Supporting information

Glypican-3-Targeted Precision Diagnosis of Hepatocellular Carcinoma on Clinical Sections with A Supramolecular 2D Imaging Probe

Hai-Hao Han,[1a] Yu-Jiao Qiu,[1a] Yuan-Yuan Shi,[2a] Wen Wen,[2] Xiao-Peng He,[1*] Li-Wei Dong,[2*] Ye-Xiong Tan,[2*] Yi-Tao Long,[1] He Tian,[1] Hong-Yang Wang[2*]

[1]Key Laboratory for Advanced Materials & Feringa Nobel Prize Scientist Joint Research Center, East China University of Science and Technology (ECUST), 130 Meilong Rd., Shanghai 200237, PR China
[2]International Cooperation Laboratory on Signal Transduction, Eastern Hepatobiliary Surgery Institute, the Second Military Medical University, Shanghai 200433, PR China
[a]These authors contributed equally to this work.

Corresponding authors:
xphe@ecust.edu.cn (X.-P. He)
donliwei@126.com (L.-W. Dong)
yxtan1214@163.com (Y.-X. Tan)
hywangk@vip.sina.com (H.-Y. Wang)

Contents list

S1. Additional figures

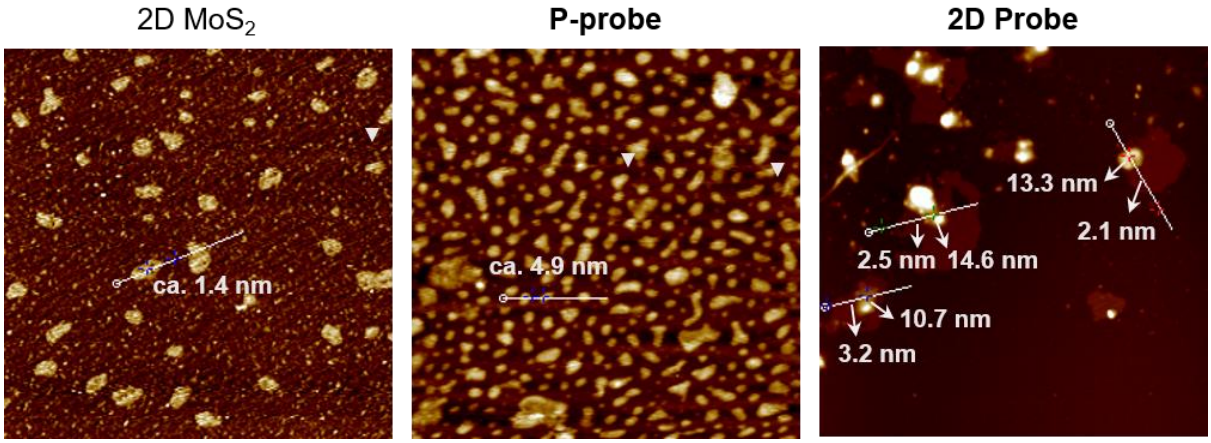

Figure S1. Representative images of 2D MoS_2, **P-probe** and **2D probe** obtained on a Veeco Nanoscope IIIA Multimode Atomic Force Microscope.

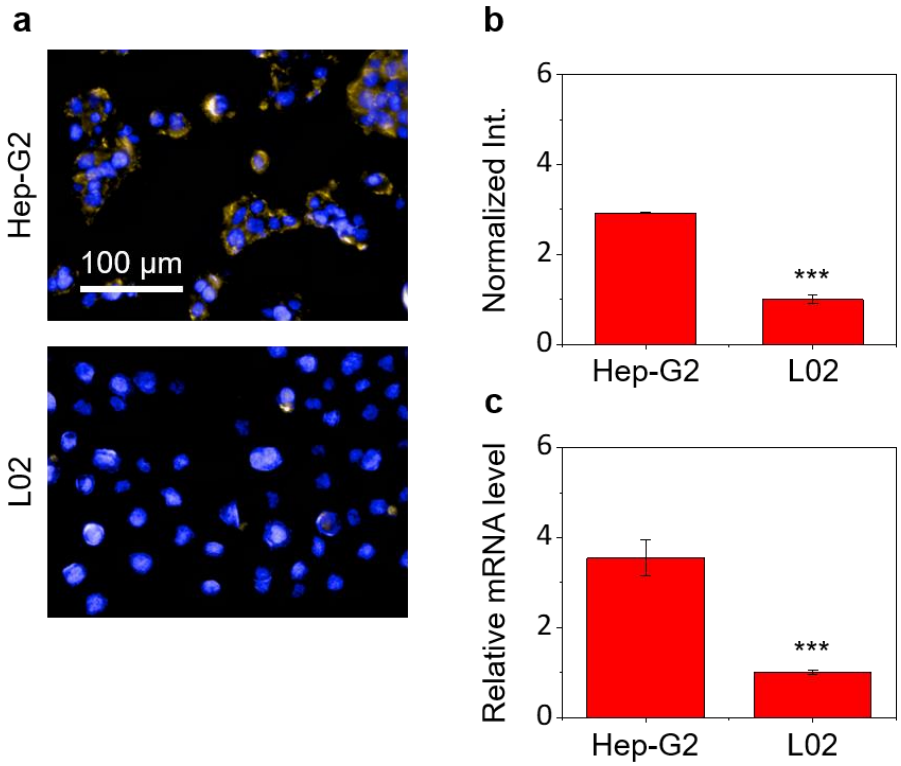

Figure S2. Fluorescence imaging (a) and (b) quantification of different cells in the presence of **2D probe** (**P-probe**/2D MoS_2 = 20 μM/10 μg mL^{-1}; (excitation channel: 520-550 nm, emission channel: 580-650 nm; the cell nuclei were stained by Hoechst 33342. (c) Relative mRNA expression level of GPC-3 in different cells determined by real-time quantitative polymerase chain reaction (***P< 0.001).

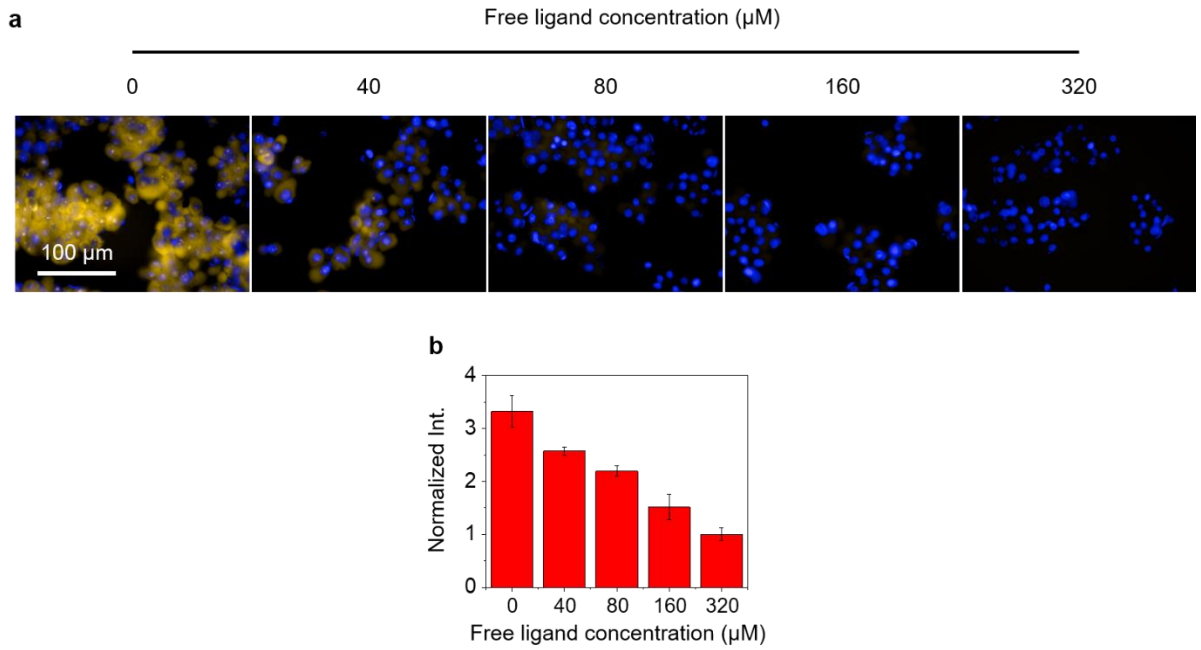

Figure S3. Fluorescence imaging (a) and quantification (b) of Hep-G2 cells with **2D probe** (**P-probe**/2D MoS_2 = 20 μM/10 μg mL^{-1}; excitation channel: 520-550 nm, emission channel: 580–650 nm; the cell nuclei were stained by Hoechst 33342). The cells were pretreated with increasing free peptide ligand (KKKRLNVGGTYFLTTRQ) of GPC-3.

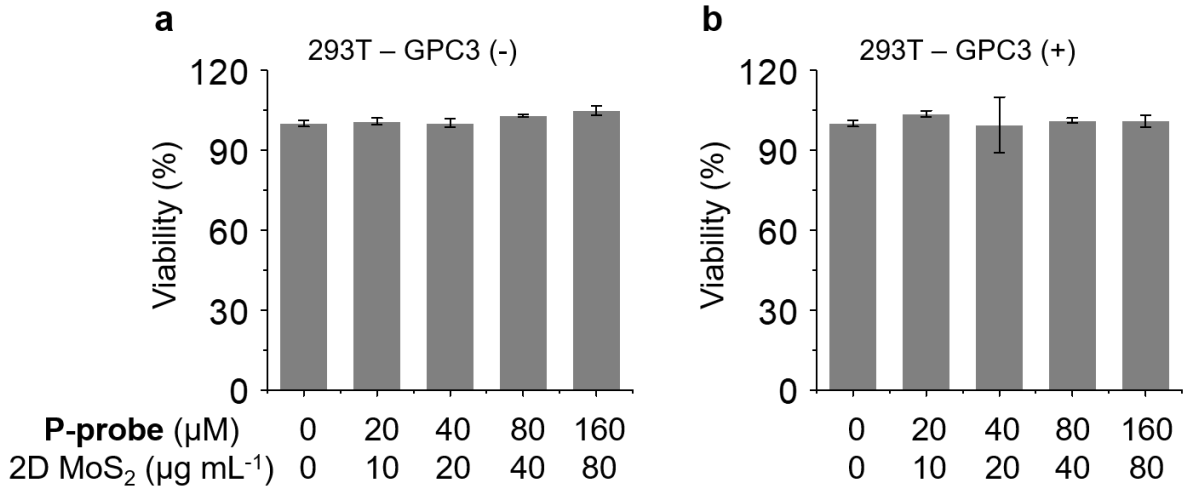

Figure S4. Viability of 293T cells without (-) and with (+) overexpression of GPC-3 in the presence of increasing **2D probe** determined by MTS assay.

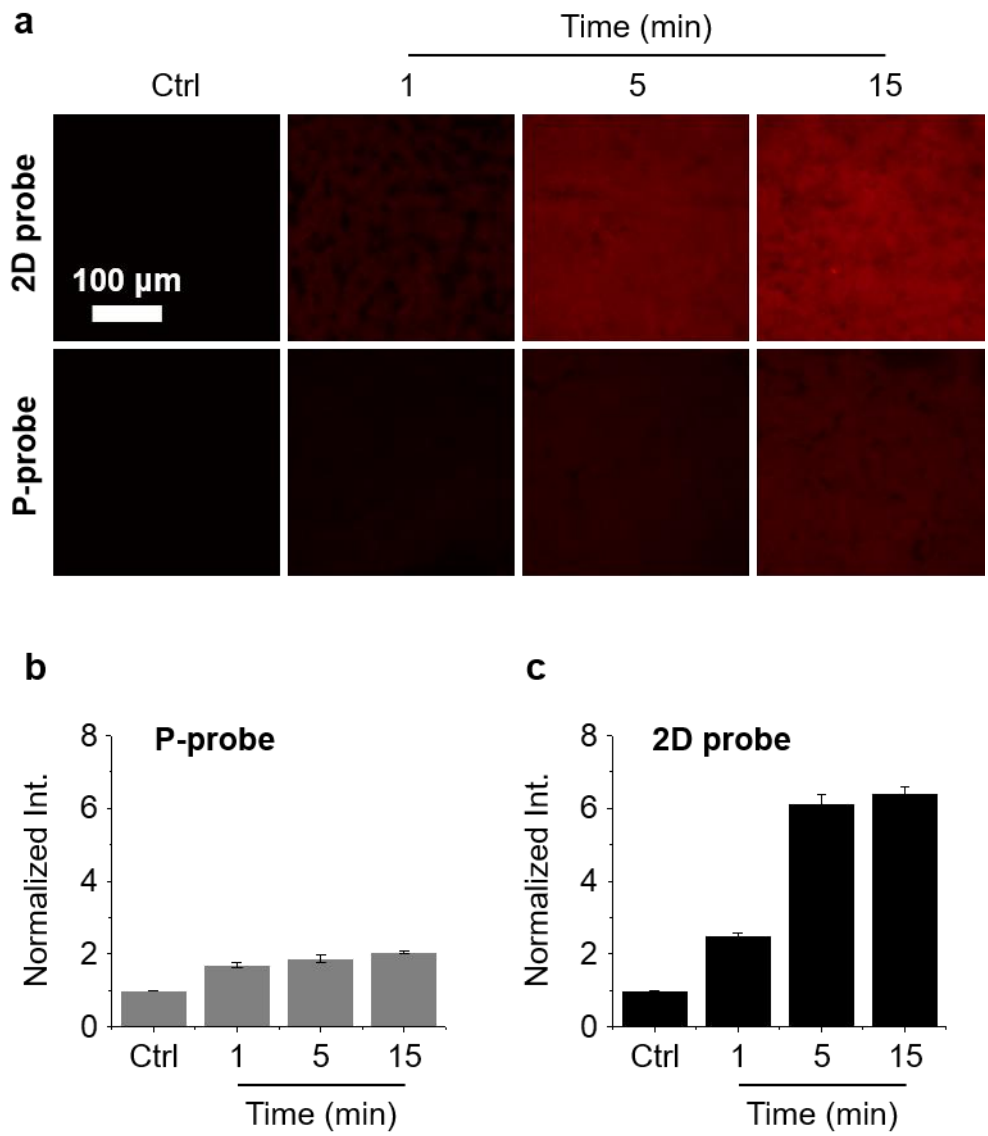

Figure S5. (a) Fluorescence imaging of frozen tissue slides of HCC in the presence of **P-probe** (40 μM) or **2D probe** (**P-probe**/2D MoS_2 = 40 μM/20 μg mL^{-1}) with time. Time-dependent fluorescence quantification of the imaged tissue slide in the presence of (b) **P-probe** (40 μM) and (c) **2D probe** (**P-probe**/2D MoS_2 = 40 μM/20 μg mL^{-1}). The fluorescence images were obtained on a fluorescence microscope (Olympus, Japan; excitation and emission channels used are 535-555 and 570-625 nm, respectively) and quantified by Image-Pro Plus.

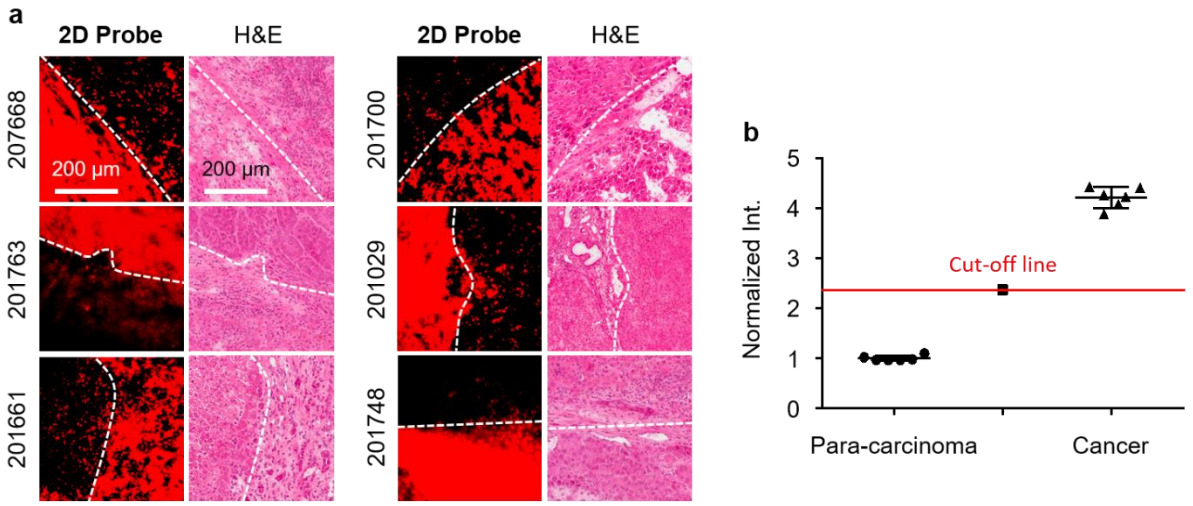

Figure S6. (a) Fluorescence imaging of HCC frozen tissue slides with **2D probe** (**P-probe**/2D MoS_2 = 40 μM/20 μg mL^{-1}) for 5 min (the dashed curves divide HCC from para-carcinoma regions). H&E (Hematoxylin-Eosin)-staining was used as a reference method. (b) Fluorescence quantification of the cancer and para-carcinoma parts of the imaged tissue slides in the presence of **2D probe** (the red line represents the cut-off value). The fluorescence images were obtained on a fluorescence microscope (Olympus, Japan; excitation and emission channels used are 535-555 and 570-625 nm, respectively).

S2. Experimental section

General remarks. All chemicals and reagents used are of the highest commercial grade available. The TAMRA-tagged peptide probe (**P-probe**) and the free peptide ligand of GPC-3 were purchased from Ziyu Biotechnology Co., Ltd. Proteins were purchased from Sigma-Aldrich Sino Biological Inc. 2D Molybdenum sulfide (MoS_2) was produced by the established liquid exfoliation approach from commercial MoS_2 powder. Ultrapure water was obtained from a Milli-Q Integral Pure/Ultrapure Water Production unit. High-resolution transmission electron microscope (HRTEM) images were obtained with JEOL 2100 equipped with a Gatan Orius charged-coupled device camera and Tridiem energy filter operating at 200 kV. Dynamic light scattering and zeta potential were carried out on a Horiba LB-550 DLS Nano-Analyzer and zeta potential Analyzer, respectively. Raman spectra were obtained using a Renishaw InVia Reflex Raman system (Renishaw plc, Wotton-under-Edge, UK) employing a grating spectrometer with a Peltier-cooled charge-coupled device (CCD) detector coupled to a confocal microscope, which were then processed with Renishaw WiRE 3.2 software. The Raman scattering was excited by an argon ion laser (I = 514.5 nm).

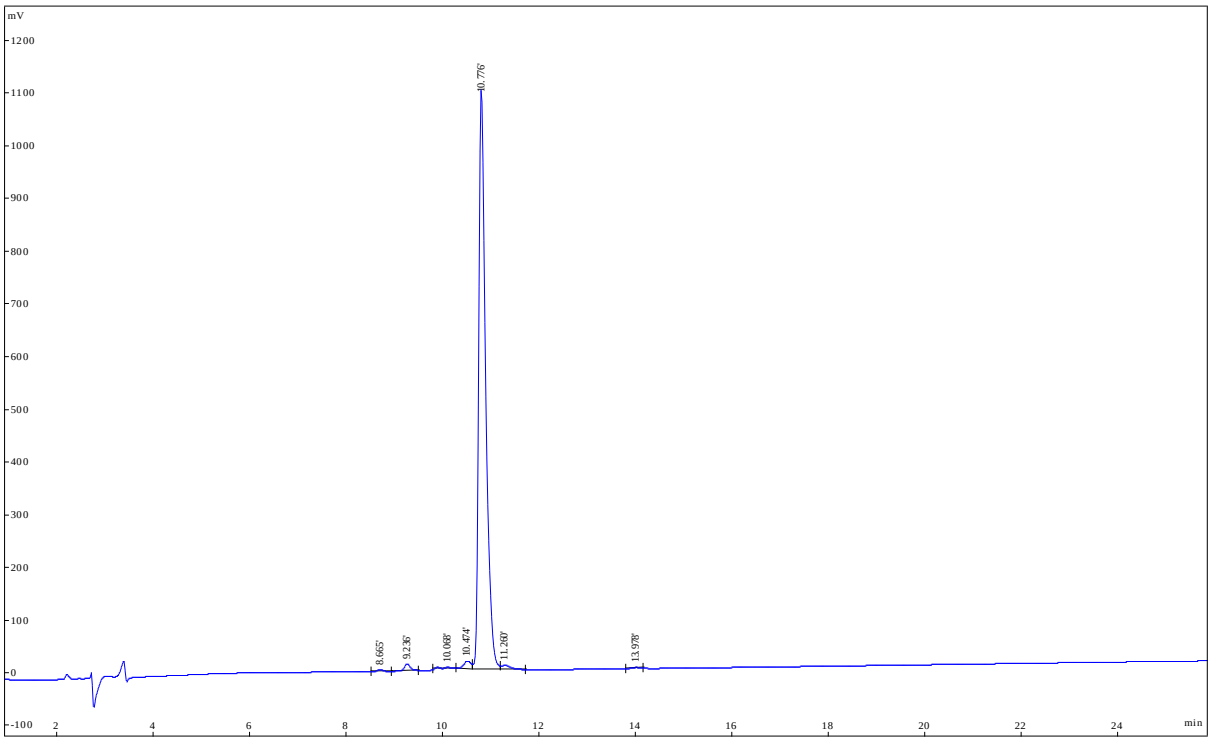

Figure S7. HPLC of **P-probe**.

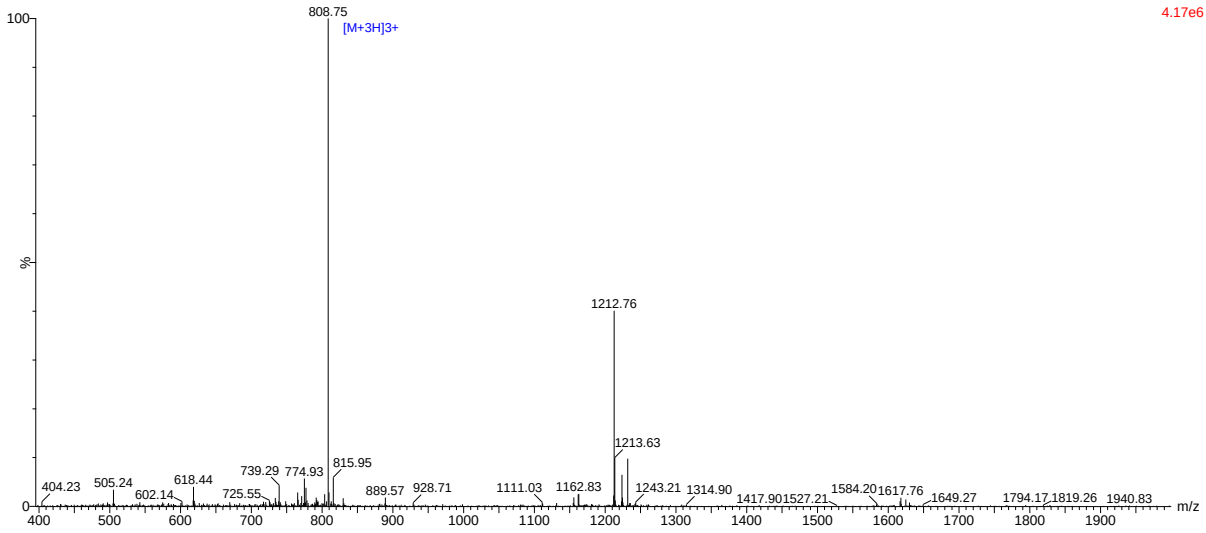

Figure S8. MS spectrum of **P-probe**.

Fluorescence spectroscopy. In a typical fluorescence quenching assay, **P-probe** (with a final concentration of 1 μM) was incubated with 2D MoS_2 of different concentrations (0-12.3 μg/mL) in phosphate buffered saline (PBS, 0.01 M, pH 7.4) for 30 s, and then the fluorescence was measured on a Varian Cary Eclipse fluorescence spectrophotometer at room temperature (rt) with an excitation wavelength of 510 nm. In a typical fluorescence assay for protein detection, to a **2D probe** solution (**P-probe**/2D MoS_2 = 1 μM/5 μg mL^{-1}) proteins dissolved in PBS were added. The resulting mixture was incubated at 37 °C for 5 min. The fluorescence measurements were carried out at rt with an excitation wavelength of 510 nm.

Cell culture. Hep-G2 cells were maintained in a Dulbecco's Modified Eagle's Medium (Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (Gibco, Gland Island, NY, USA) and L02 cells were cultured in RPMI 1640 (Gibco Laboratoties, Grand Island, NY) supplemented with 10% fetal bovine serum (Gibco, Gland Island, NY, USA) in a humidified atmosphere of 5% CO_2 and 95% air at 37 °C and split when the cells reached 90% confluency.

Cell viability assay. Cells were plated overnight on 96-well plates in growth medium. After seeding, cells were treated with **P-probe** and 2D MoS_2 of different concentrations for 48 hours. Then, a solution of MTS/PMS (20:1, Promega Corp) (10 μL per well) was added to each well containing 100 μL of growth medium. After incubation at 37°C under 5% CO_2 for 2 h, the absorbance of the solutions was measured at 490 nm using

an M5 microplate reader (Molecular Device, USA). The optical density of the result in MTS assay was directly proportional to the number of viable cells.

Fluorescence imaging of cells. Cells were seeded on a black 96-well microplate with optically clear bottom (Greiner bio-one, Germany) overnight. The cells were incubated with **2D probe** for 15 min. For the competition assay, cells were preincubated with free peptide ligand (sequence: KKKRLNVGGTYFLTTRQ) (0, 40, 80, 160 and 320 μM) for 1 h, gently washed with phosphate buffered saline (PBS), and then incubated with **2D probe** for 15 min. The cells nuclei were stained with Hoechst 33342 (5 μg mL^{-1}) at 37 °C in a humidified atmosphere of 5% CO_2 in air for 5 min. Then, cells were washed with PBS three times. The fluorescence images were recorded using an Operetta high-content imaging system (Perkinelmer, US), and was quantified and plotted by Columbus analysis system (Perkinelmer, US).

Real-time quantitative polymerase chain reaction (RT qPCR). Total RNA was isolated from cells using TRIzol Reagent (Invitrogen) according to the manufacturer's protocol. Complementary DNA generated using a PrimeScript® RT reagent kit (TaKaRa, Dalian, China) was analyzed by quantitative PCR using SYBR® Premix Ex TaqTM. RT q-PCR was carried out by Stratagene Mx3005P (Agilent Technologies). GAPDH was detected as the housekeeping gene. Primers for qPCR are as follows:

GAPDH forward, 5'-ATCACTGCCACCCAGAAGAC-3'
and reverse, 5'-ATGAGGTCCACCACCCTGTT-3'
GPC-3 sequences: sense, 5'-GTTACTGCAATGTGGTCATGC-3'
and antisense, 5'-CTGGTGCCCAGCACATGT-3'.

Gene transient transfection assay. 293T cells were centrifuged and resuspended in fresh serum-free DMEM at a density of 1.0×10^6 cells mL^{-1} before transfection. The cell suspension (500 μL) was distributed per well in a 12-well plate. DNA was diluted in fresh serum-free DMEM (in a volume equivalent to one-tenth of the culture to be transfected), PEI was added, and the mixture immediately vortexed and incubated for 10 min at room temperature prior to adding to the cells. Following an incubation with DNA-PEI complexes for 3 h, culture medium was completed to 1 mL by the addition of DMEM supplemented with 10% FBS.

Western blot. Plasmids including GPC3, GPC1, GPC5, GOLM1, and DKKL1 were

transfected into 293T cells using a cationic polymer (polyethylenimine) according to the manufacturers' protocol. The transfected cells were lysed in lysis buffer containing 100 mM Tris (pH 7.5), 1% Triton X-100, a 1:100 dilution of a protease inhibitor cocktail and 3 mM phenylmethyl sulphonyl fluoride. The cell lysates were subject to SDS-PAGE and western blot analyses using the corresponding antibodies (anti-His-tag antibody and GAPDH mouse monoclonal antibody).

Fluorescence imaging of frozen sections. Successive frozen pathological specimen sections of 4 μm were made with freezing microtome (Leica, Germany). Frozen sections were returned to room temperature and washed with PBS. Then, the sections were treated with **2D probe** (40 μM/20 μg mL^{-1}) for 5 min. Subsequently, the sections were washed and the nucleus stained with Hoechst 33342. The fluorescence was detected and photographed with fluorescence microscope (Olympus, Japan) or confocal laser scanning microscopy (Olympus, Japan).

Clinical patient samples. Cancer tissues and adjacent tissue samples of patients with hepatocellular carcinoma or cavernous hemangioma were obtained from Eastern Hepatobiliary Surgery Hospital (Shanghai, China).

H&E staining. Frozen sections of fresh human samples were stained with Hematoxylin-Eosin (H&E) according to standard protocols.