Supporting Information for:

Ultrasensitive Fluorescent Probes Reveal an Adverse Action of Dipeptide Peptidase IV and Fibroblast Activation Protein during Proliferation of Cancer Cells

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Table of Contents

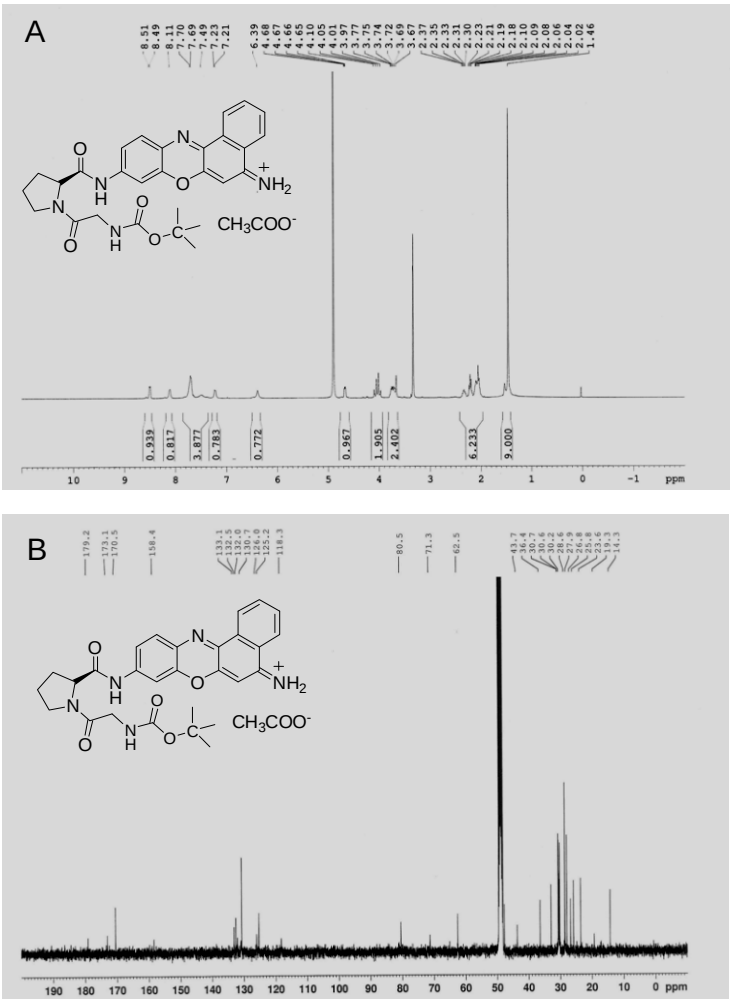

Figure S1. ^{1}H NMR and ^{13}C NMR spectra of probe **1**. (A) ^{1}H NMR (400 MHz, CD_3OD, 298 K) and (B) ^{13}C NMR (100 MHz, CD_3OD, 298 K) spectra of probe **1**.

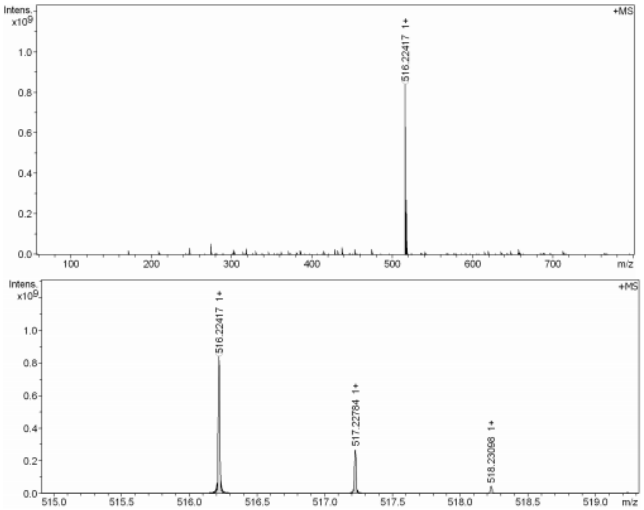

Figure S2. The HR-ESI-mass spectra of probe **1**.

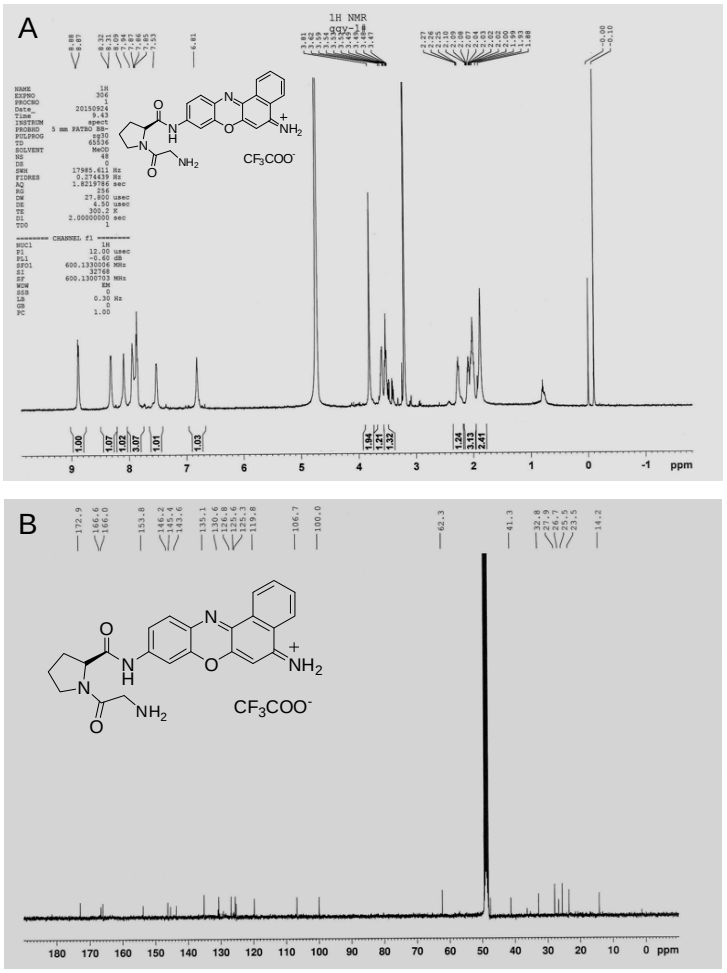

Figure S3. ^{1}H NMR and ^{13}C NMR spectra of probe **2**. (A) ^{1}H NMR (600 MHz, CD_3OD, 298 K) and (B) ^{13}C NMR (100 MHz, CD_3OD, 298 K) spectra of probe **2**.

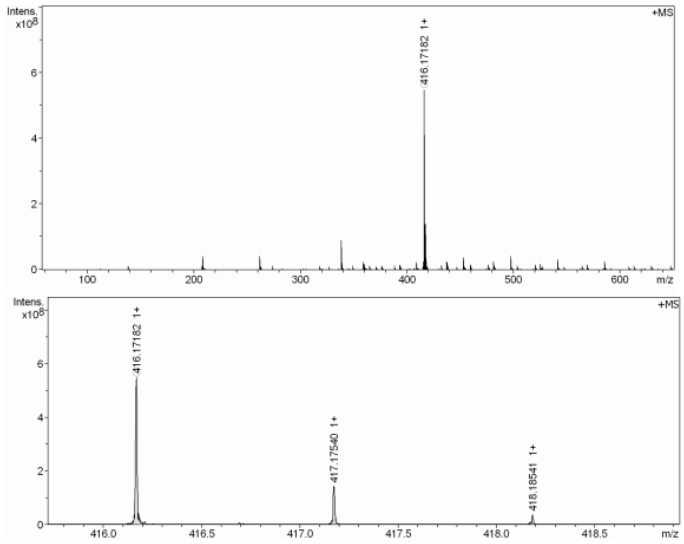

Figure S4. The HR-ESI-mass spectra of probe **2**.

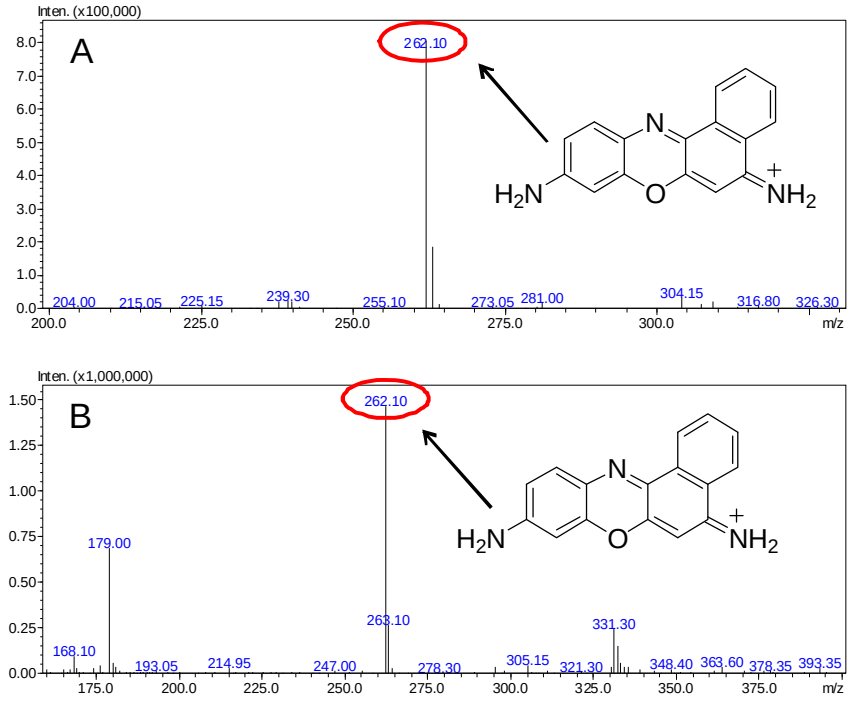

Figure S5. ESI mass spectra of the reaction products. (A) **1** with FAP and (B) **2** with DPPIV.

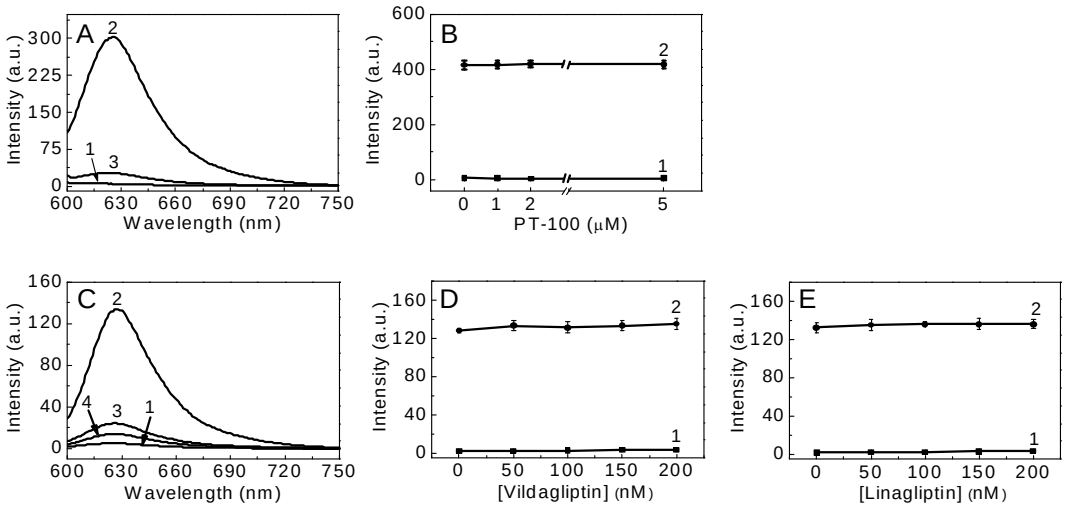

Figure S6. Inhibitor experiments. (A) Fluorescence spectra of probe **1** in different reaction systems: (1) probe **1** only (5 μM); (2) probe **1** (5 μM) + FAP (0.6 μg/mL); (3) system 2 + 5 μM PT-100. (B) The effects of PT-100 at varied concentrations on the fluorescence intensity of (1) probe **1** (5 μM) and (2) cresyl violet (5 μM). (C) Fluorescence spectra of probe **2** in different reaction systems: (1) probe **2** only (10 μM); (2) probe **2** (10 μM) + DPPIV (0.2 μg/mL); (3) system 2 + 100 nM vildagliptin; (4) system 2 + 100 nM linagliptin. The effects of (D) vildagliptin and (E) linagliptin at varied concentrations on the fluorescence intensity of (1) probe **2** (10 μM) and (2) cresyl violet (10 μM). Linagliptin was chosen for cell experiments below. The reactions were performed at 37 °C in PBS (pH 7.4). $\lambda_{ex/em}$ = 585/625 nm.

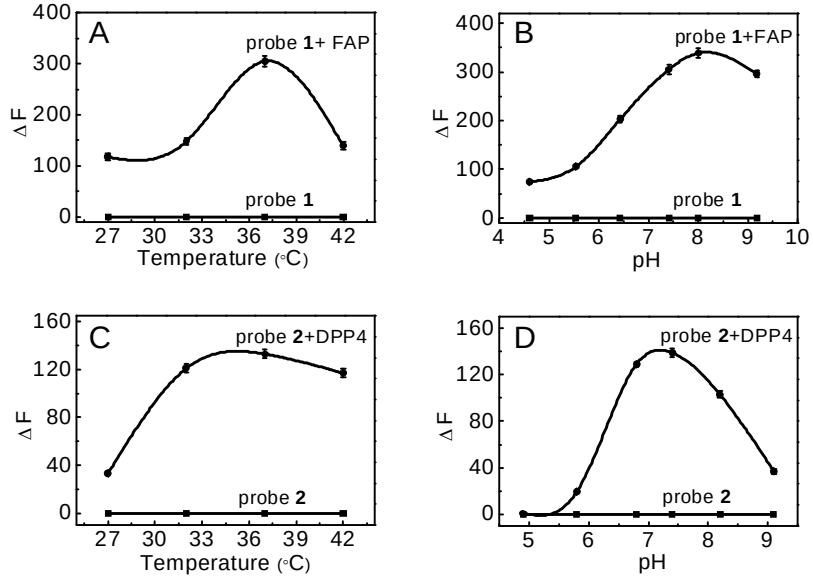

Figure S7. Effects of reaction temperature and pH on the fluorescence of reaction systems. Effects of reaction temperature (A) and pH (B) on the fluorescence of probe **1** (5 μM) in the absence and presence of FAP (0.6 μg/mL). Conditions: the reaction was performed in PBS (pH 7.4) for 3 h (A) at different temperatures and (B) at different pH values adjusted with dilute HCl or NaOH. Effects of reaction temperature (C) and pH (D) on the fluorescence of probe **2** (10 μM) in the absence and presence of DPPIV (0.2 μg/mL). Conditions: the reaction was performed in PBS (pH 7.4) for 30 min (c) at different temperatures and (d) at different pH values adjusted with dilute HCl or NaOH. $\lambda_{ex/em}$ = 585/625 nm. From this figure it can be seen that DPPIV and FAP function well under physiological conditions (pH 7.4 and 37 °C).

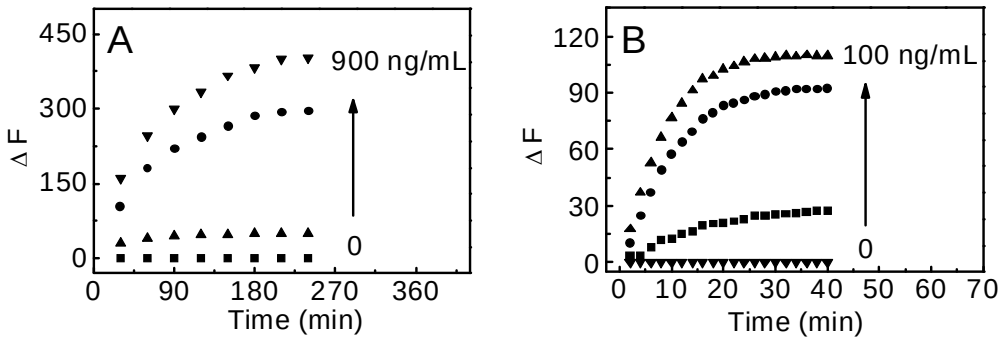

Figure S8. Effects of reaction time on the fluorescence of reaction systems. (A) A plot of fluorescence enhancement of probe **1** (5 μM) versus the reaction time in the presence of varied FAP concentrations. (B) A plot of fluorescence enhancement of probe **2** (10 μM) versus the reaction time in the presence of varied DPPIV concentrations. The reaction was performed at 37 °C in PBS (pH 7.4). $\lambda_{ex/em}$ = 585/625 nm. As seen, a reaction time of 3 h may be chosen for FAP, and 30 min for DPPIV.

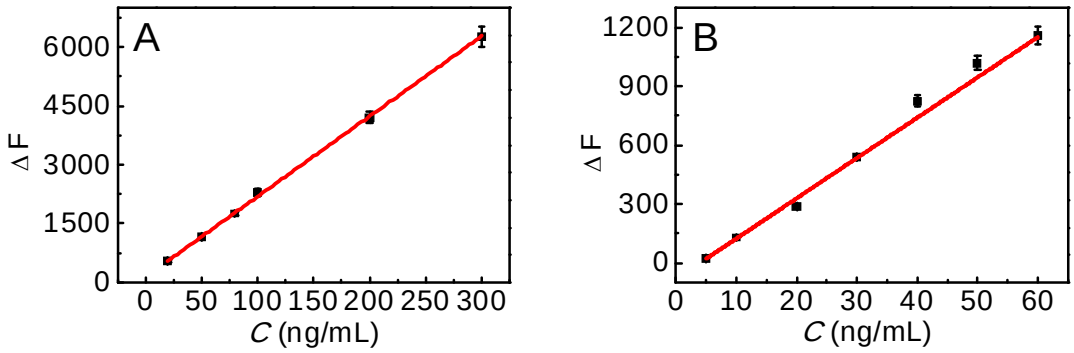

Figure S9. The plots of fluorescence enhancements of commercial probes versus the enzymes concentrations. (A) A plot of fluorescence enhancement of commercial FAP probe (5 μM) versus the FAP concentration. The reactions were performed at 37 °C in PBS (pH 7.4) for 3 h; $\lambda_{ex/em}$ = 340/440 nm. (B) A plot of fluorescence enhancement of commercial DPPIV probe (10 μM) versus the DPPIV concentration. The reactions were performed at 37 °C in PBS (pH 7.4) for 30 min; $\lambda_{ex/em}$ = 340/440 nm. The detection limits of the commercial probes were determined to be 9.8 ng/mL FAP and 1.35 ng/mL DPPIV.

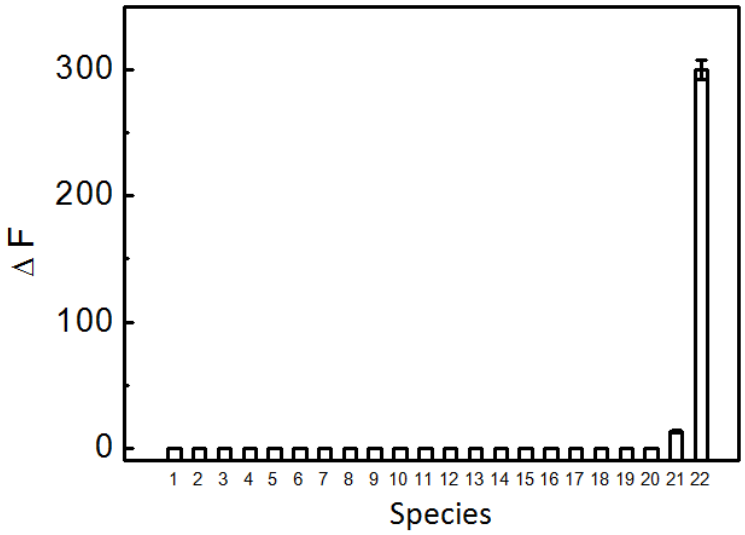

Figure S10. Fluorescence change of probe **1** in the presence of various species. (1) probe **1** only (5 μM; control); (2) 150 mM KCl; (3) 2.5 mM $CaCl_2$; (4) 100 μM $ZnCl_2$; (5) 2.5 mM $MgCl_2$; (6) 100 μM $CuSO_4$; (7) 10 mM glucose; (8) 1 mM vitamin C; (9) 1 mM glutamic acid; (10) 1 mM cysteine; (11) 5 mM glutathione; (12) 100 μM OCl^-; (13) 100 μM human serum albumin; (14) 4 ng/mL LAP; (15) 2 μg/mL V8 protease; (16) 2 μg/mL prolidase; (17) 2 μg/mL esterase; (18) 2 μg/mL trypsin; (19) 2 μg/mL thrombin; (20) 2 μg/mL MMP-2; (21) 0.9 μg/mL DPPIV; (22) 0.6 μg/mL FAP.

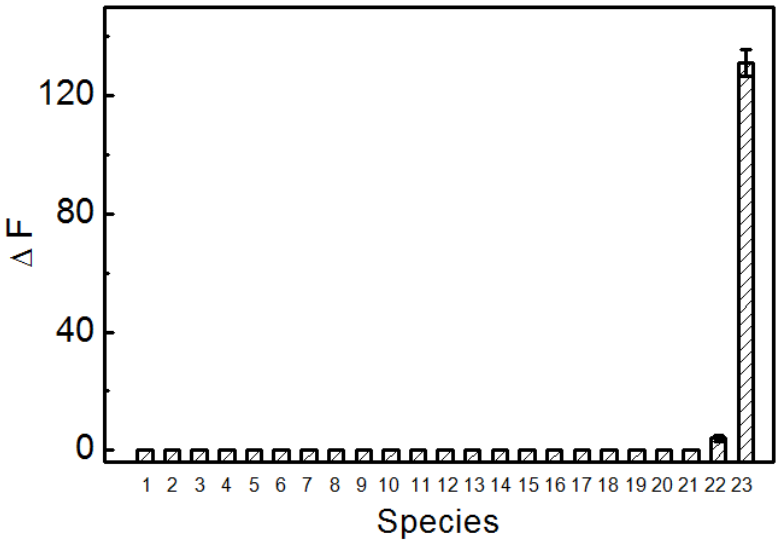

Figure S11. Fluorescence change of probe **2** in the presence of various species. (1) probe **2** onlyonly (10 μM; control); (2) 150 mM KCl; (3) 2.5 mM $CaCl_2$; (4) 100 μM $ZnCl_2$; (5) 2.5 mM $MgCl_2$; (6) 100 μM $CuSO_4$; (7) 10 mM glucose; (8) 1 mM vitamin C; (9) 1 mM glutamic acid; (10) 1 mM cysteine; (11) 5 mM glutathione; (12) 100 μM OCl^-; (13) 100 μM human serum albumin; (14) 4 ng/mL LAP; (15) 2 μg/mL V8 protease; (16) 2 μg/mL prolidase; (17) 2 μg/mL esterase; (18) 2 μg/mL trypsin; (19) 2 μg/mL thrombin; (20) 2 μg/mL PGP-1; (21) 2 μg/mL MMP-2; (22) 0.8 μg/mL FAP; (23) 0.2 μg/mL DPPIV.

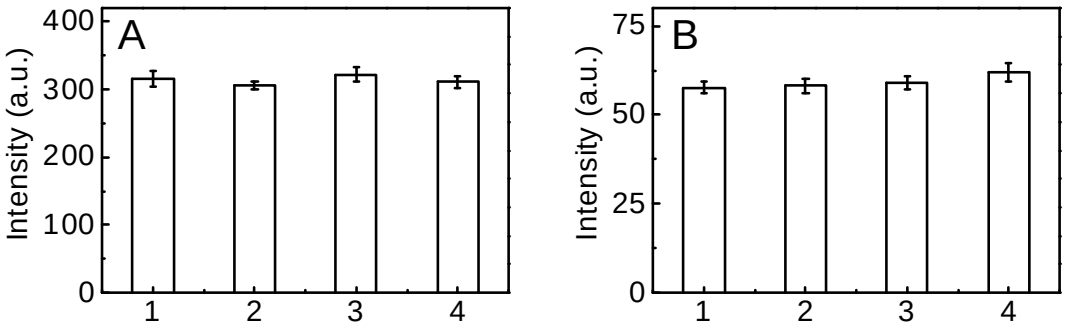

Figure S12. The influence of enzymes on each other. (A) The influence of DPPIV at varied concentrations on FAP. (1) Probe **1** (5 μM) + 0.6 μg/mL FAP; (2) system 1 + 0.6 μg/mL DPPIV; (3) system 1 + 1.2 μg/mL DPPIV; (4) system 1 + 2.4 μg/mL DPPIV. The reactions were performed at 37 °C in PBS (pH 7.4) for 3 h. (B) The influence of FAP at varied concentrations on DPPIV. (1) Probe **2** (10 μM) + 0.02 μg/mL DPPIV; (2) system 1 + 0.02 μg/mL FAP; (3) system 1 + 0.04 μg/mL FAP; (4) system 1 + 0.08 μg/mL FAP. The reactions were performed at 37 °C in PBS (pH 7.4) for 30 min. $\lambda_{ex/em}$ = 585/625 nm. As seen, the two enzymes scarcely interfere with each other.

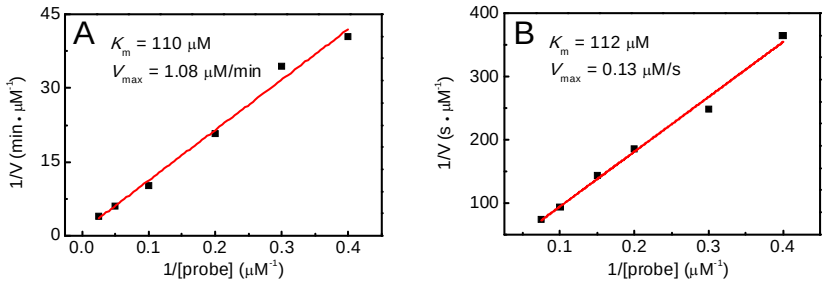

Figure S13. The Michaelis-Menten equations of the reaction systems. The Michaelis-Menten equation was described as: $V = V_{max}[probe]/(K_m+[probe])$, where V is the reaction rate, [probe] is the probe concentration, and K_m is the Michaelis constant. Conditions: (A) 600 ng/mL FAP, 2.5-40 μM probe **1**. (B) 30 ng/mL DPPIV, 2.5-13 μM probe **2**. pH 7.4, temperature 37 °C. $\lambda_{ex/em}$ = 585/625 nm.

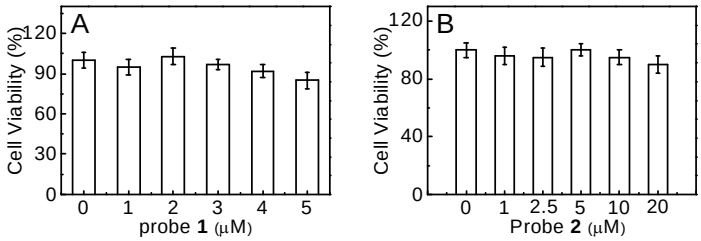

Figure S14. Effects of probes at varied concentrations on the cell viability of MGC803 cells. Effects of (A) probe **1** and (B) probe **2** at varied concentrations on the cell viability of MGC803 cells. The cell viability without the probe is defined as 100%.

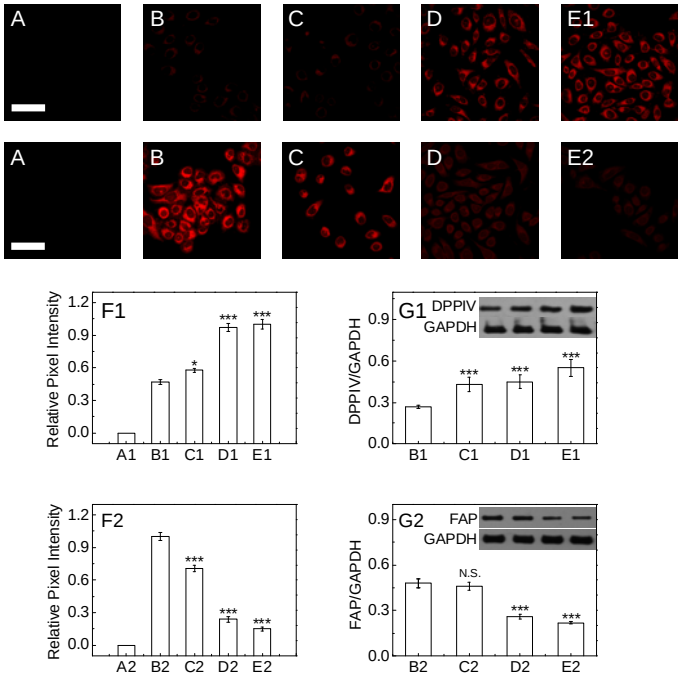

Figure S15. The changes of DPPIV and FAP in HO8910PM cells with varied genistein. (A1) HO8910PM cells only; (B1-E1) the cells were pretreated at 37 °C for 24 h with genistein at

varied concentrations (0, 5, 10, 20 μg/mL, respectively), and then incubated with probe **2** (10 μM). (A2) HO8910PM cells only; (B2-E2) the cells were pretreated at 37 °C for 24 h with genistein at varied concentrations (0, 5, 10, 20 μg/mL, respectively), and then incubated with probe **1** (5 μM). Scale bar, 20 μm. (F1, F2) Relative pixel intensity measurements (n = 3) from the above corresponding fluorescence images by using software ImageJ (the pixel intensities from images e1 and b2 are defined as 1.0). (G1, G2) The changes of DPPIV and FAP in the above corresponding cells determined by western blot (GAPDH as a protein standard; N.S. No significance, * $p<0.05$, *** $p<0.001$, as compared with the control, two-sided Student's *t*-test).

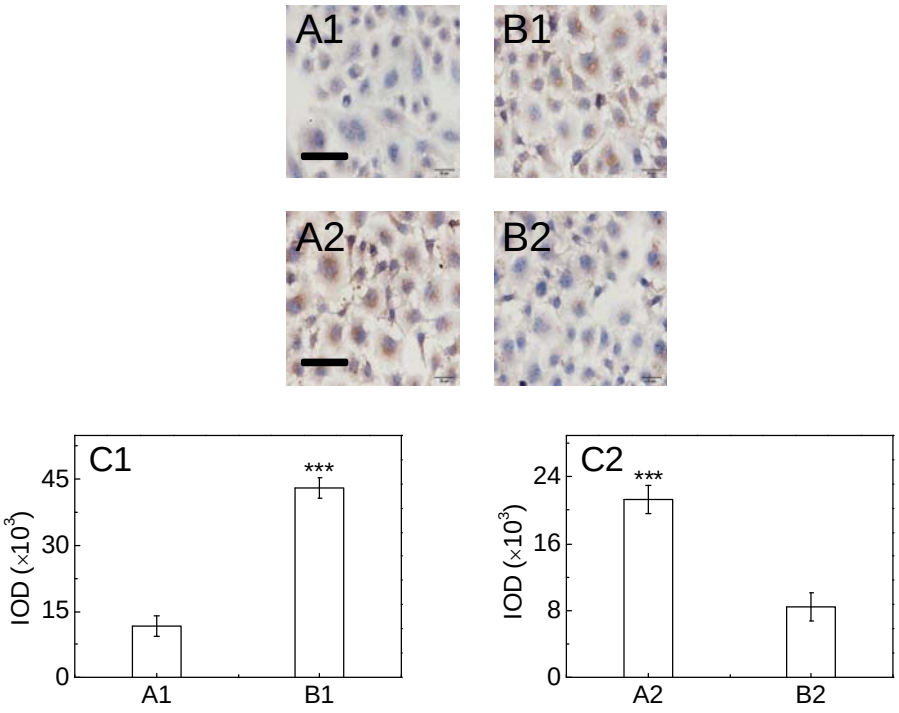

Figure S16. Representative IHC images of HO8910PM cells. HO8910PM cells were immunolabeled with (A1) anti-DPPIV antibody (control); HO8910PM cells were pre-incubated with genistein (20 μg/mL) for 24 h, and then immunolabeled with (B1) anti-DPPIV antibody. HO8910PM cells were immunolabeled with (A2) anti-FAP antibody (control); HO8910PM cells were pre-incubated with genistein (20 μg/mL) for 24 h, and then immunolabeled with (B2) anti-FAP antibody. Scale bar, 20 μm. (C1, C2) The integral optical density (IOD) values from the above corresponding IHC images; the IOD values represent the relative concentrations of intracellular DPPIV and FAP, respectively (*** $p<0.001$, two-sided Student's *t*-test).

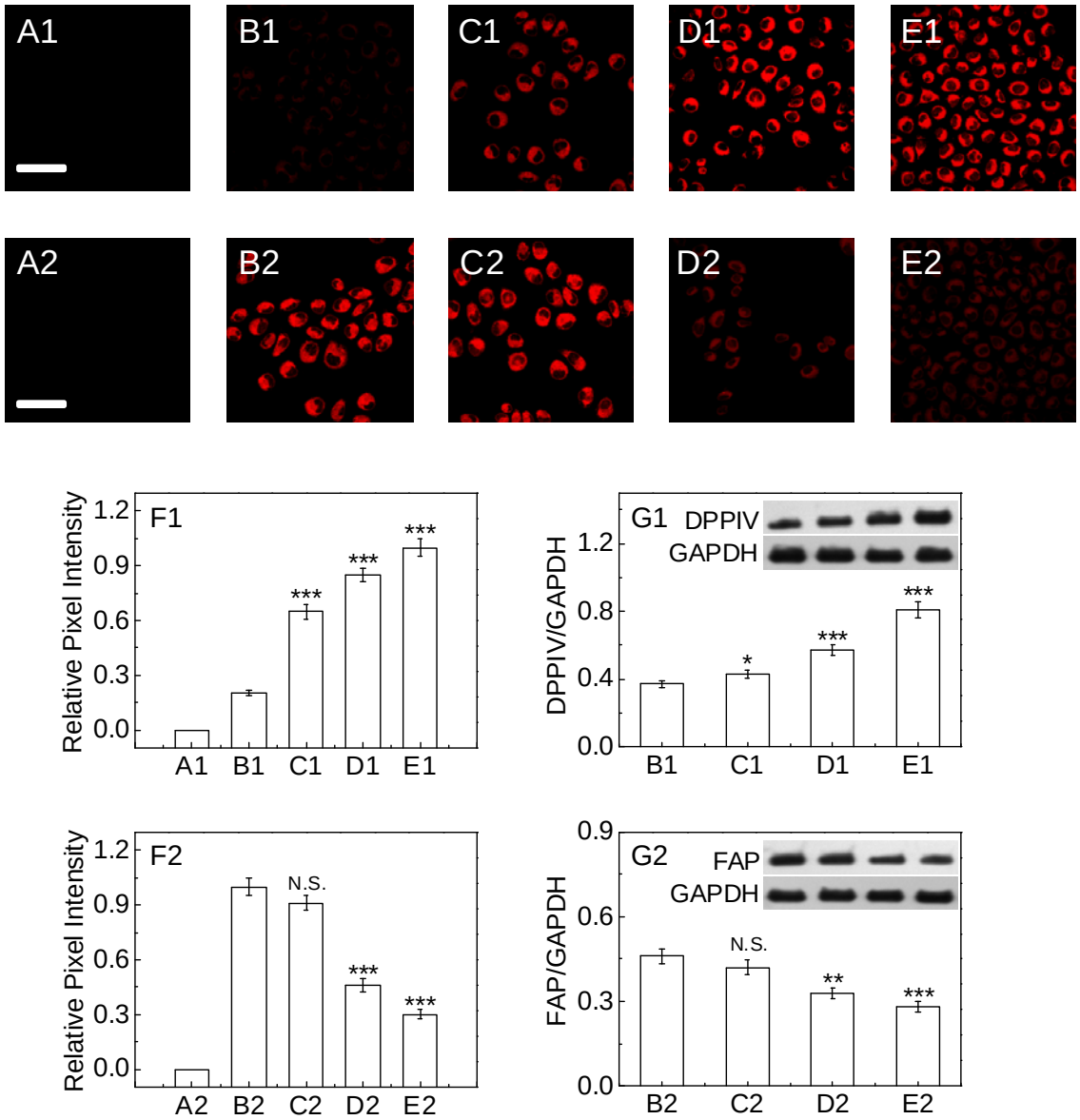

Figure S17. Fluorescence images of MKN28 cells. (A1,A2) MKN28 only. (B1-E1) The MKN28 cells were pretreated with 0, 5, 10, and 20 μg/mL of genistein, respectively, at 37 °C for 24 h, and then incubated with probe **2** (10 μM). (B2-E2) The MKN28 cells were pretreated with 0, 5, 10, and 20 μg/mL of genistein, respectively, at 37 °C for 24 h, and then incubated with probe **1** (5 μM). Scale bar 20 μm. (F1, F2) Relative pixel intensity measurements (n = 3) from the above corresponding images by using the software ImageJ (the pixel intensities from images E1 and B2 are defined as 1.0). (G1, G2) The changes of DPPIV and FAP in the above corresponding cells determined by western blot (GAPDH as a protein standard; N.S. No significance, * $p<0.05$, ** $p<0.01$, *** $p<0.001$, as compared with the control, two-sided Student's *t*-test).

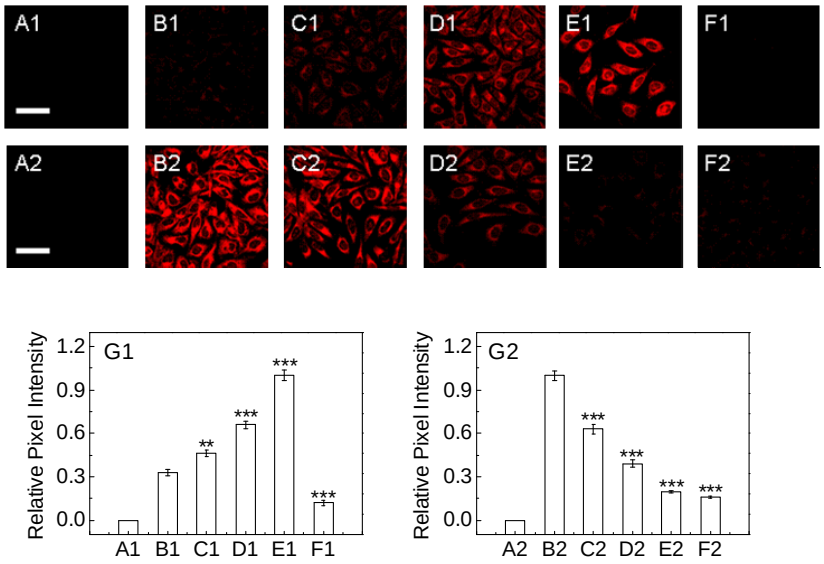

Figure S18. Fluorescence images of MGC803 cells. (A1) MGC803 only. (B1-E1) The MGC803 cells were pretreated with 20 μg/mL genistein at 37 °C for 0, 6, 12 and 24 h, respectively, and then incubated with probe **2** (10 μM). (F1) MGC803 cells were pretreated with 20 μg/mL genistein for 24 h, then treated with 100 nM linagliptin for 1 h, and finally incubated with probe **2** (10 μM). (A2) MGC803 cells only. (B2-E2) MGC803 cells were pretreated with 20 μg/mL genistein at 37 °C for 0, 6, 12 and 24 h, respectively, and then incubated with probe **1** (5 μM). (F2) MGC803 cells were pretreated with 5 μM PT-100 for 1 h, and finally incubated with probe **1** (5 μM). Scale bar 20 μm. (G1, G2) Relative pixel intensity measurements (n = 3) from above corresponding images by using the software ImageJ (the pixel intensities from images E1 and B2 are defined as 1.0; ** $p<0.01$, *** $p<0.001$, as compared with the control, two-sided Student's *t*-test).

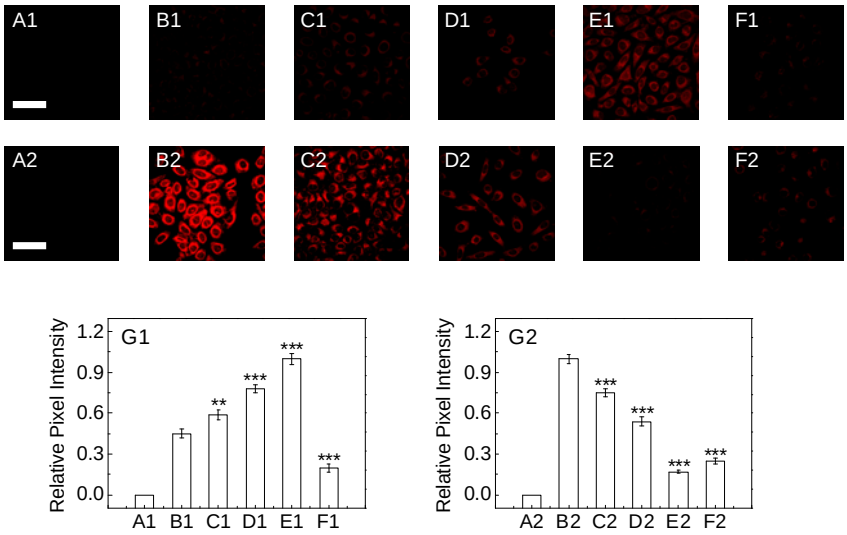

Figure S19. Fluorescence images of HO8910PM cells. (A1) HO8910PM only. (B1-E1) The HO8910PM cells were pretreated with 20 μg/mL genistein at 37 °C for 0, 6, 12 and 24 h,

respectively, and then incubated with probe **2** (10 μM). (F1) cells were pretreated with 20 μg/mL genistein for 24 h, then treated with 100 nM linagliptin for 1 h, and finally incubated with probe **2** (10 μM). (A2) HO8910PM cells only. (B2-E2) HO8910PM cells were pretreated with 20 μg/mL genistein at 37 °C for 0, 6, 12 and 24 h, respectively, and then incubated with probe **1** (5 μM). (F2) HO8910PM cells were pretreated with 5 μM PT-100 for 1 h, and then incubated with probe **1** (5 μM). Scale bar 20 μm. (G1, G2) Relative pixel intensity measurements (n = 3) from above corresponding images by using the software ImageJ (the pixel intensities from images E1 and B2 are defined as 1.0; ** $p<0.01$, *** $p<0.001$, as compared with the control, two-sided Student's *t*-test).

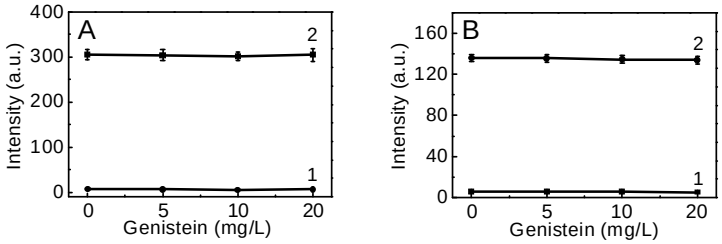

Figure S20. Effects of genistein at varied concentrations on the fluorescence of reaction systems. (A) Effects of genistein at varied concentrations on the fluorescence of (1) probe **1** (5 μM) and (2) its reaction product with FAP (0.6 μg/mL). The reactions were performed in PBS (pH 7.4) at 37 °C for 3 h. (B) Effects of genistein at varied concentrations on the fluorescence of probe **2** (10 μM) and (2) its reaction product with DPPIV (0.2 μg/mL). The reactions were performed in PBS (pH 7.4) at 37 °C for 30 min. $\lambda_{ex/em}$ = 585/625 nm. As seen, genistein hardly affects the fluorescence of probes **1** and **2** and their reaction products as well as the activity of the two enzymes.

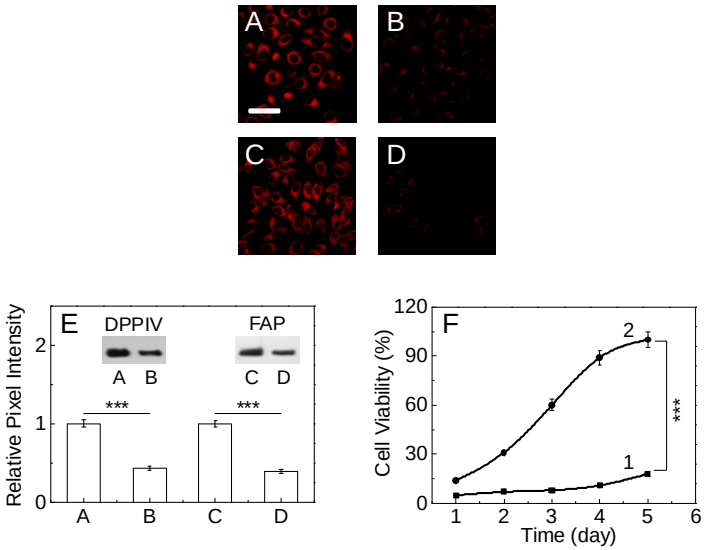

Figure S21. The relationship between enzymes concentrations and cell viabilities. (A, B) Fluorescence images of SGC7901 and MGC803 cells incubated with probe **2** (10 μM),

respectively. (C, D) Fluorescence images of MGC803 and SGC7901 cells incubated with probe **1** (5 μM), respectively. Scale bar 20 μm. (E) Relative pixel intensity measurements (n = 3) from the above corresponding images by using the software ImageJ (the pixel intensities from images a and c are defined as 1.0). The insets show the western blot analysis of the corresponding cells. (F) The viabilities of (1) SGC7901 and (2) MGC803 cells (the highest cell viability is defined as 100%). *** $p<0.001$, two-sided Student's *t*-test.

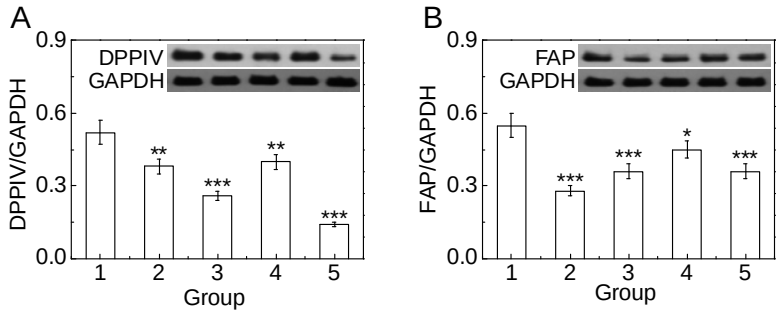

Figure S22. Western blot analysis of normal and siRNA-transfected MGC803 cells. (A) DPPIV and (B) FAP in normal (control) and different siRNA-transfected MGC803 cells. Note that in both panels group 1 is the control, and groups 2-5 are the cells transfected with different siRNAs (sequences I-IV for DPPIV, and sequences I'-IV' for FAP, respectively). As seen, group 5 in panel a and group 2 in panel b can be chosen for the cells with the inhibition of DPPIV and FAP, respectively (* $p<0.05$, ** $p<0.01$, *** $p<0.001$, two-sided Student's *t*-test).