Supporting Information

An innovative peptide with high affinity to GPC3 for hepatocellular carcinoma diagnosis

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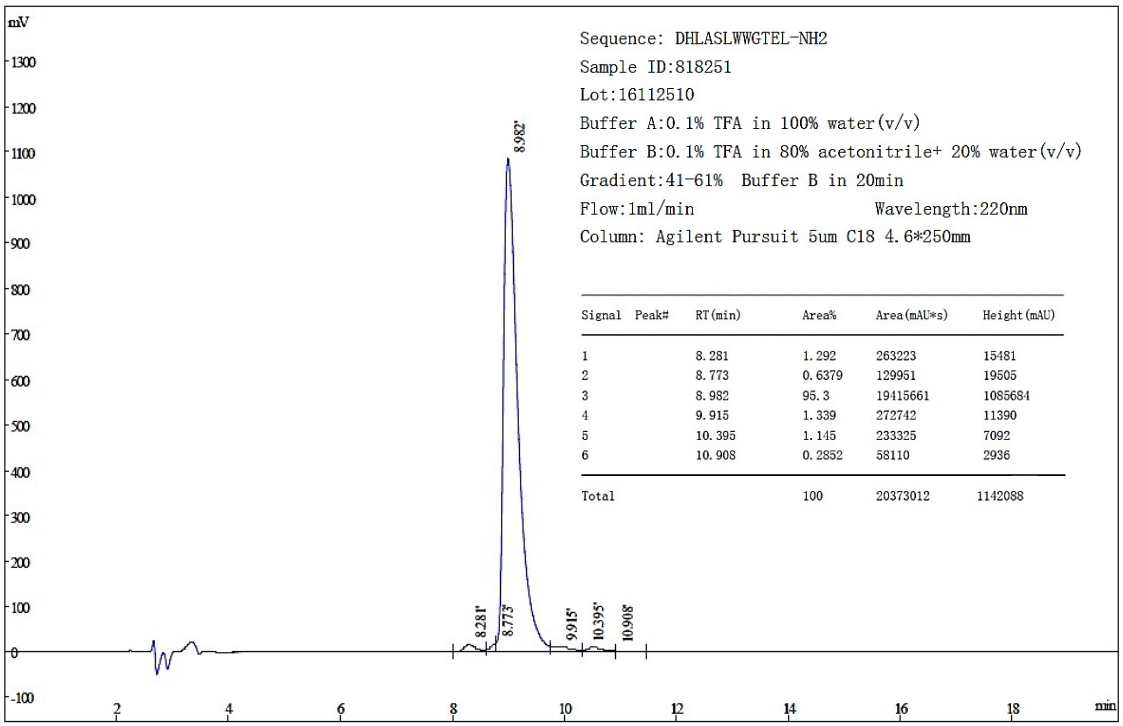
Contents:



Signal	Peak#	RT(min)	Area%	Area(mAU*s)	Height(mAU)
1		8.281	1.292	263223	15481
2		8.773	0.6379	129951	19505
3		8.982	95.3	19415661	1085684
4		9.915	1.339	272742	11390
5		10.395	1.145	233325	7092
6		10.908	0.2852	58110	2936
Total			100	20373012	1142088

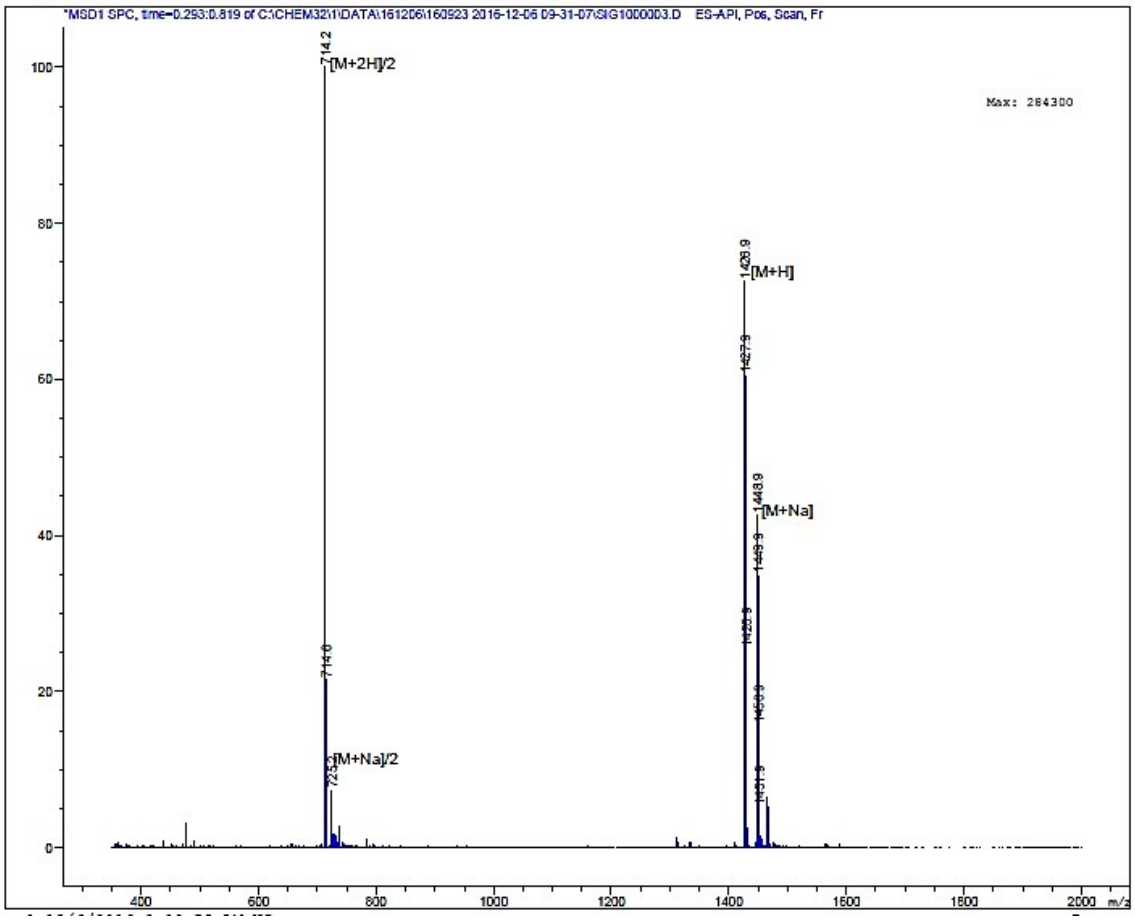
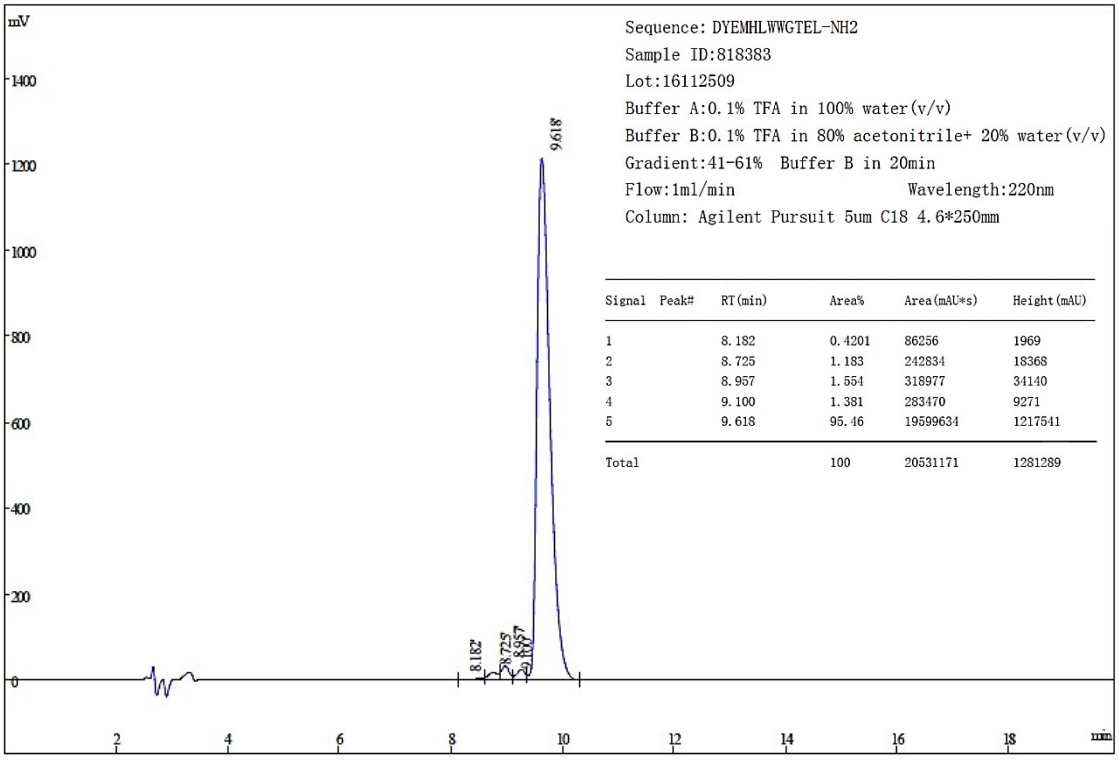


Figure S1-1. HPLC analysis and MS spectrum of YP (Sequence: DHLASLWWGTEL-NH2, MW: 1426.61)



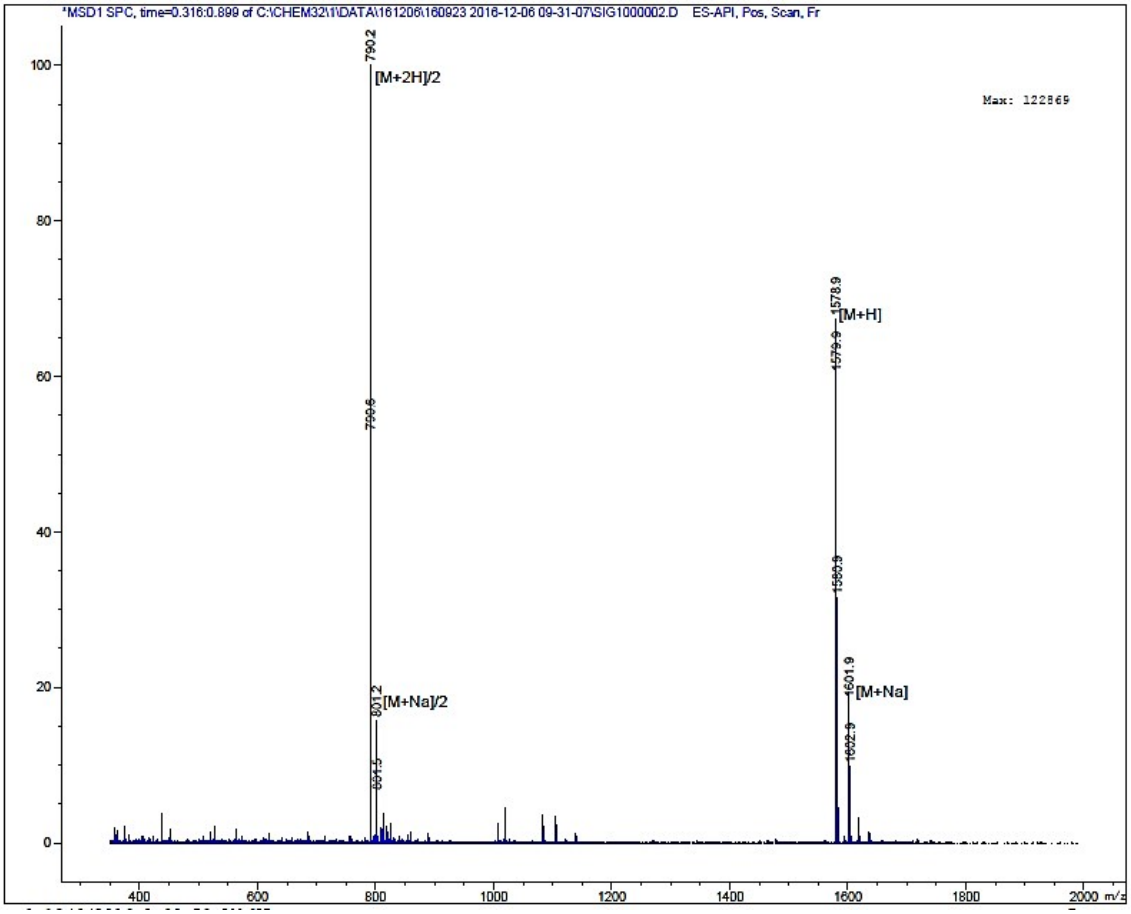
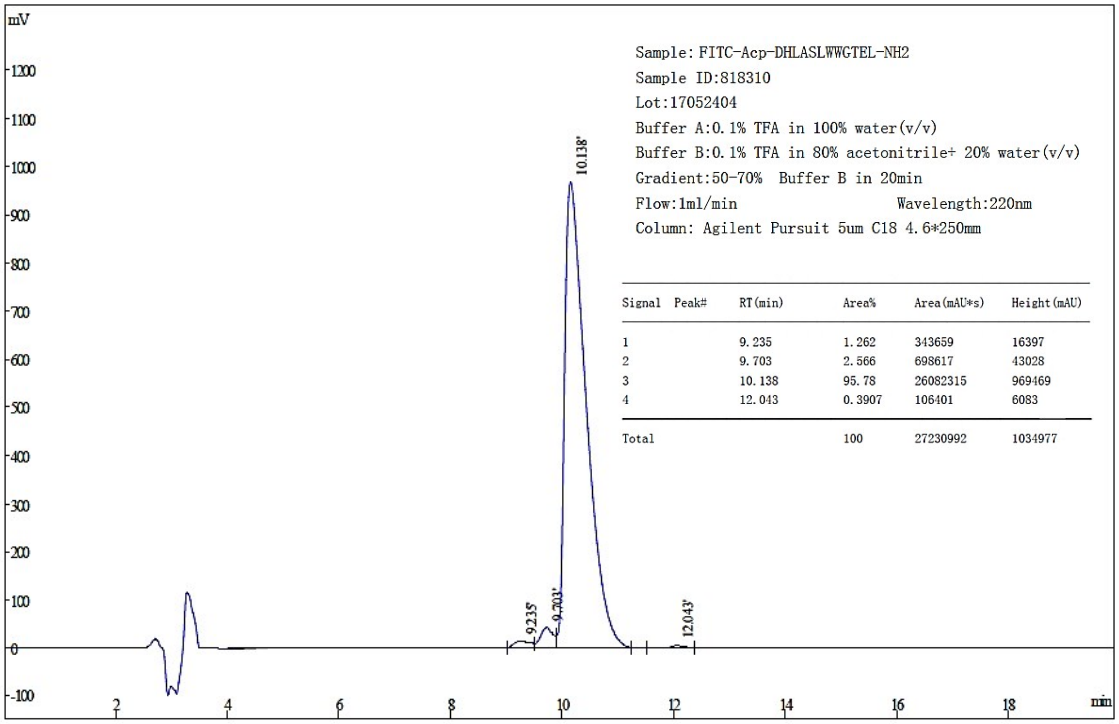


Figure S1-2. HPLC analysis and MS spectrum of IPA (Sequence: DYEMHLWWGTEL-NH2, MW: 1578.78)



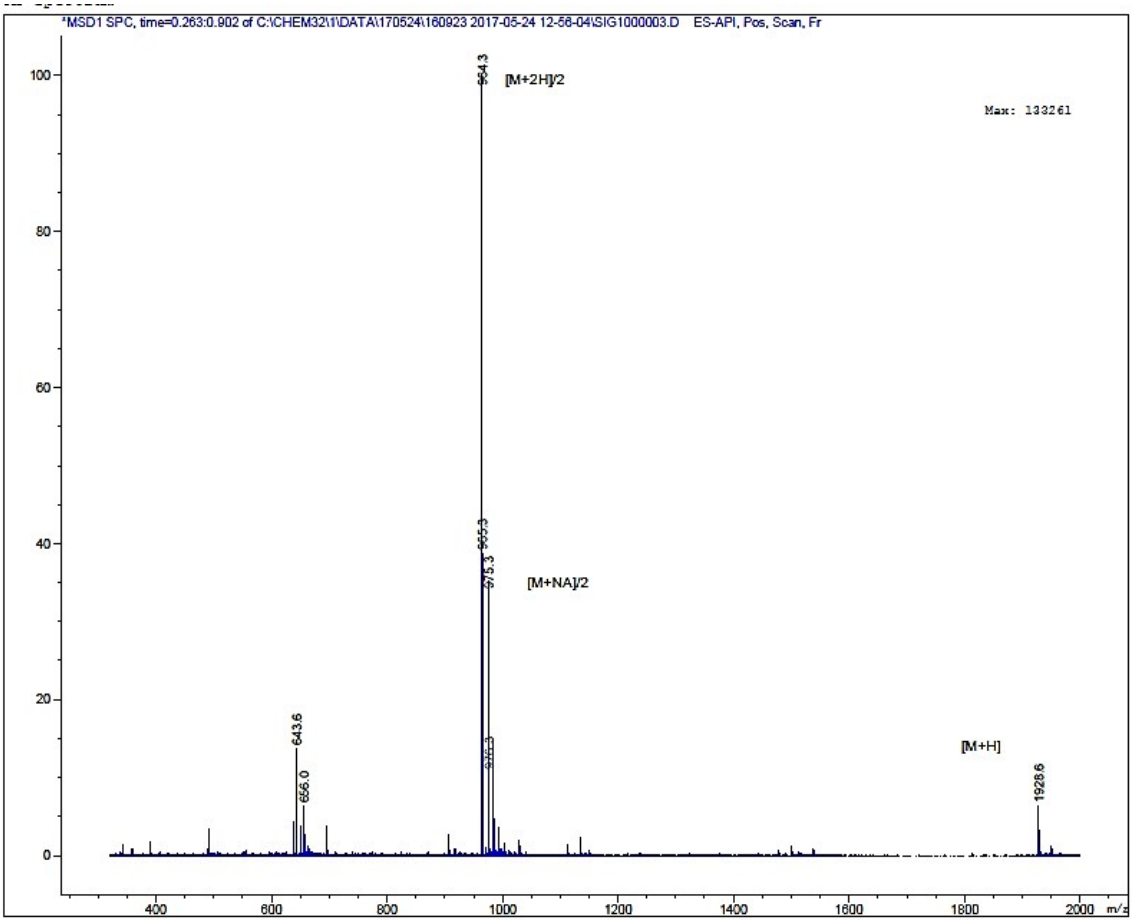


Figure S1-3. HPLC analysis and MS spectrum of YP-FITC (Sequence: FITC-Acp-DHLASLWWGTEL-NH2, MW: 1929.03)

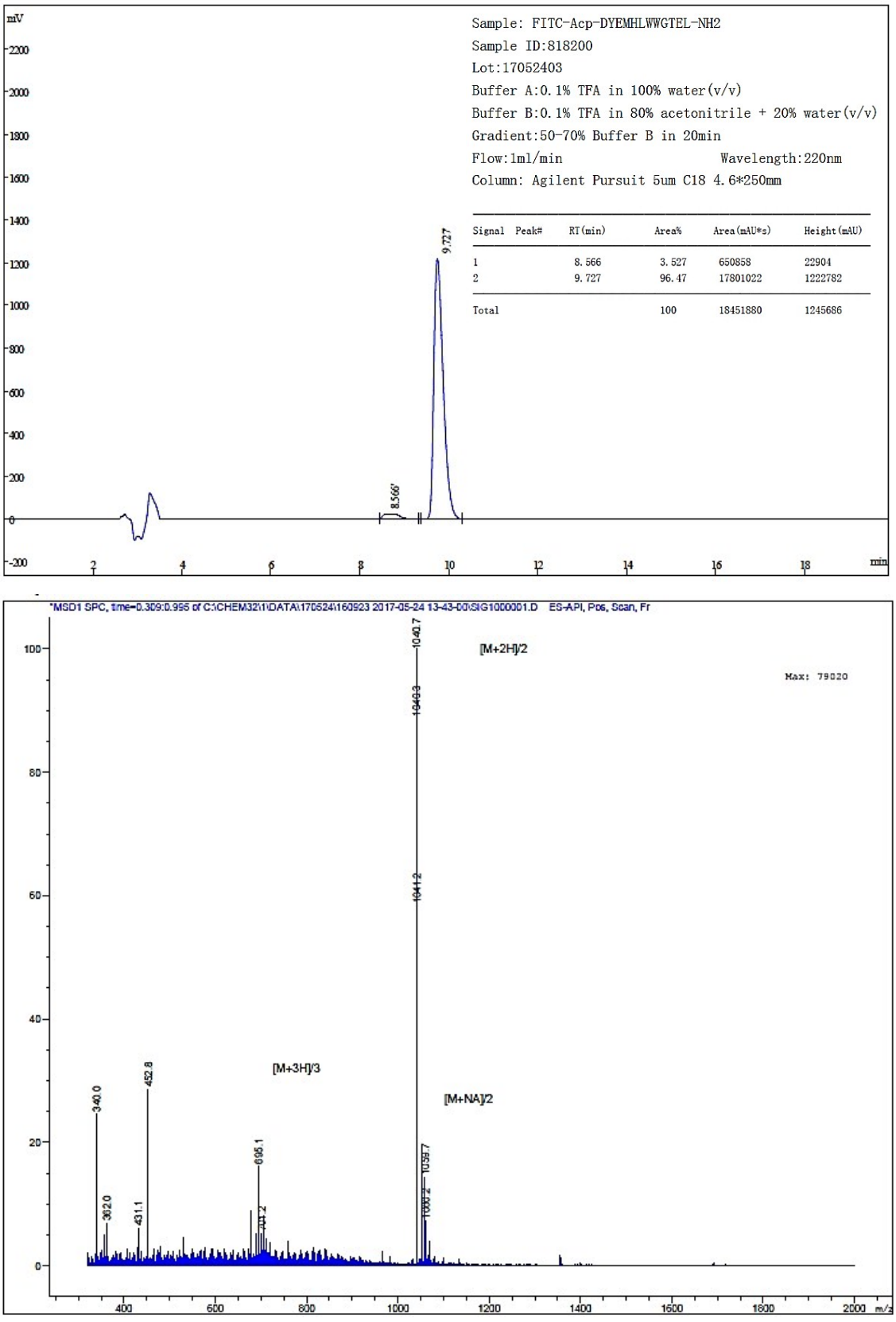


Figure S1-4. HPLC analysis and MS spectrum of IPA-FITC (Sequence: FITC-Acp-DYEMHLWWGTEL-NH2, MW: 2081.21)

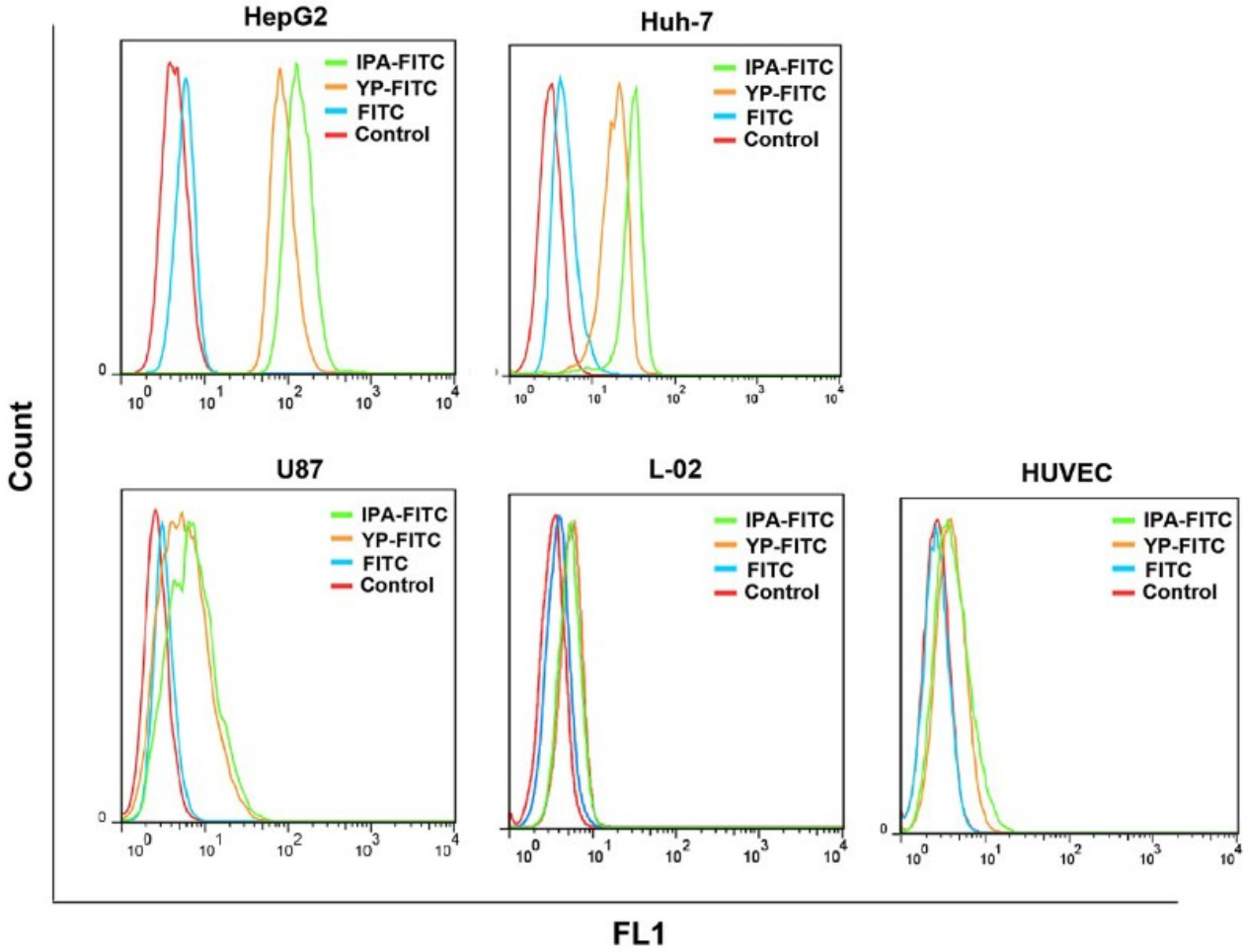


Figure S2. Flow cytometric examination of YP-FITC and IPA-FITC binding to these cell lines after 4 h incubation. The cells treated with PBS or FTIC use as a negative control.

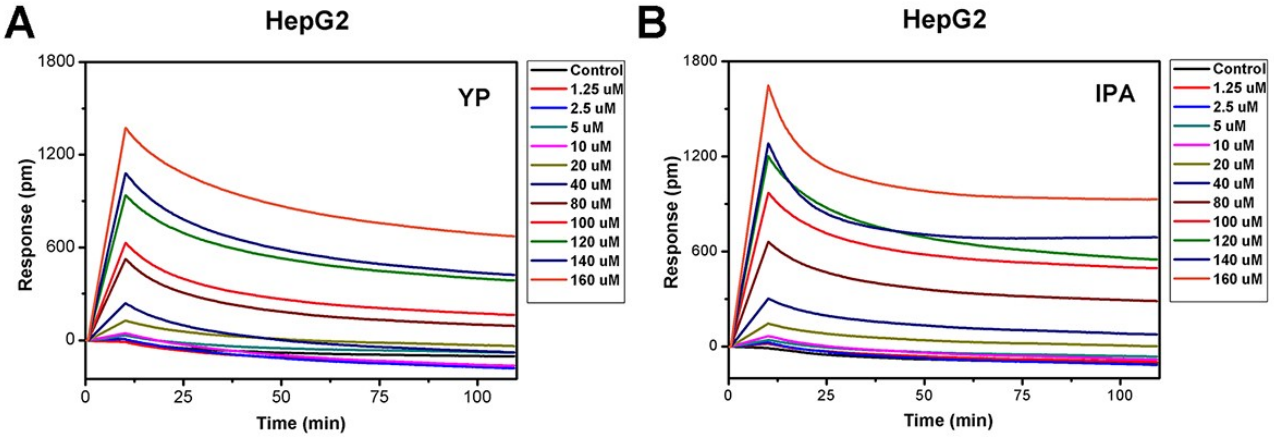


Figure S3. Real time DMR dose response. The response of addition with increasing concentrations of YP (A) or IPA (B) on HepG2 cells.

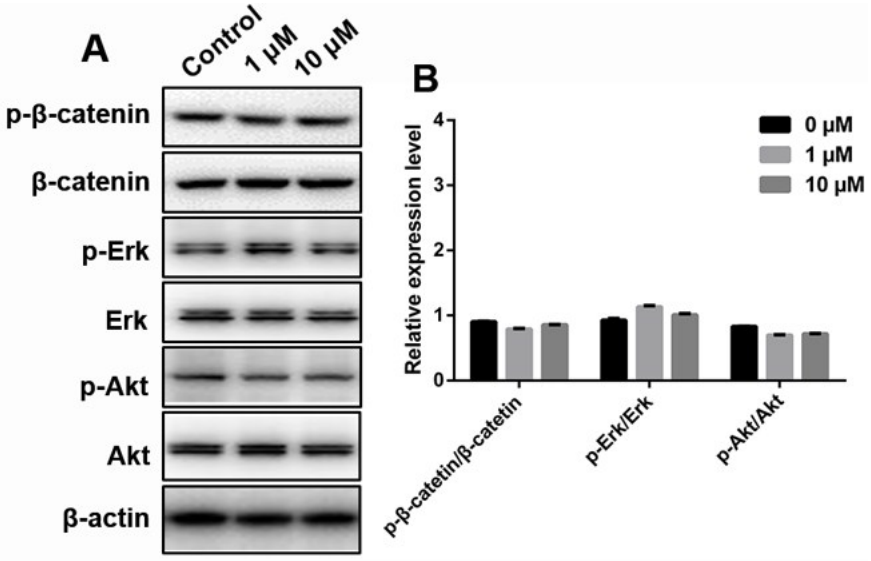


Figure S4. Pharmacological effects. (A) The cells were incubated with different concentrations (0, 1, 10 μM) of IPA for 4h. Subsequently, the proteins of cells were extracted for electrophoresis and transfer onto membranes. The protein expression levels of GPC3 relevant signaling pathways on HepG2 cells were analyzed, including total proteins (β-catenin, Erk and Akt) and phosphorylated proteins (p-β-catenin, p-Erk and p-Akt). (B) Graphical representation for semi-quantification of western blot analysis.

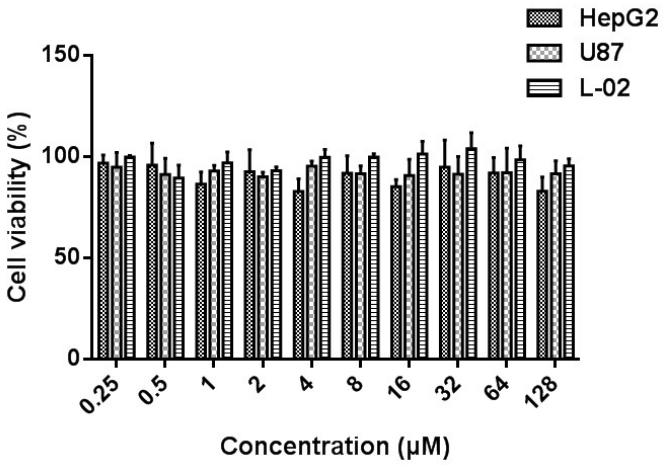


Figure S5. The cytotoxicity of IPA in HepG2, U87 and L-02 were examined by MTT assay. Briefly, different cell lines were added to each well of 96-well plates and were respectively cultured with IPA at different concentrations for 24 h. Then the medium was replaced with 200 μL of fresh culture medium and the MTT (20 μL, 5.0 mg/mL) solution was added to each well for 4 h incubation. Then 150 μL of DMSO was added to dissolve the insoluble blue-violet crystals. The plates were shaken slowly on the horizontal shaker for 10 min to facilitate dissolution. The optical density (OD) at 570 nm was measured using a multi-well plate reader. The relative cell viability was counted as: cell viability (%) = $(OD_{sample} - OD_{blank}/OD_{control} - OD_{blank}) \times 100\%$.

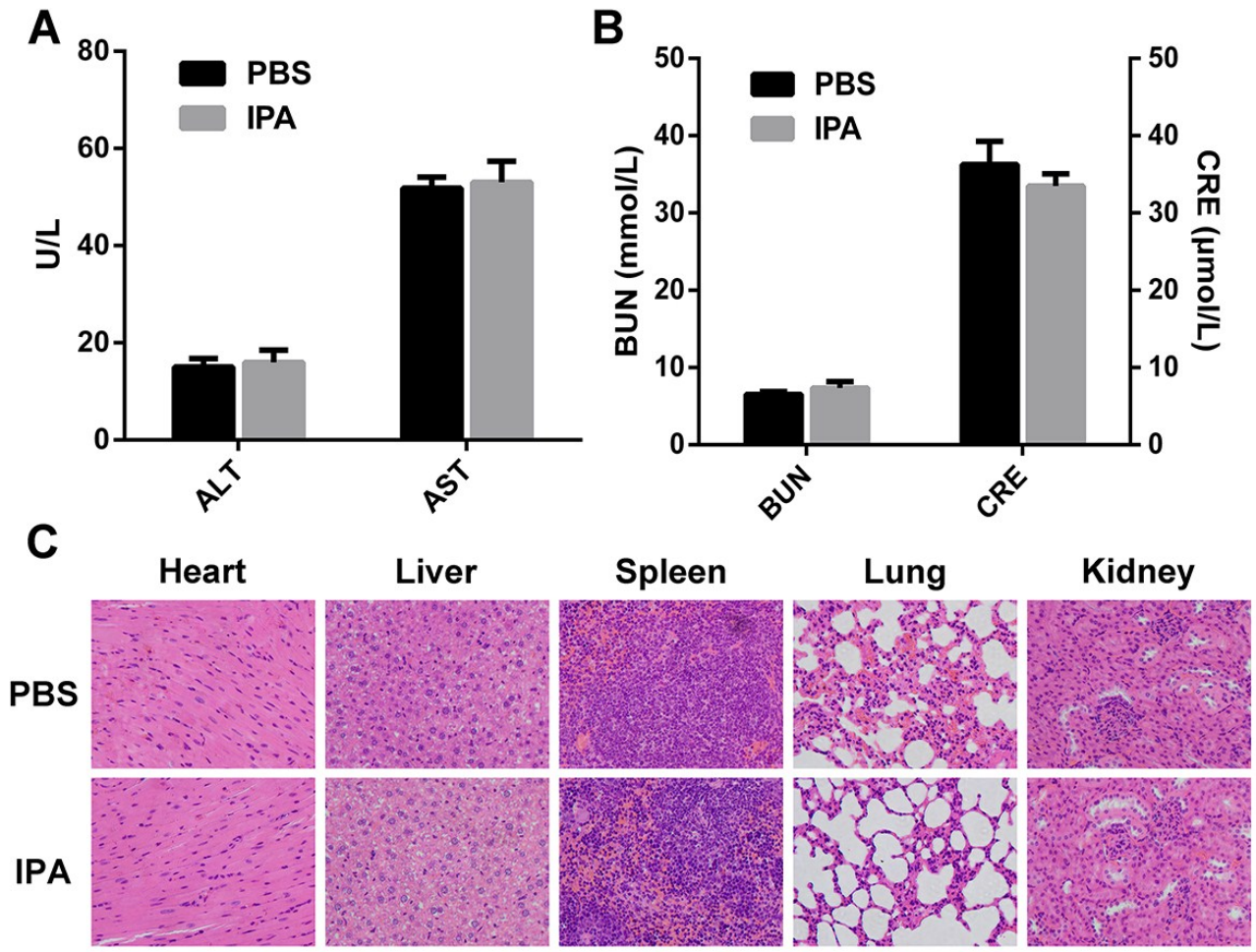


Figure S6. Acute toxicity test in mice: the mice (5 per group) were injected with IPA (150 μL, 100 mg/kg) and PBS (150 μL) via the tail vein. Blood samples were obtained from the eye socket of the mice at 7 days postinjection. (A-B) Serum biochemical data of the mice: alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), and creatinine (CRE). (C) H&E staining was used to stain the excised organs observed for histology analysis.

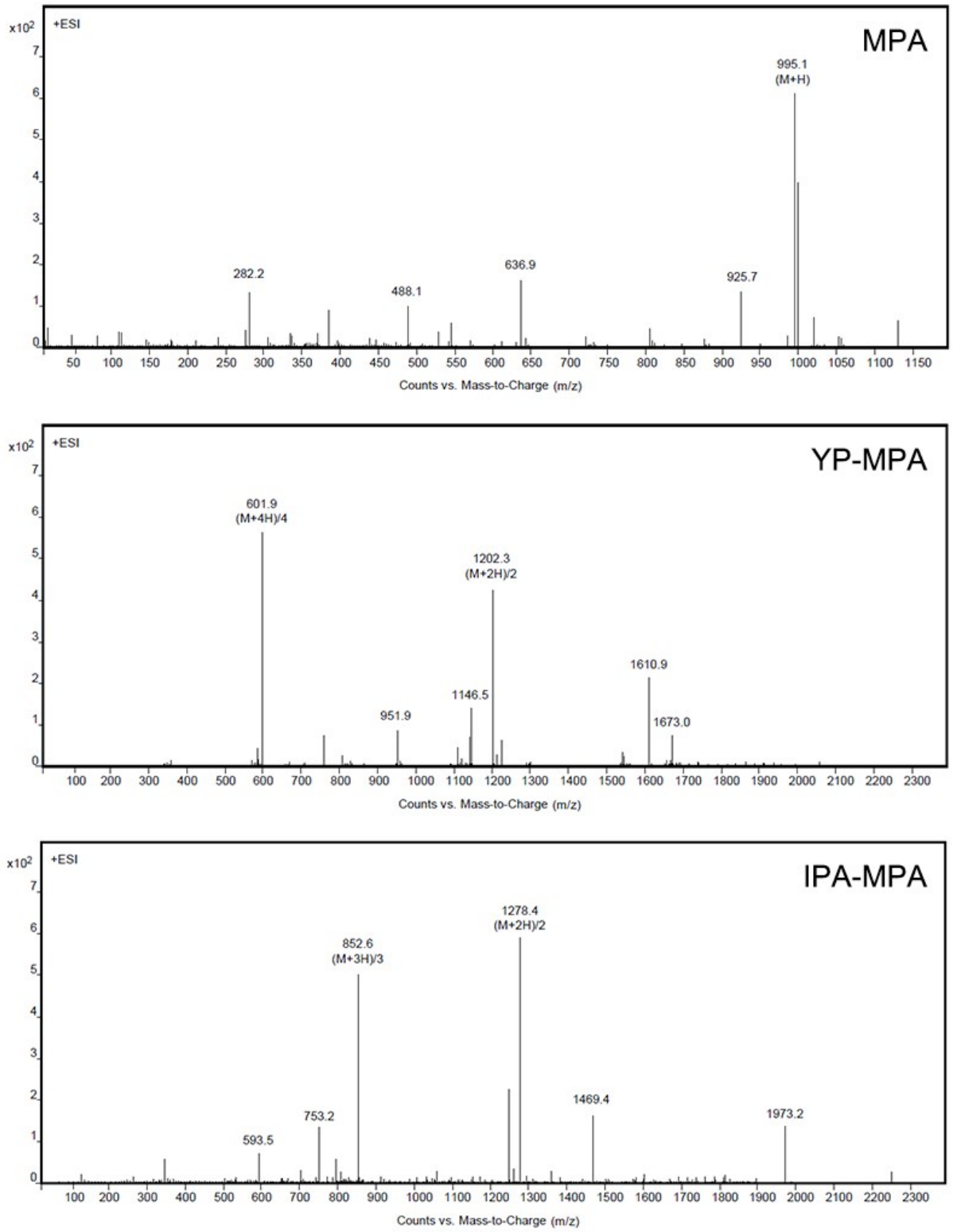


Figure S7. The MS spectrum of MPA, YP-MPA and IPA-MPA.

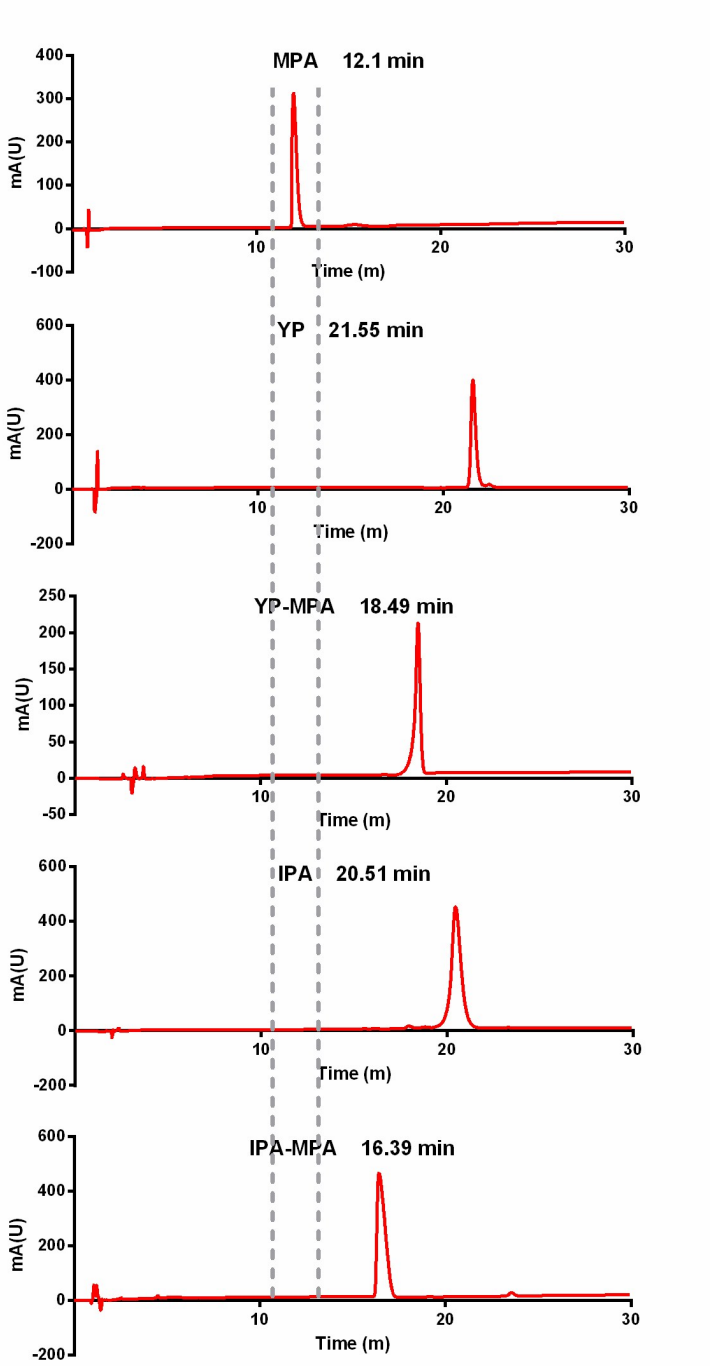


Figure S8. The HPLC analysis of MPA, YP-MPA and IPA-MPA. Mobile phase: Buffer A: 0.1% TFA in 100% water (v/v), Buffer B: 0.1% TFA in 100% acetonitrile (v/v). The gradients of Buffer B in 50 minutes: 0-30 min, 0-54% B; 31-50 min, 55-100% B. Flow rate: 1 mL/min, Wavelenth:220 nm.

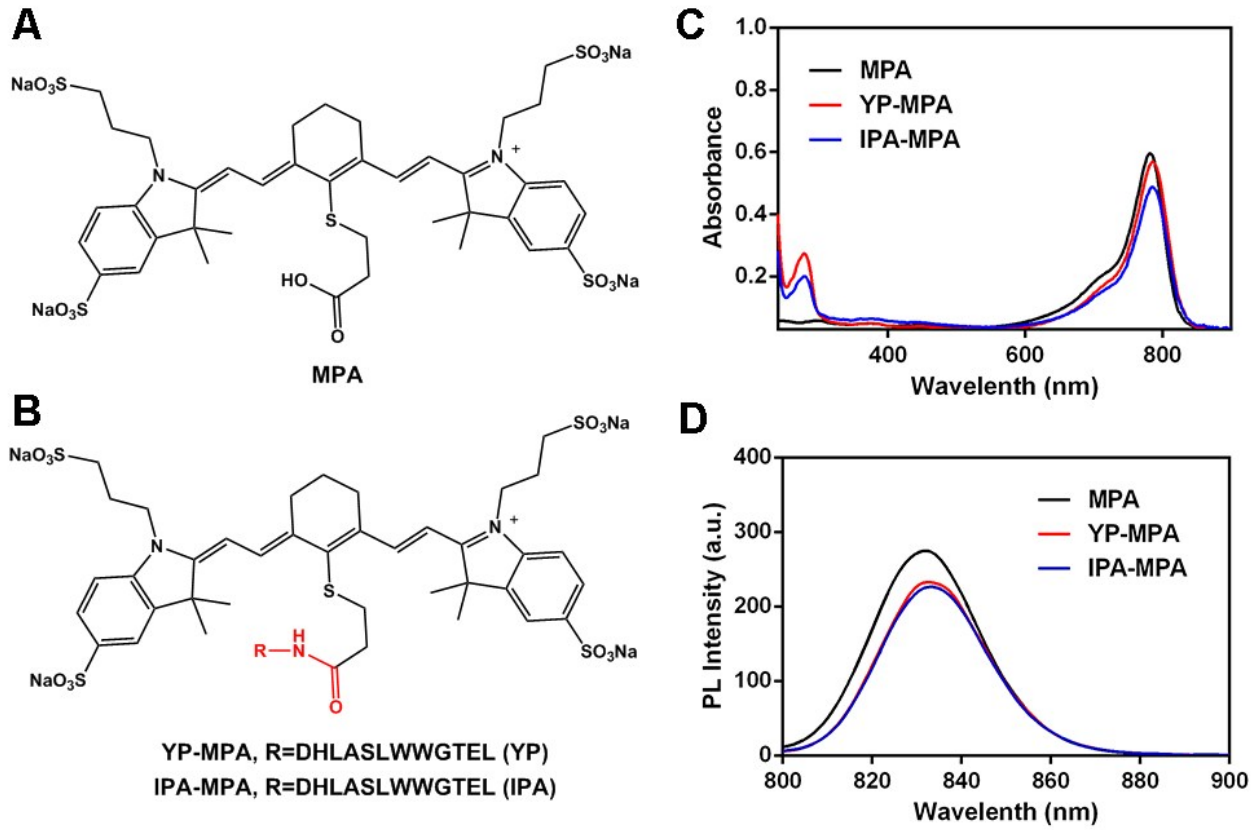


Figure S9. The structure and optical characterization. (A) the structure of MPA, (B) the structure of YP-MPA and IPA-MPA, (C) absorption spectra and (D) fluorescence spectra of free MPA, YP-MPA and IPA-MPA.

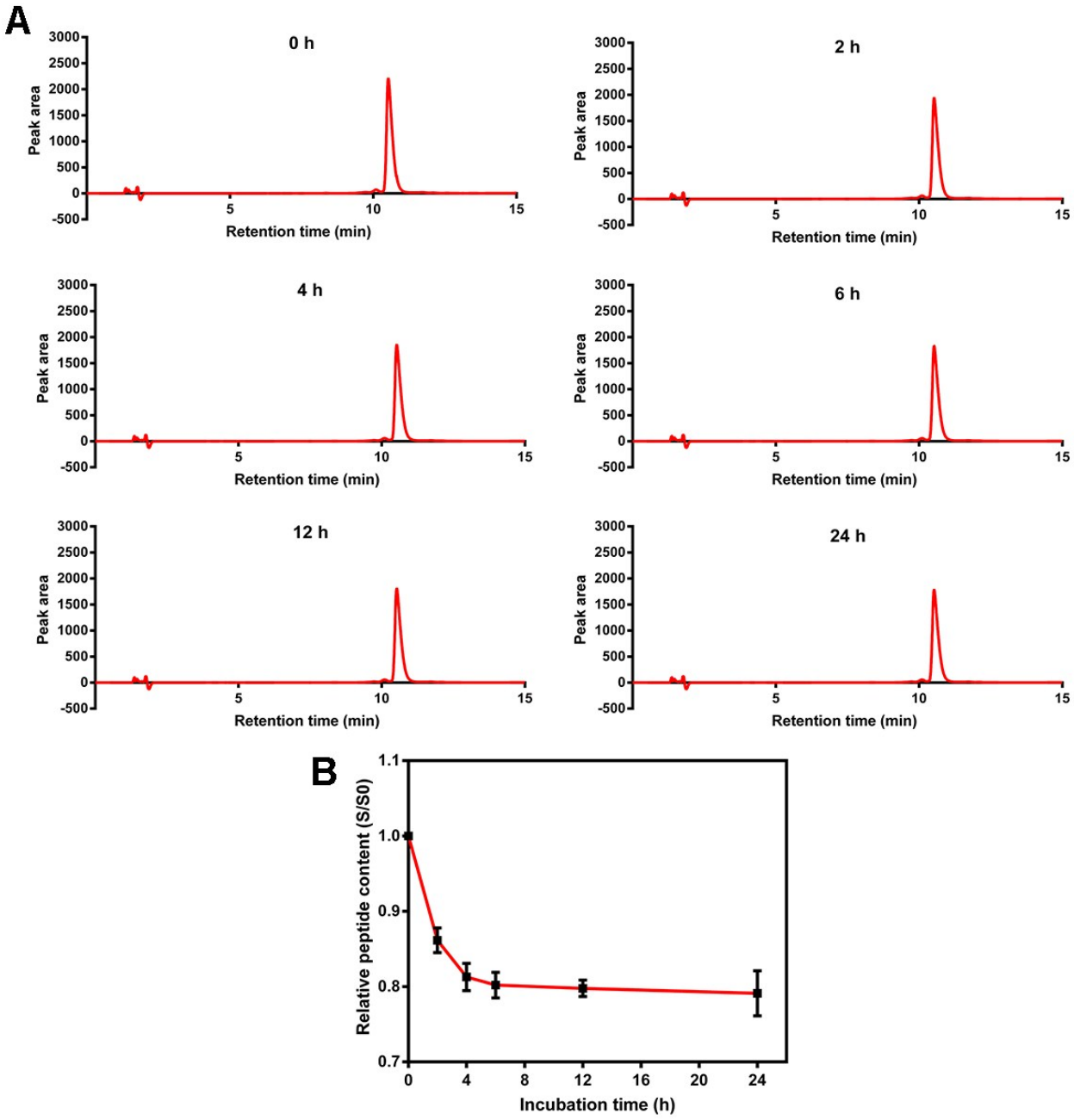


Figure S10. (A) The remaing peptide IPA in rat plasma at different times examined by liquid chromatography. (B) Plasma stability of the peptide IPA in rat plasma over 24 h. Mobile phase: Buffer A: 0.1% TFA in 100% water (v/v), Buffer B: 0.1% TFA in 100% acetonitrile(v/v). The gradients of Buffer B in 20 minutes: 0-15 min, 25-40% B; 15-15.1 min, 40-100% B; 15.1-20 min, 100% B; 20-27 min, post time.

Table S1. The current application of GPC3 targeting materials for HCC imaging

Name	Category	Kd (Binding to protein)	Probe Name	Application
IPA	Peptide	225.1 ± 1.33 nM	IPA-MPA	NIR Imaging
TJ12P1[1]	Peptide	280.4 ± 33.51 nM	Cy5.5-TJ12P1	NIR Imaging Fluorescent Staining
L5[2,3,4]	Peptide	44.7 nM	^{18}F-AlF-NODA-MP-6-Aoc-L5	PET Imaging Fluorescent Staining
GBP[5]	Peptide	735.2 ± 53.60 nM	Cy5.5-GBP	NIR Imaging Fluorescent Staining
AP613-1[6,7]	Aptamer	59.85 ± 15.39 nM	AF750 labeled AP613-1 Apt-USPIO	NIR Imaging MRI Imaging
1G12[8]	Antibody	0.41 ± 0.05 nM	^{89}Zr-DFO-1G12	PET Imaging
αGPC3-F(ab′)2[9]	Antibody	0.03 nM	^{89}Zr-αGPC3-F(ab′)2	PET Imaging
GC33[10]	Antibody	0.67 nM	No	No Application
YP7[11]	Antibody	0.30 nM (for cell)	No	No Application
HN3[12]	Antibody	0.27 nM	No	No Application

Table S2. The predicted physicochemical properties of peptides

(from an online server HLP: http://crdd.osdd.net/cgibin/hlp/pep_both.pl)

Name	Sequence	Relative Stability	Half-life [hours]	Hydrophobicity (KJ/mol)	Molecular weight	Isoelectric point	Surface Accessibility	Flexibility	Charge	Polarity	Free Energy of Solution (in water,kcal/mole)	Heat Capacity
IPA	DYEMHLWWGTEL	3.556	1.860	5.000	1579.930	5.298	44.208	4.840	-2.500	201.920	15.981	570.270
TJ12P1[1]	DHLASLWWGTEL	3.342	1.512	1.342	1427.760	5.551	39.250	4.860	-1.500	151.090	8.823	517.030
L5[2,3]	RLNVGGTYFLTTRQ	3.099	2.039	0.108	1341.710	6.179	41.533	5.230	1.000	63.350	6.431	454.350
GBP[5]	THVSPNQGGLPS	2.420	3.915	0.875	1193.460	6.013	42.650	5.580	0.500	67.250	-4.376	450.020