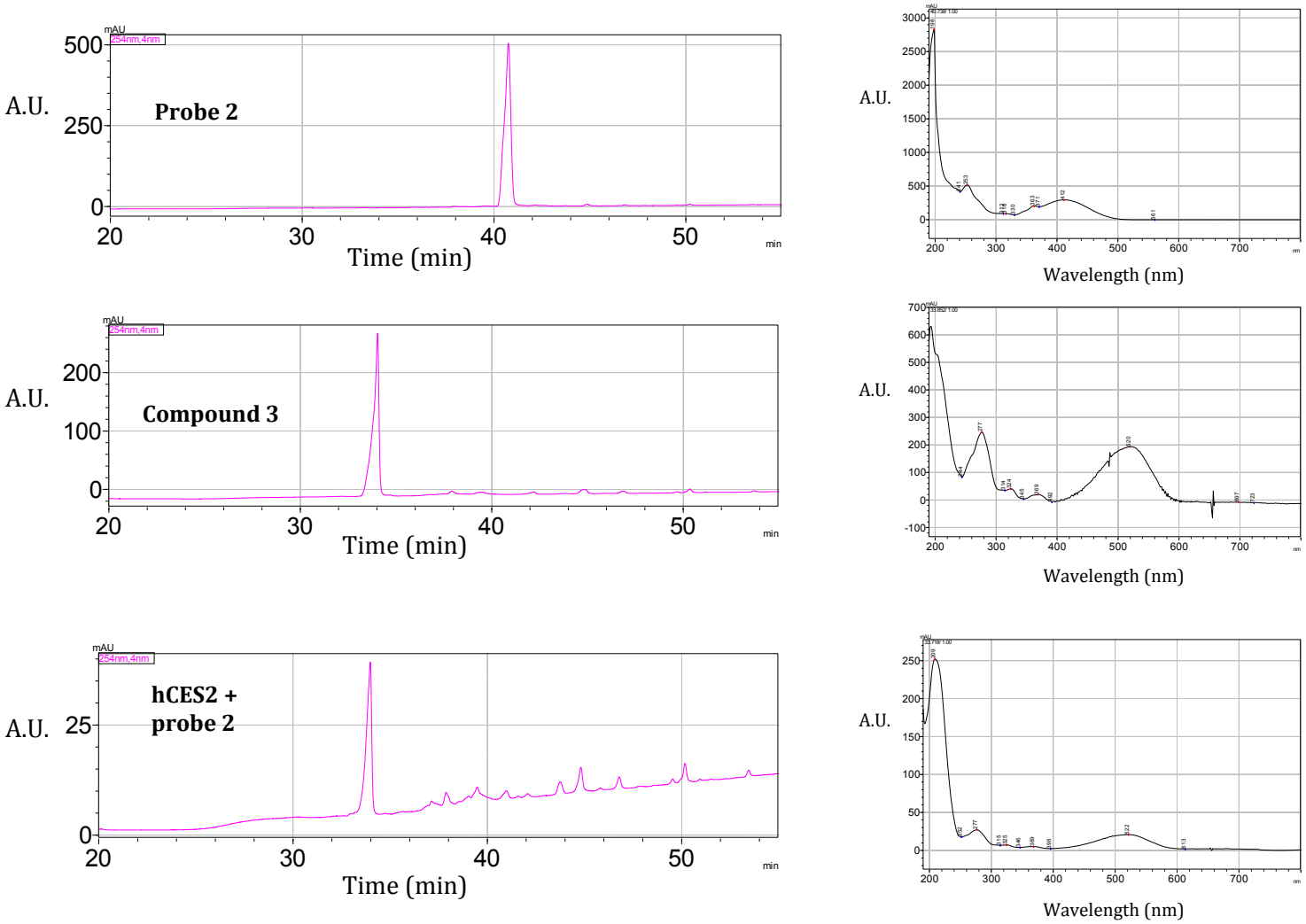


Figure S3. HPLC analysis of Probe **2** (top), **3** (middle), and post-hCES2 reaction with probe **2** (bottom). For the latter, 11 µM Probe **2** and 110 nM hCES2 were incubated in DPBS at 37 °C for 3 h then injected. Left, HPLC chromatograms (monitored at 254 nm); right, UV-Vis spectra of the strongest peaks. Post-hCES2 reaction (bottom) shows a peak at 34 min, matching the retention time and absorption spectrum of purified compound **3** (middle). No peak at 40.5 min corresponding to probe **2** (top) was observed in the reaction mixture.

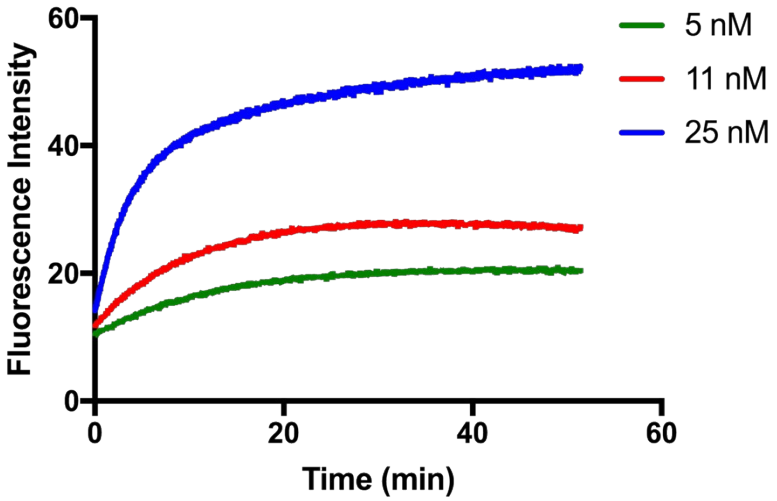


Figure S4. Time-dependent fluorescence intensity at 605 nm (Ex 548 nm) changes of probe **2** (20 μM) with different concentrations (5, 11, 25 nM) of hCES2 at 37°C.

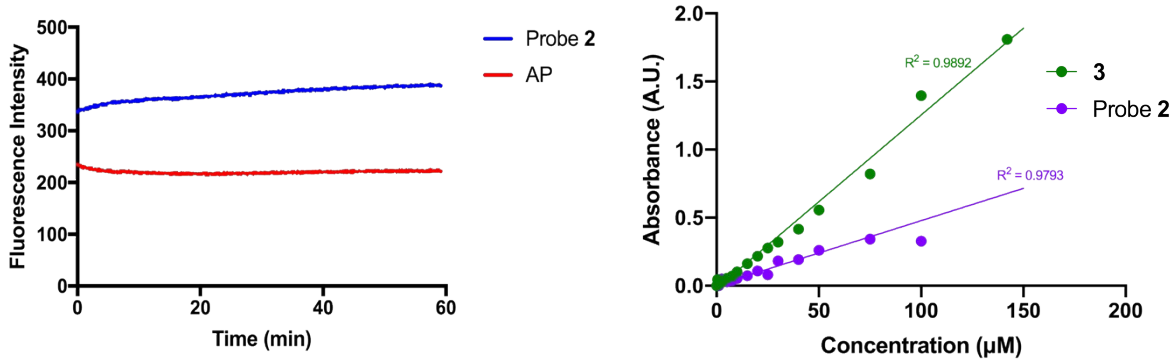


Figure S5. Left, photostability of probe **2** and AP (11 μM). Spectra were recorded in DPBS with 413 nm excitation for probe **2** and 548 nm excitation for AP. Right, plot of absorbance at λmax (413 nm for Probe **2**, 548 nm for **3**) against concentration for Probe **2** (purple trace) and **3** (green trace). The maximum linear response was taken as the solubility limit in PBS, pH 7.4, 25 °C. Probe **2** has solubility up to 75 μM while **3** is soluble to at least 140 μM; further concentrations were not tested since higher absorbance values may deviate from linearity due to detection by the spectrophotometer rather than insolubility.

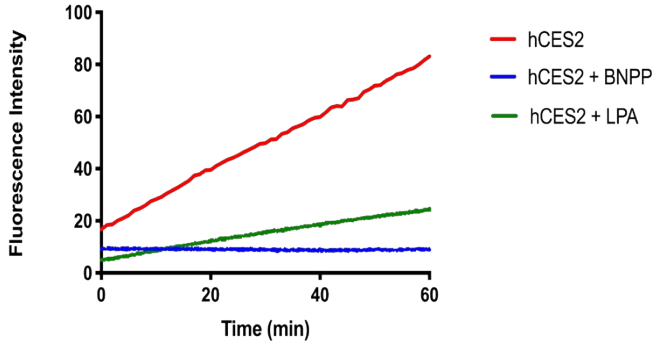


Figure S6. Initial rates monitored by fluorescence intensity at 605 nm (Ex. 548 nm) of 11 µM probe **2** with 110 nM CES2. Blue and green traces indicate pretreatment of CES2 with 1 µM inhibitor before addition of probe **2**.

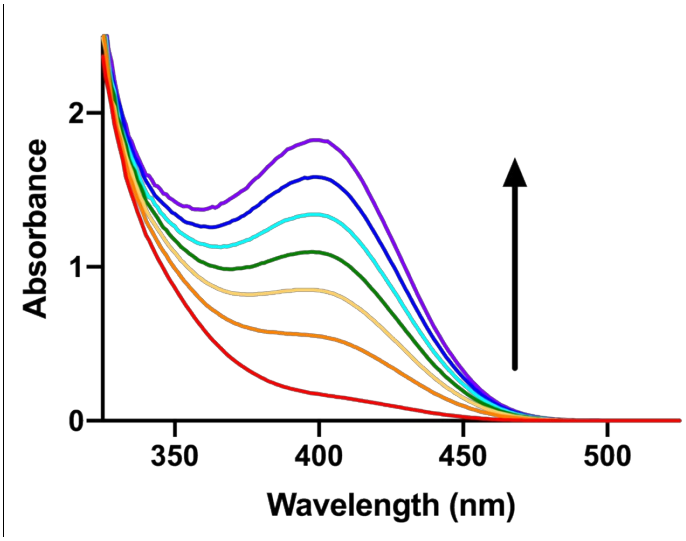


Figure S7. UV-Vis spectra changes of *para*-nitrophenyl acetate (p-NPA) (2 mM) upon addition of 110 nM hCES1. Spectra were recorded in 5 min intervals in DPBS at 37^{o}C.

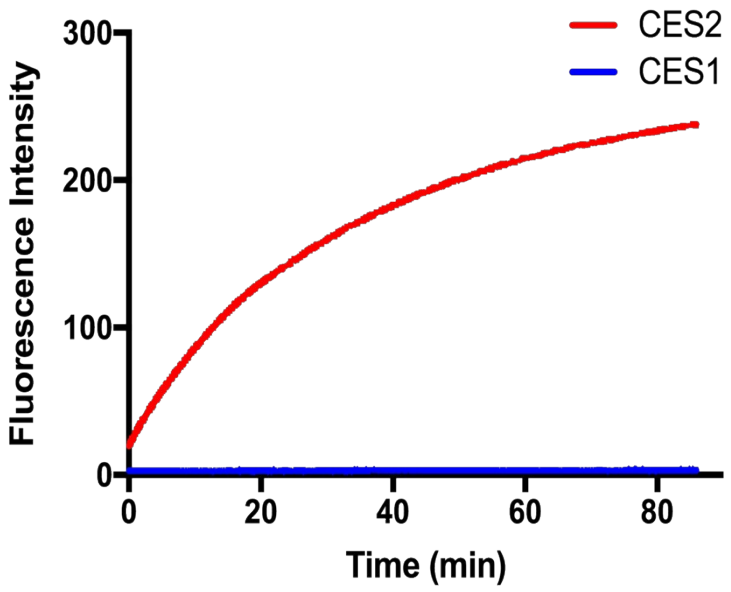


Figure S8. Fluorescence intensity at 605 nm (Ex 548 nm) of 11 µM probe **2** with 110 nM CES1 or CES2.

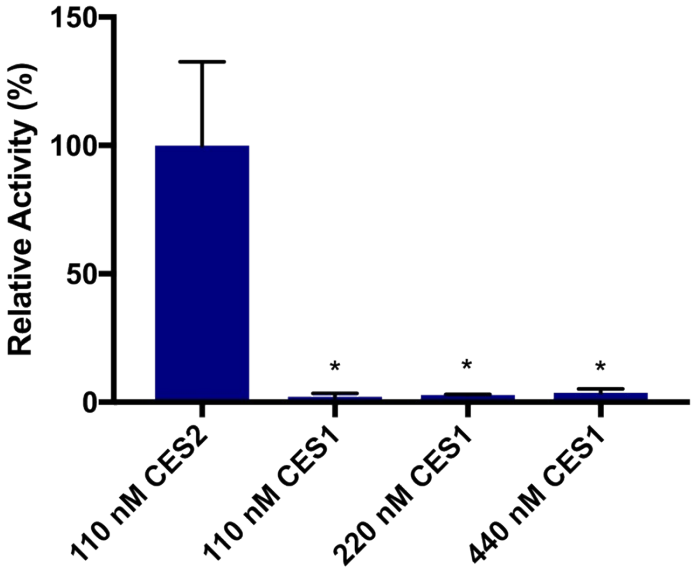


Figure S9. Relative activity of probe **2** (11 µM) with CES1. Fluorescence emission spectra at 605 nm were acquired in DPBS at 37°C for one hour with excitation at 548 nm. Data are shown as mean ± standard deviation of triplicate experiments. Statistical significance was calculated by student's t-test (*$p<0.05$).

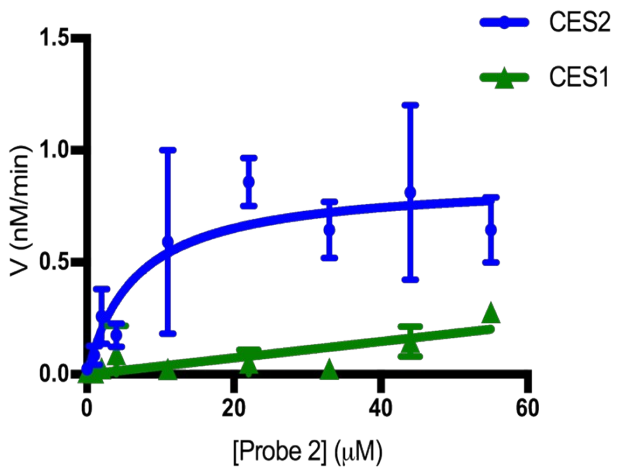


Figure S10. Michaelis-Menten kinetic curve for probe **2** with 55 nM CES2 and CES1. For CES2, K_m = 6.6 ± 3.7 μM, k_{cat} = 0.26 ± 0.04 s^{-1}. Michaelis-Menten parameters could not be determined for CES1.

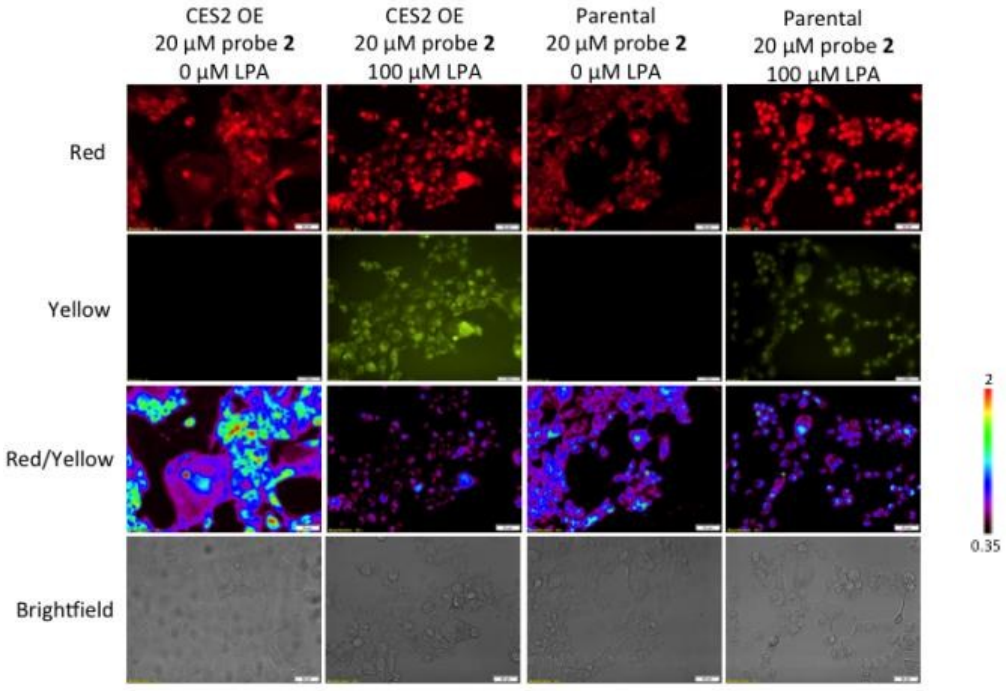


Figure S11. Ratiometric measurement of hCES2 activity in pancreatic cancer cells. Red fluorescence and yellow fluorescence of 20 μM probe **2** in SU.86.86 cells were measured in the presence or absence of LPA (100 μM) to produce ratio overlay images.

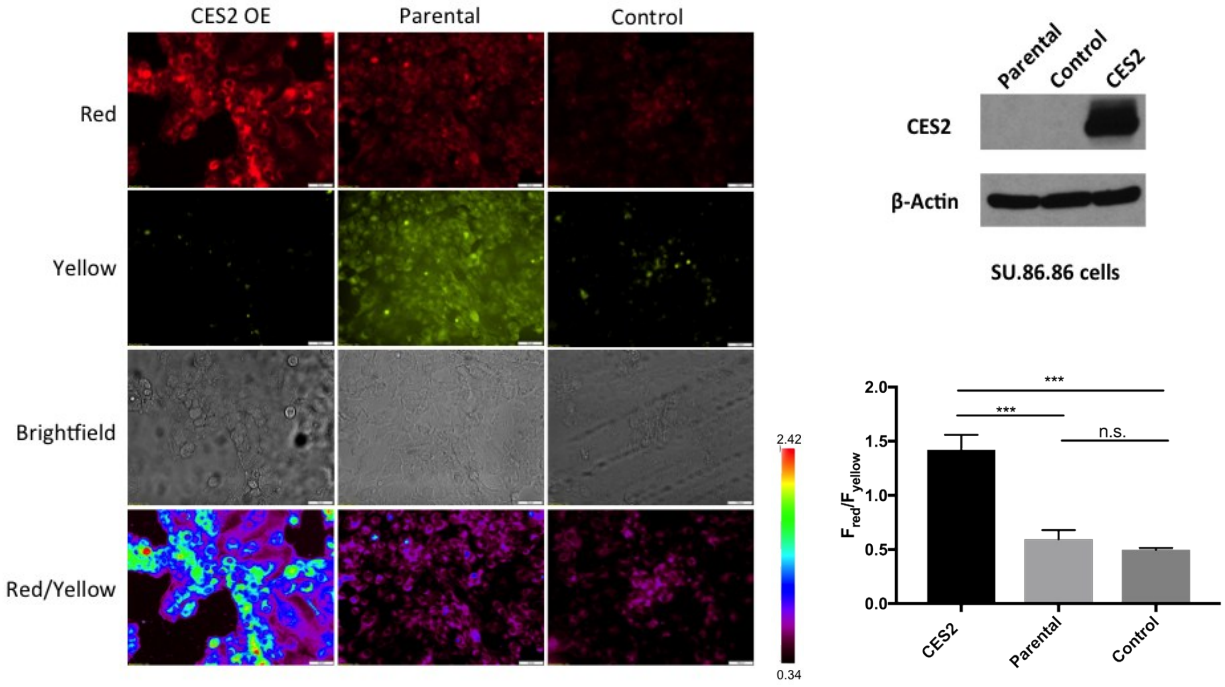


Figure S12. Ratiometric measurement of hCES2 activity in pancreatic cancer cells. Red fluorescence and yellow fluorescence of probe **2** (20 μM) in SU.86.86 cells (CES2 OE, parental, and control vector) were measured to produce ratio overlay images (left). Western blot (top right) displays hCES2 expression levels. Bar graph (bottom right) quantifies ratio of red over yellow fluorescence. Scale bars = 50 μ m. Statistical significance is calculated using unpaired t test compared with CES2 (***p<0.001, n.s. = no significance).

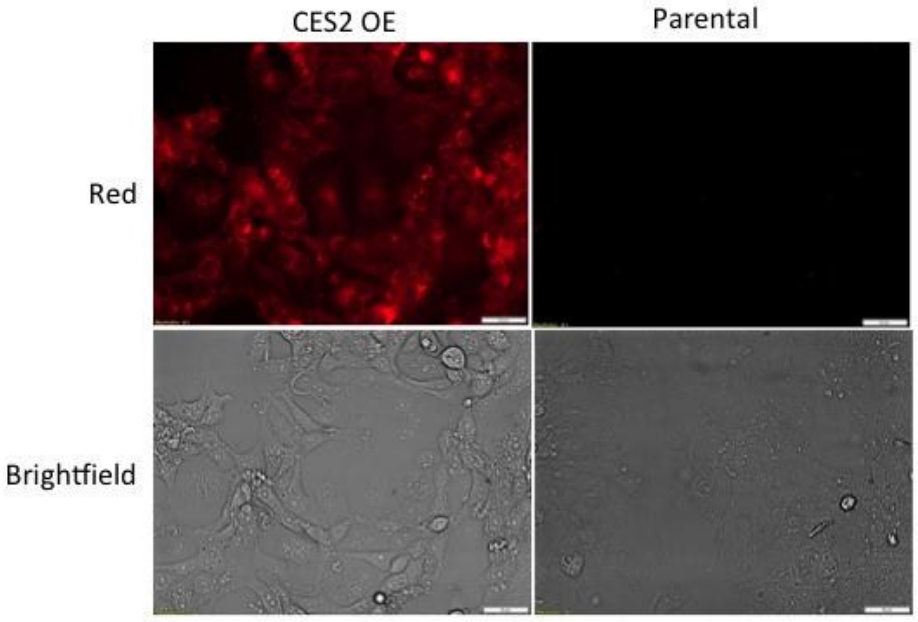


Figure S13. Red fluorescence measurement of hCES2 activity in pancreatic cancer cells treated with 20 μM probe **2**.

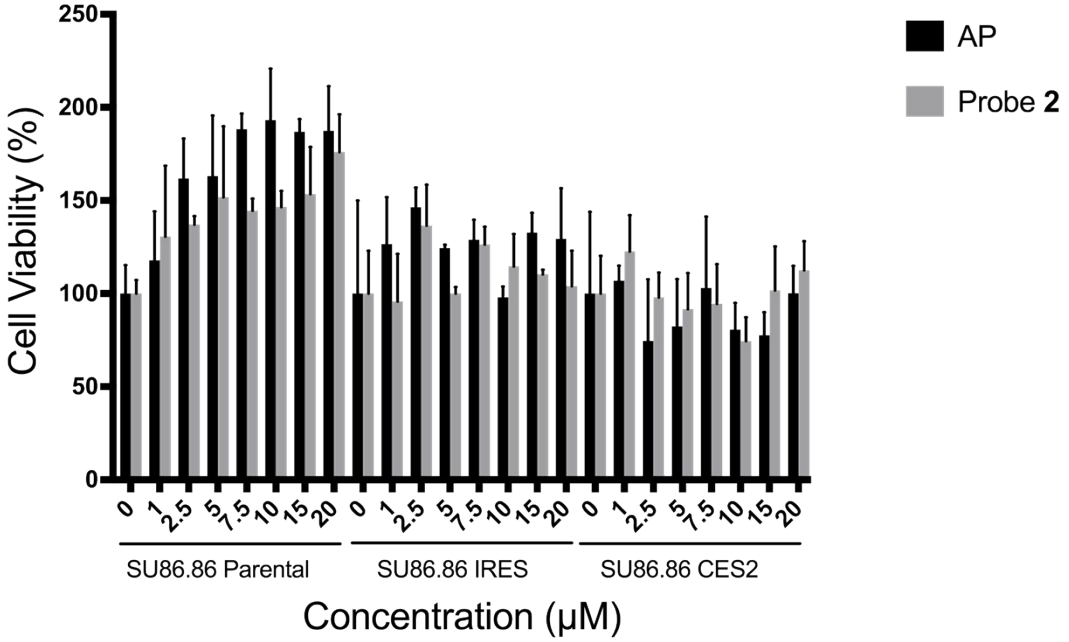


Figure S14. Cell viability assay of SU.86.86 Parental, control vector (IRES), and CES2 OE (CES2) cells following 3h incubation with AP and Probe **2.** Data are shown as mean ± standard deviation of triplicate experiments.

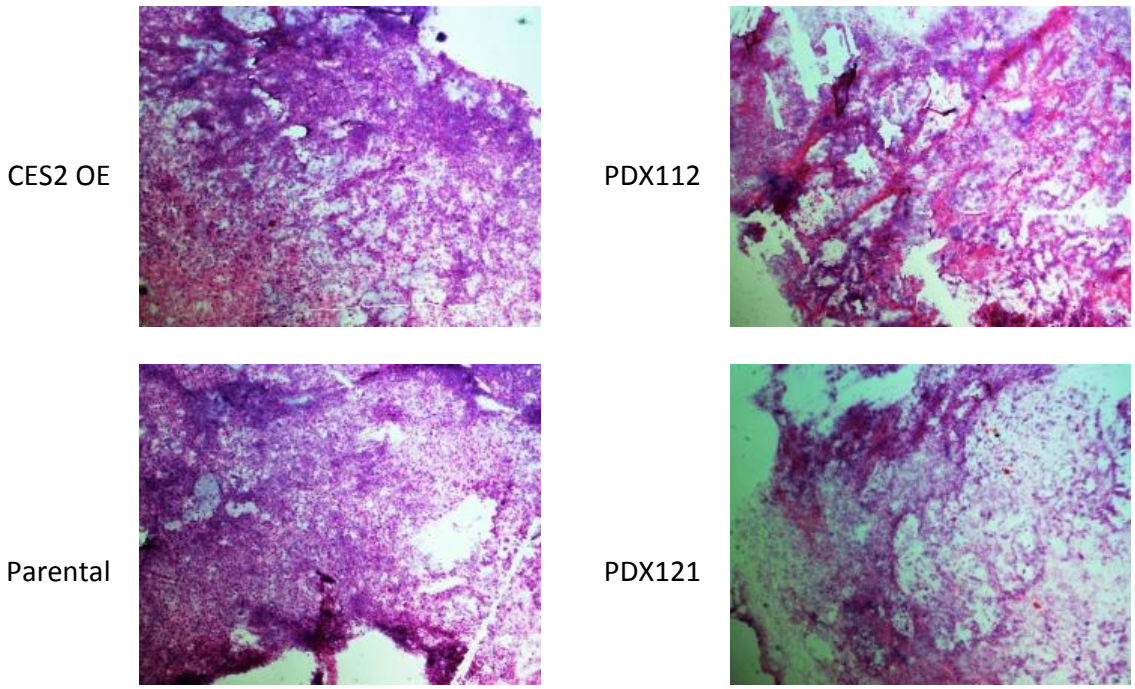


Figure S15. H&E Staining of SU.86.86 Orthotopic (left) and Patient-Derived Xenografts (right).

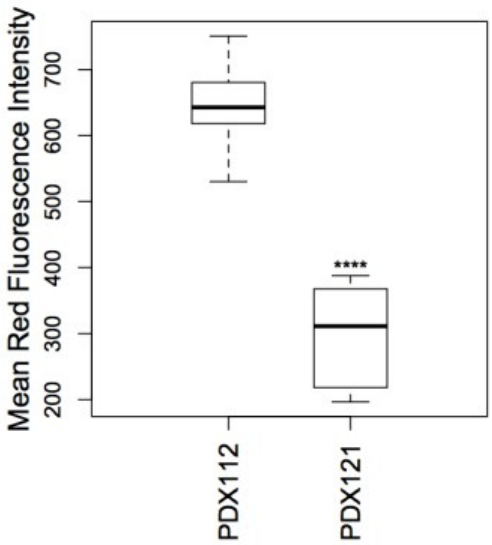


Figure S16. Single Channel Measurements of Red Fluorescence of PDX112 and PDX121 upon 3 h incubation with probe **2** (20 μM). Statistical significance is calculated using unpaired t test compared with CES2 (****p<0.0001).

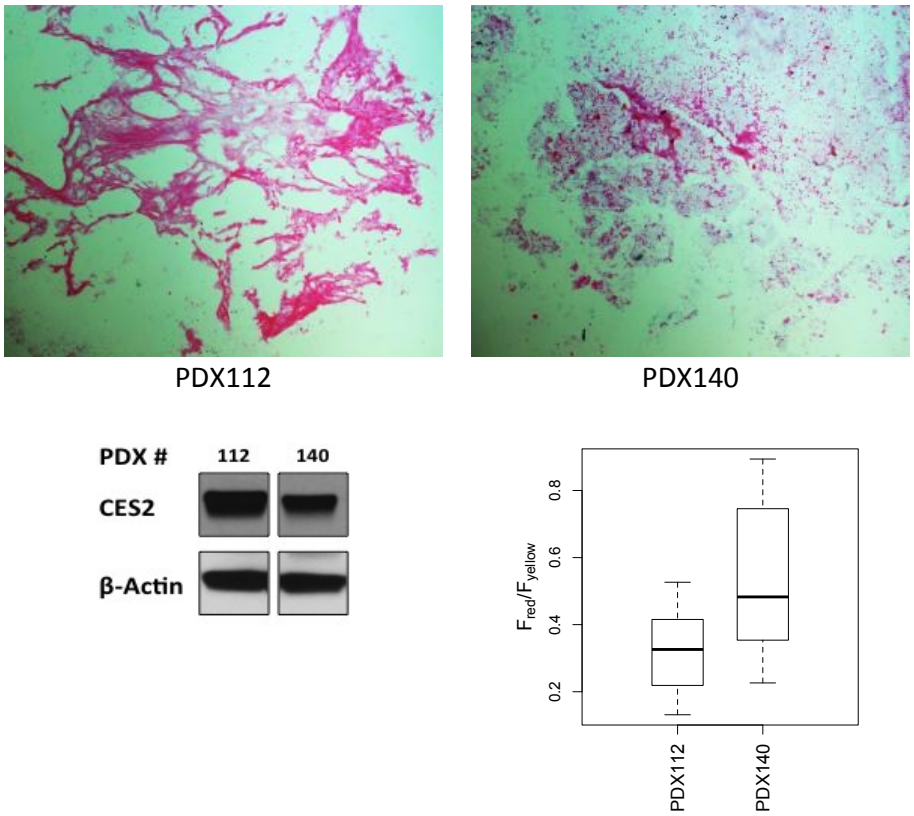


Figure S17. Top, H&E staining on a slide showing uniquely stromal components or necrotic cells, respectively. Bottom left, Western blot of hCES2 expression levels. Bottom right, ratiometric measurements of PDX112 (tissue slice 2) and PDX140. Right,

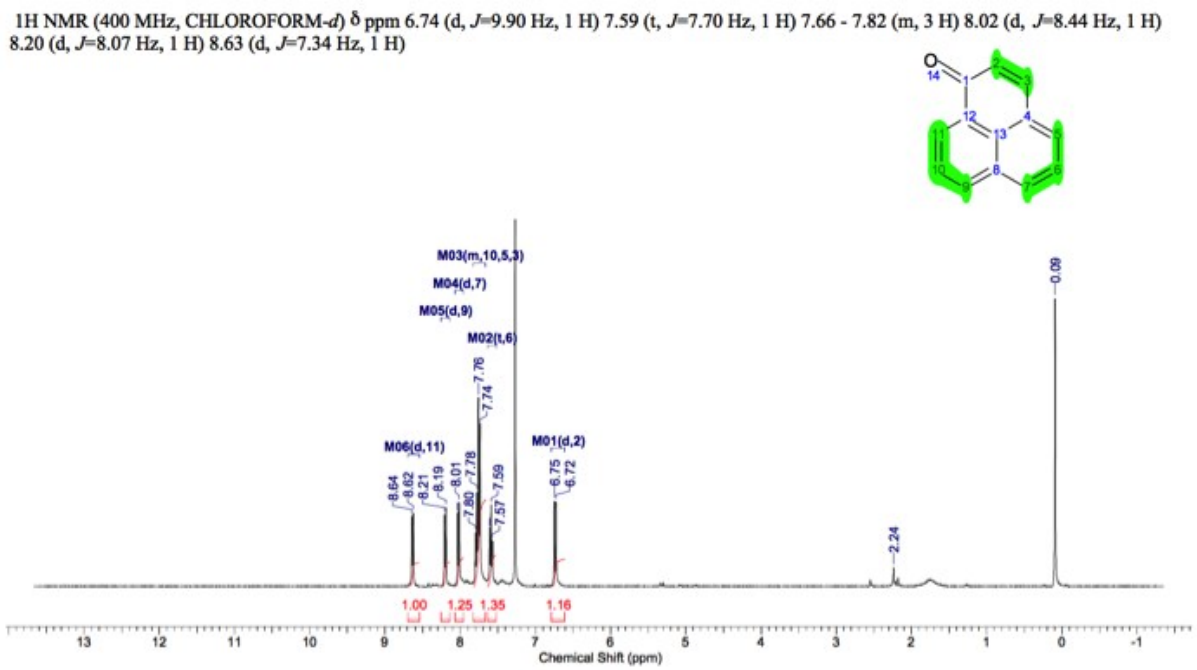


Figure S18. ^{1}H NMR of phenalenone.

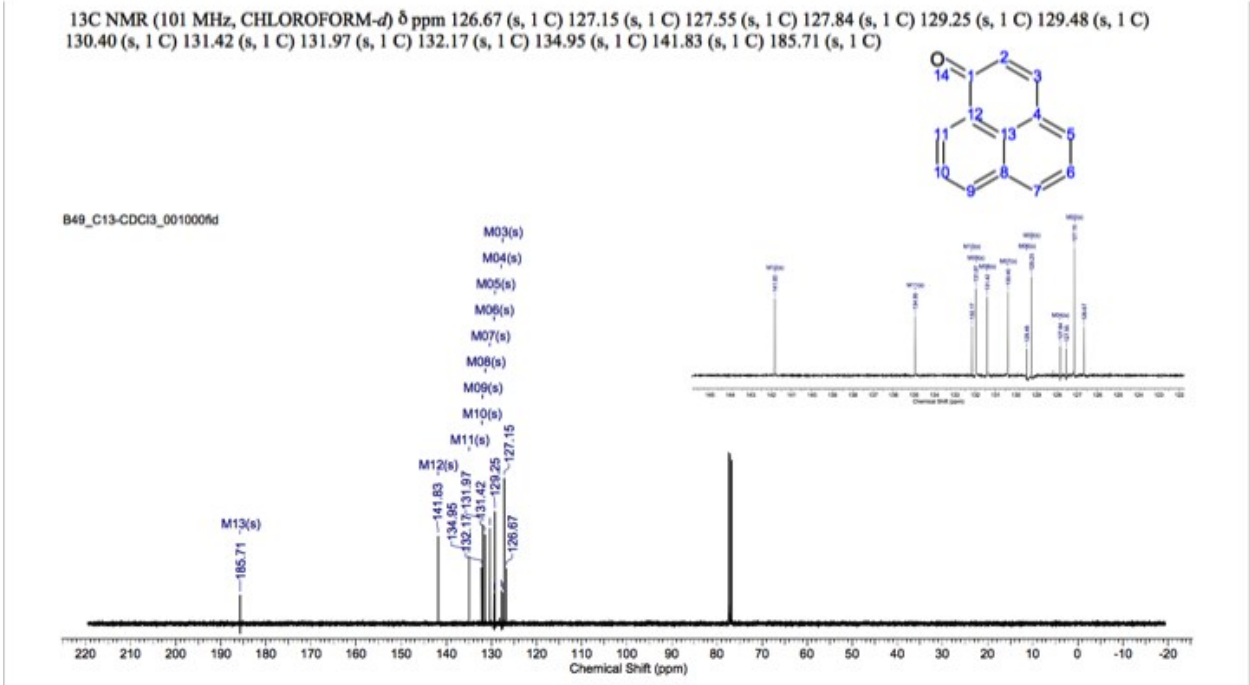


Figure S19. ^{13}C NMR of phenalenone.

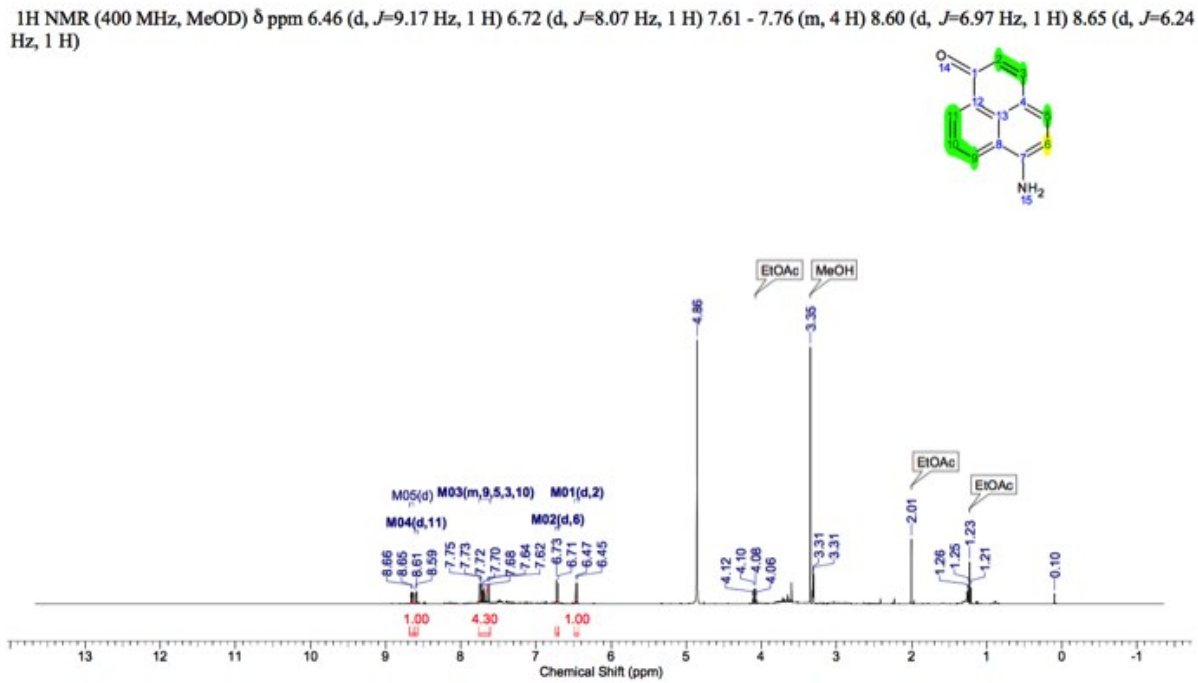


Figure S20. ^{1}H NMR of 6-amino-1-phenalenone (**3**).

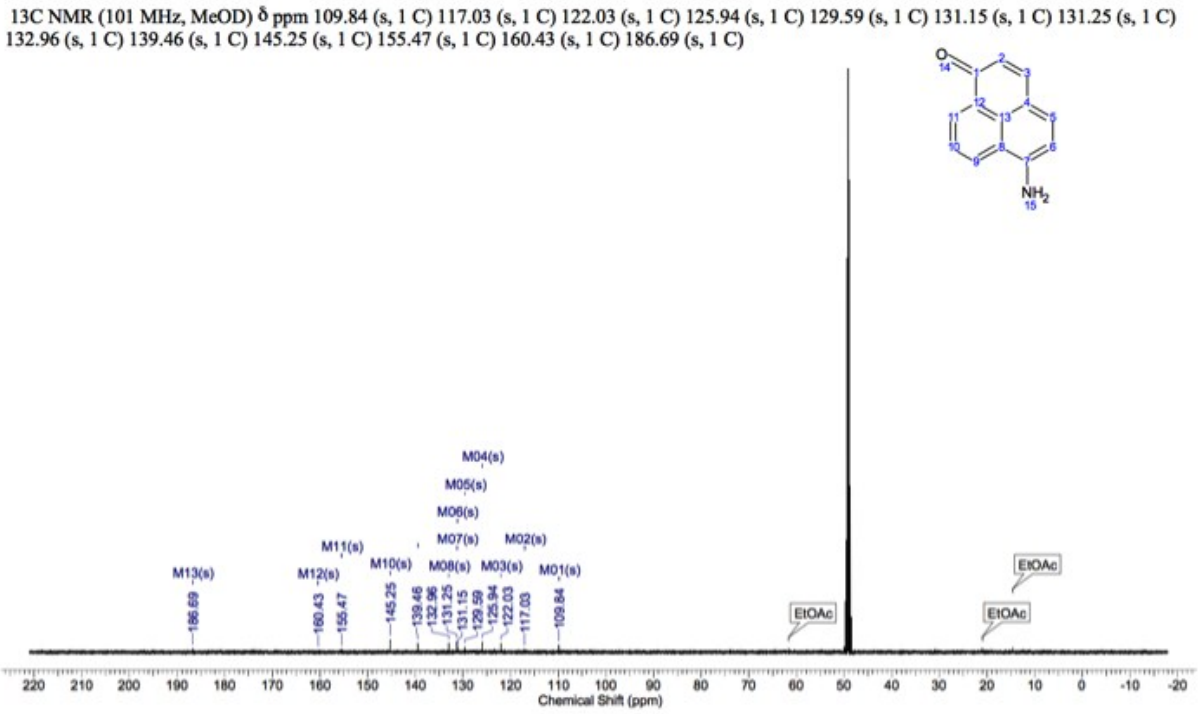


Figure S21. ^{13}C NMR of 6-amino-1-phenaleone (**3**).

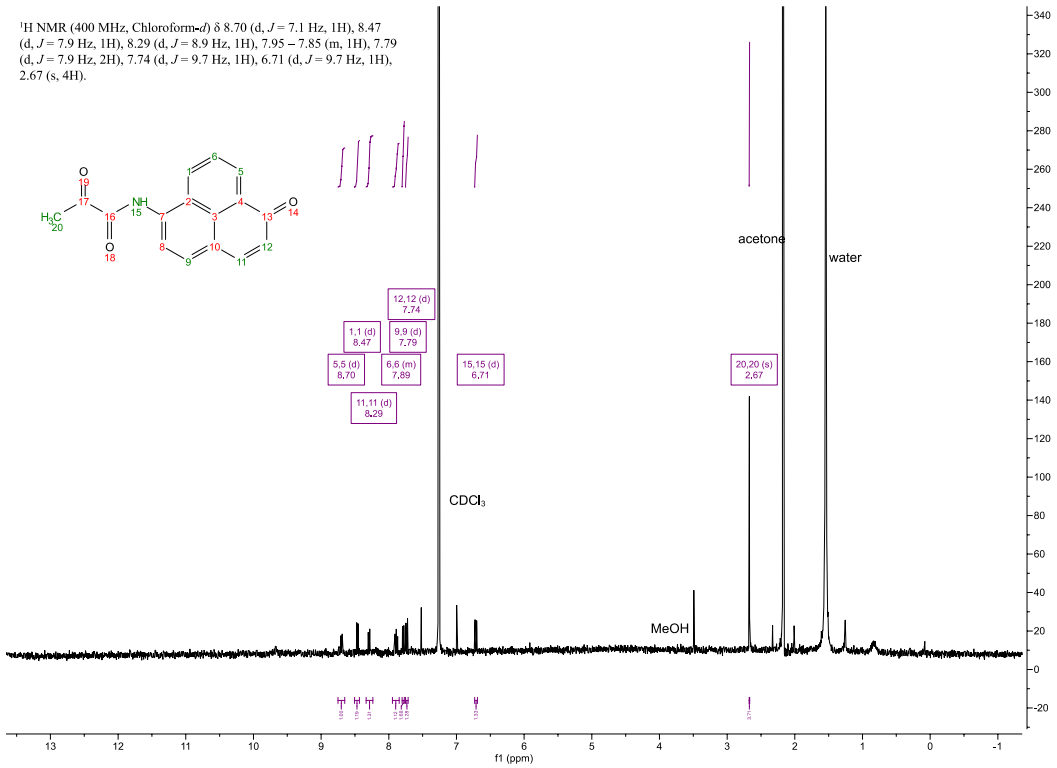


Figure S22. ^{1}H NMR of Probe **1**.

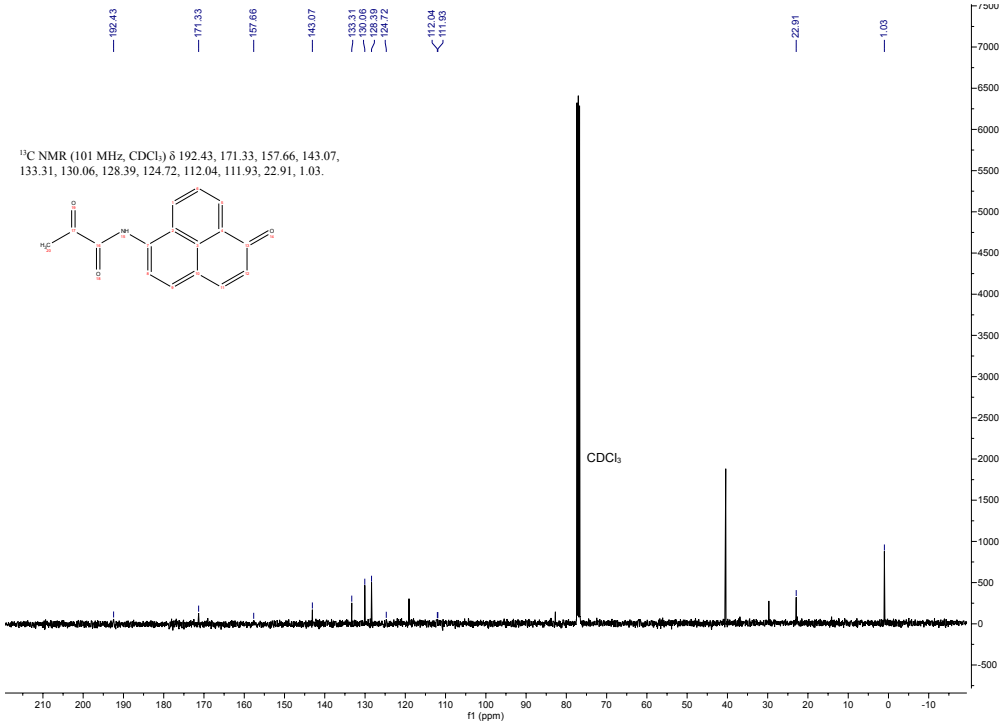


Figure S23. ^{13}C NMR of Probe **1**.

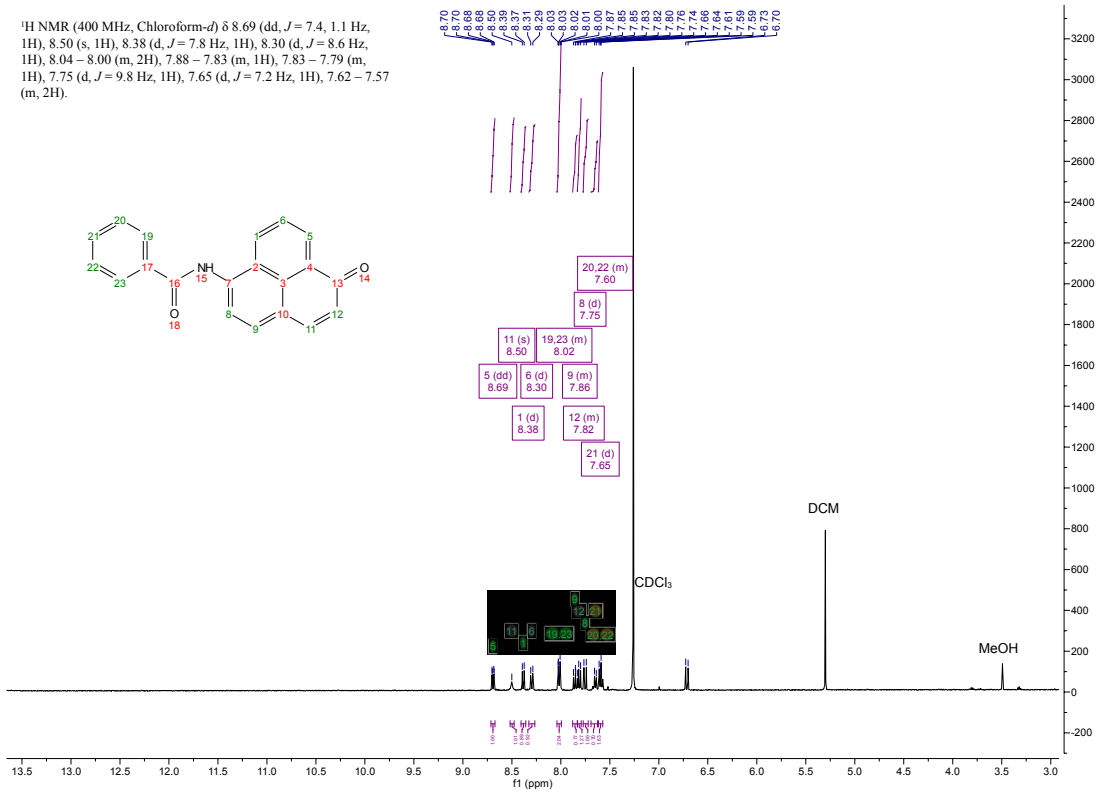


Figure S24. ^{1}H NMR of Probe **2.**

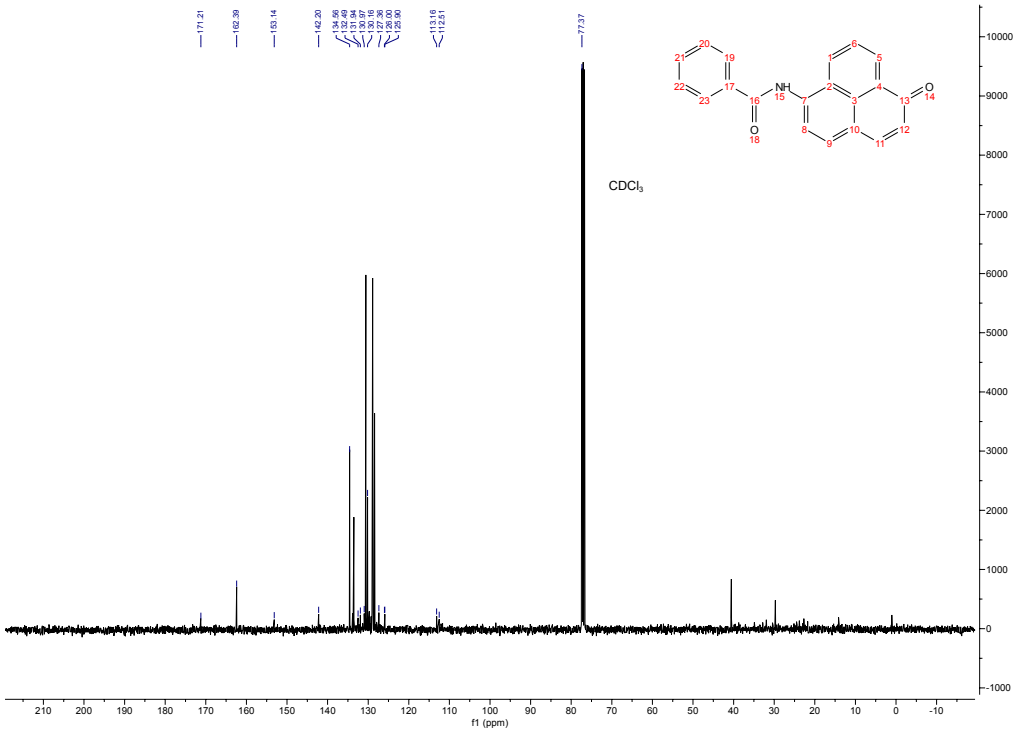


Figure S25. ^{13}C NMR of Probe **2.**

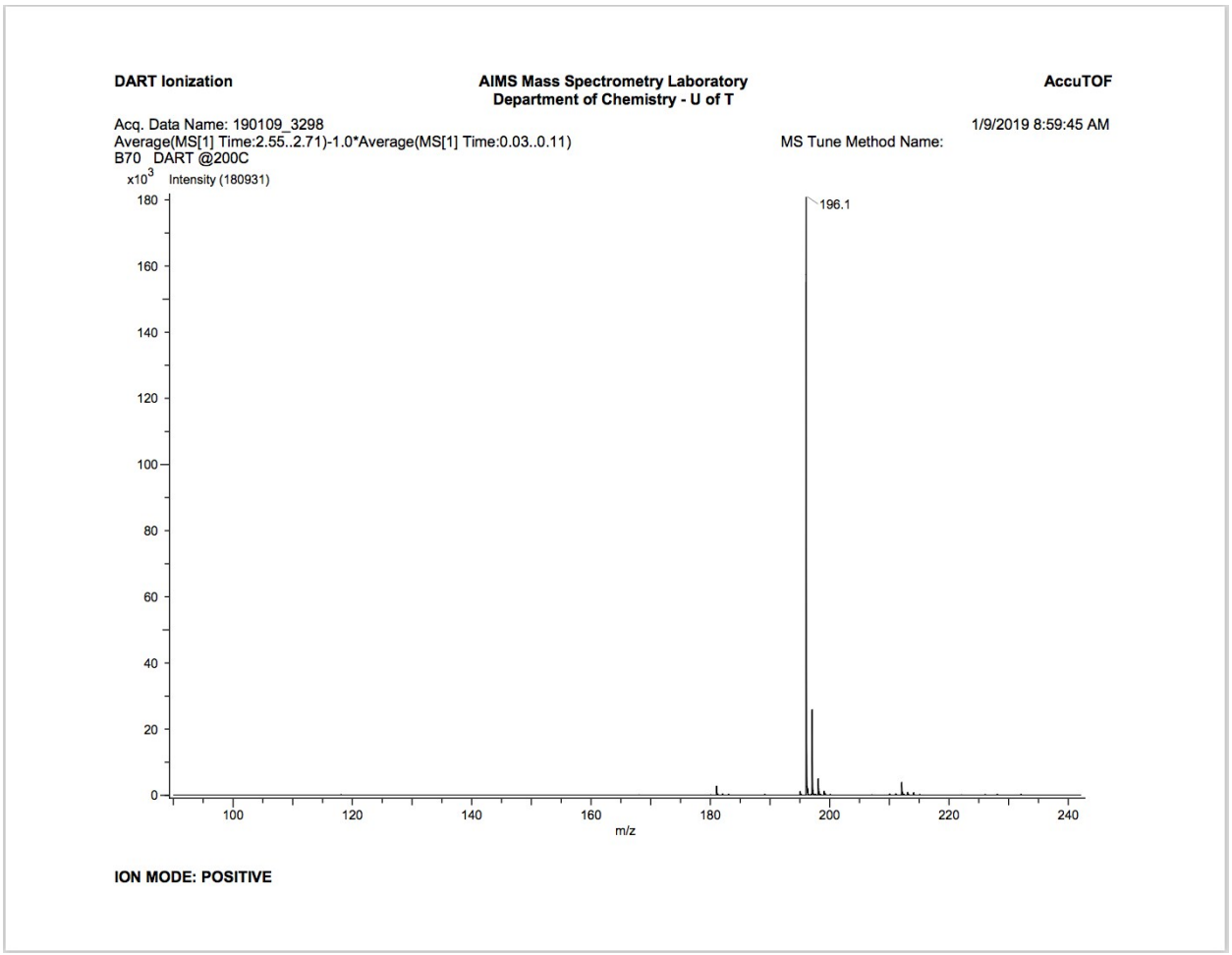


Figure S26. DART-MS of **3**.

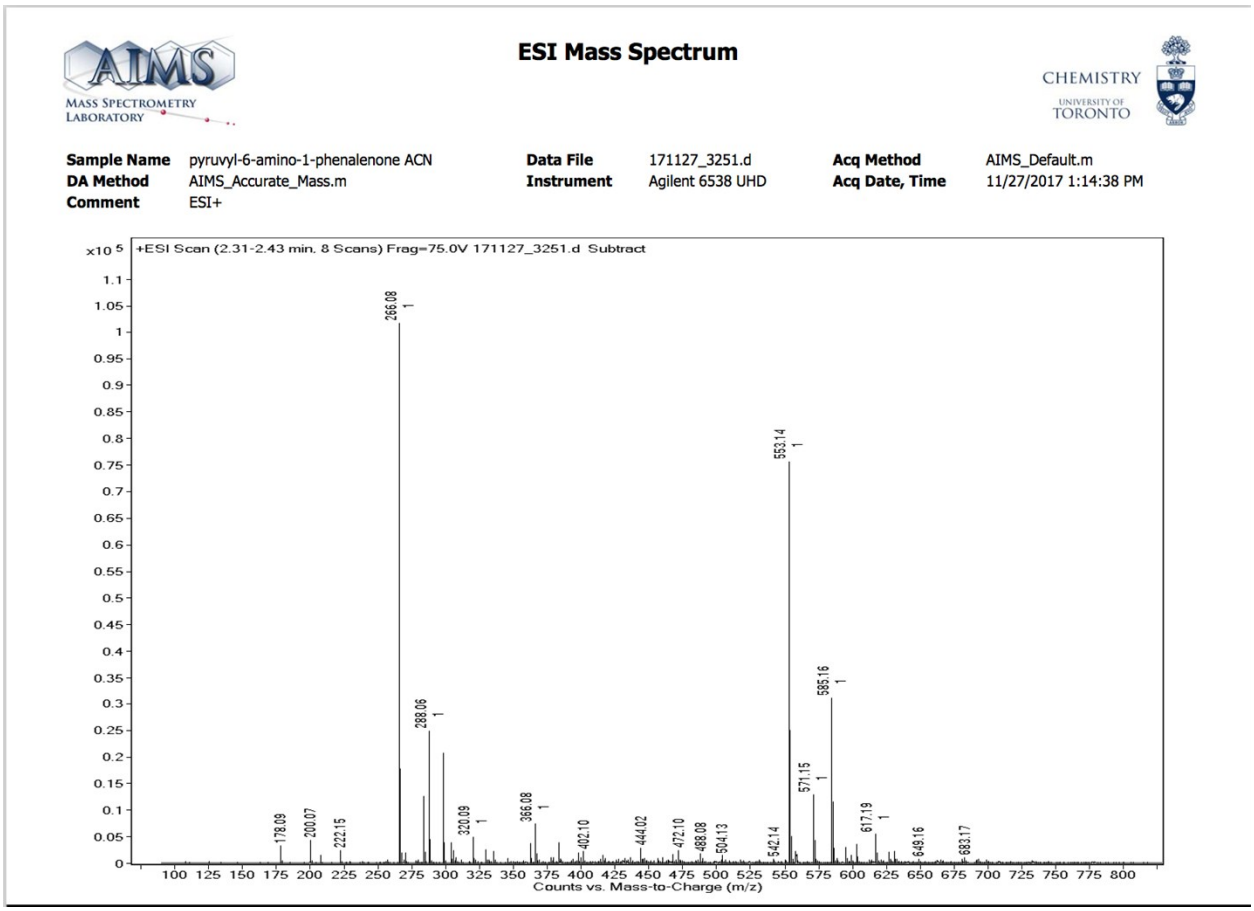


Figure S27. HRMS of Probe **1**.

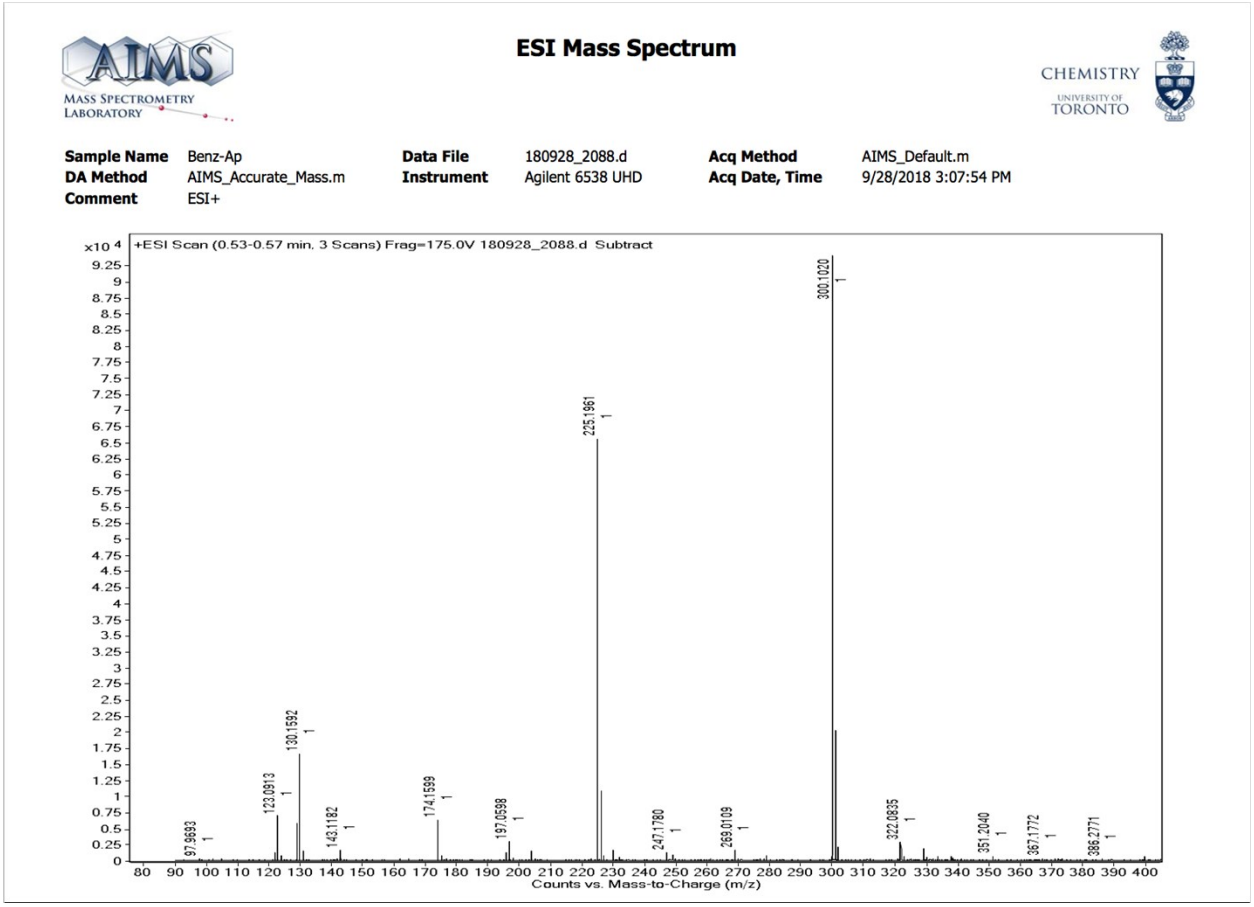


Figure S28. HRMS of Probe **2**.