Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Identification of Annexin A2 as a target protein for plant alkaloid matrine

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1. Abbreviations.

DCC: dicyclohexylcarbodiimide

DCM: dichloromethane

DIPEA: *N,N'*-diisopropylethylamine

DMEM: Dulbecco's modified eagle medium

DMF: *N,N'*-dimethylformamide

EDC: 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride

FBS: fetal bovine serum

Fmoc: 9-fluorenylmethyloxycarbonyl

HBTU: *O*-benzotriazol-1-yl-*N, N, N', N'*-tetramethyluronium hexafluorophosphate

NHS: *N*−hydroxysuccinimide

PBS: phosphate−buffered saline

PAGE: polyacrylamide gel electrophoresis

SDS: sodium dodecyl sulfate

2. Materials and General Methods.

Unless otherwise noted, all reagents and solvents were purchased from commercial sources and used as received. Hep3B cell line was purchased from ATCC. Water was purified with a Thermo Scientific Barnstead Nanopure system. Photo-cleavage experiments were conducted by a UV LED Ultraviolet crosslinker at 365 nm wavelength with an intensity of approximately 100 μJ /cm^2. Proteins and protein-probe conjugates were analyzed by SDS-PAGE (12%) and silver-stained with commercial kits. All gel images were captured by a Bio-Rad Chemidoc system. Synthesized small molecules were characterized by ^{1}H-NMR (500 MHz, Bruker AVAMCE III). Proteins were identified by matrix-assisted laser desorption/ionization time-of-flight/time-of-flight mass spectrometry (MALDI-TOF/TOF-MS) using the service of Shanghai Sangon Biotech (Shanghai, China).

3. Probe Synthesis, Purification, and Characterization.

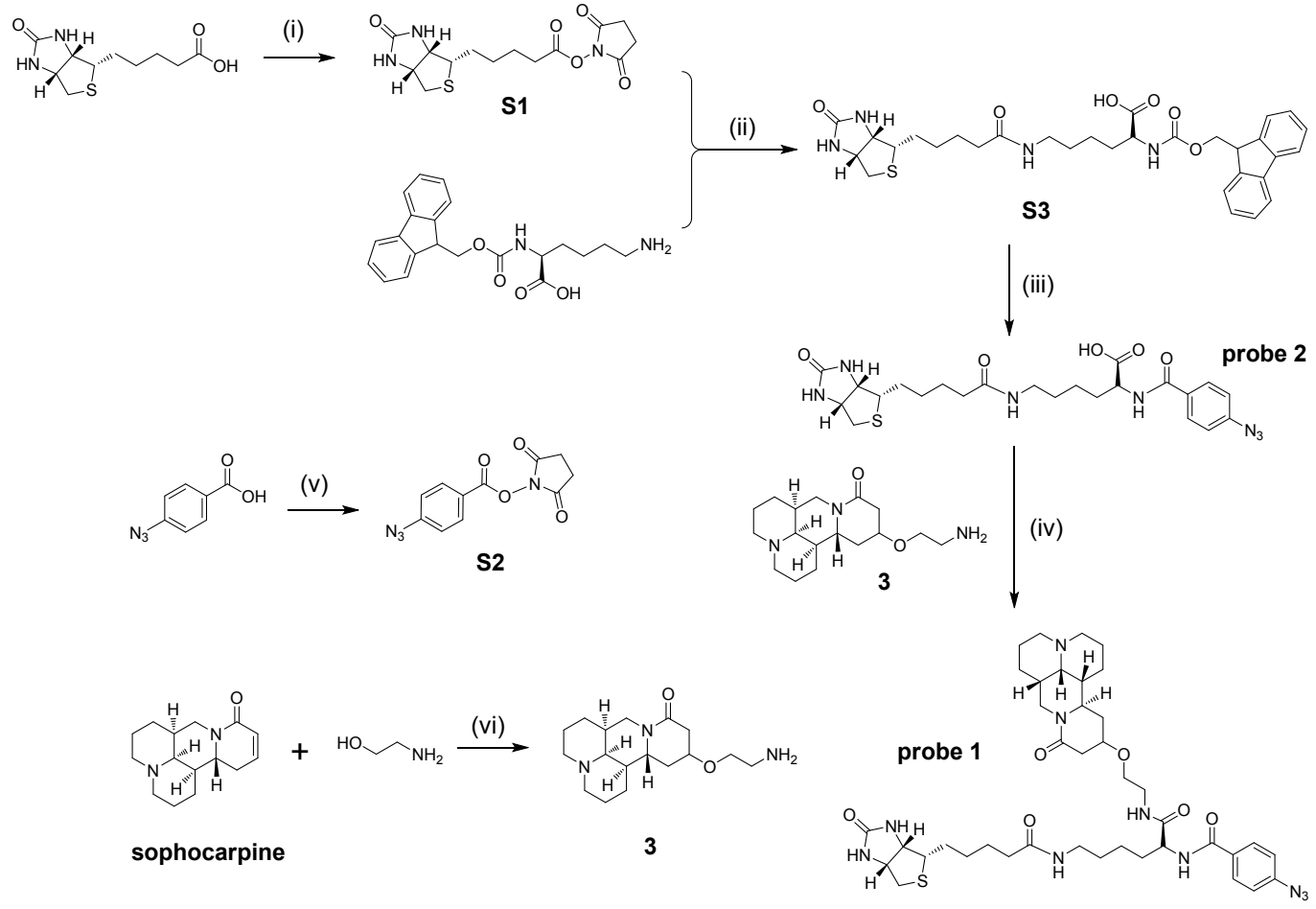


Figure S1: Synthetic scheme for compound **1-3**. (i) NHS, EDC, DMF, yield: 86.7%; (i) dioxane/water (1:1), pH = 8.0-8.5, crude yield: 60%; (iii) 3% DBU in DMF, and then **S2**, DIPEA, yield: 30.4%; (iv) amine-modified matrine, HBTU, DIPEA, DMF, yield: 26.8%; (v) NHS, EDC, DCM, crude yield: 89%; (vi) sodium, 60 °C, 30 hours, yield: 71%. See experimental details below.

(a) Biotin-NHS ester (S1).

To a solution of *L*-biotin (4.886 g, 20 mmol) in anhydrous DMF, NHS (2.762 g, 24 mmol) and EDC (4.6 g, 24 mmol) was added. The reaction was allowed to proceed overnight at room temperature. The resulting mixture was dried under vacuum to remove DMF. The gel-like residue was recrystallized from EtOH/acetic acid/H_2O (95:1:4) to afford the crude product as a white solid (crude yield: 86.7%).[S1] The crude product was directly used in the next step of synthesis without further purification.

(b) Azide-NHS ester (S2).

To a solution of 4-azidobenzoic acid (3.26 g, 20 mmol) in anhydrous DCM, *N*-hydroxysuccinimide (2.762 g, 24 mmol) and EDC (4.60 g, 24 mmol) was added. The reaction was allowed to proceed overnight at room temperature. The resulting mixture was dried under vacuum to remove DCM. The gel-like residue was recrystallized from ethyl acetate to afford azide-NHS ester as a light yellow solid (4.56 g; crude yield: 89%), which was directly used in the next step of synthesis with further purification.

(c) Fmoc-Lys(biotin)-OH (S3).

Fmoc-Lys-OH (5.185 g, 8.5 mmol) was dissolved in 28 ml of 1:1 dioxane /water, and then the pH of the solution was adjusted to 8~8.5 with 4 M NaOH solution at 0° C. Biotin-NHS (**S1**, 2.9 g, 8.5 mmol) was added and the reaction mixture was stirred at room temperature overnight. The resulting gelatinous mixture formed was added with ethyl ether and then stirred for 5 minutes. The supernatant ether was decanted followed by the addition of acetone and 2 M HCl was added to adjust the pH of the residue to ~3 at 0° C. The formed precipitation was filtered and washed several times with methanol to afford the product a white solid (crude yield: 60%), which was directly used in the next step of synthesis with further purification.[S2]

(d) Azide-Lys(biotin)-OH (probe 2).

S3 (5.10 g, 8.5 mmol) was dissolved in 10.0 mL of anhydrous DMF containing 3% DBU, the reaction mixture was stirred at room temperature for 1 hour to remove the Fmoc protection group. Azide-NHS ester (**S2**, 2.21 g, 8.5 mmol) and DIPEA (1.5 mL, 8.5 mmol) were directly added to the mixture and the reaction was stirred at room temperature overnight. The product was purified by column chromatography and used for the next step of reaction without further characterization (yield: 30.4%). ^{1}H-NMR (400 MHz, DMSO-d_6): δ 12.56 (br, 1H), 8.60 (d, J = 7.6 Hz, 1H), 8.04 – 7.91 (m, 2H), 7.79 (s, 1H), 7.33 – 7.16 (m, 2H), 6.44 (s, 2H), 4.32 (td, J = 7.8, 7.1, 5.1 Hz, 2H), 4.12 (dd, J = 7.8, 4.4 Hz, 1H), 3.06 (dd, J = 21.1, 4.3 Hz, 3H), 2.83 (dd, J = 12.4, 5.1 Hz, 1H), 2.58 (d, J = 12.4 Hz, 1H), 2.04 (t, J = 7.4 Hz, 2H), 1.80 (s, 2H), 1.59 (dt, J = 12.0, 7.3 Hz, 1H), 1.52 – 1.19 (m, 9H). The mass calculated for $C_{22}H_{31}N_7O_4S$ $[M+H]^+$: 489.2158, found 489.2350.

(e) Azide-Lys(biotin)-matrine (probe 1).

Azide-Lys(biotin)-OH (probe **2**, 980 mg, 1.5 mmol) was dissolved in 6.0 mL anhydrous DMF. HBTU (1.0 g, 2.0 mmol), matrine analogue **3** (615 mg, 2.0 mmol, prepared with the reported method in Ref. 31 of the main text) and DIPEA (0.5 mL, 2.0 mmol) was added to the solution. The reaction mixture was stirred at room temperature overnight, washed with saturated $NaHCO_3$ solution, and extracted with 1-butanol (25 ml for 3 times). The organic phase was dried over anhydrous Na_2SO_4, concentrated under vacuum, and then

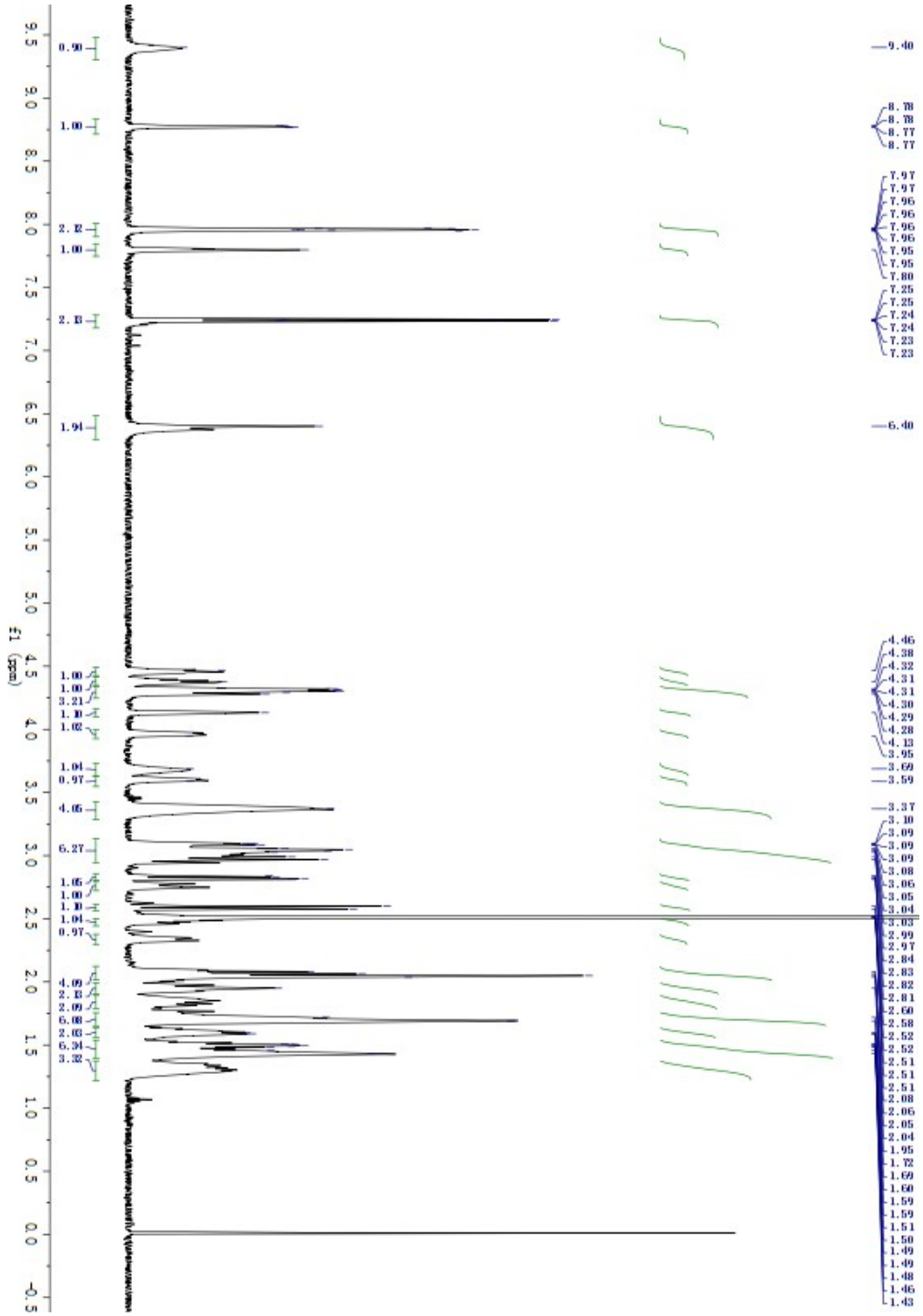
purified by chromatography. The final product of probe **1** was obtained as a white solid (yield: 26.8%). ^{1}H-NMR (600 MHz, DMSO-d_6): δ 9.40 (s, 1H), 8.77 (dd, J = 7.1, 1.7 Hz, 1H), 8.01 – 7.90 (m, 2H), 7.80 (s, 1H), 7.28 – 7.18 (m, 2H), 6.40 (s, 2H), 4.46 (s, 1H), 4.38 (s, 1H), 4.34 – 4.25 (m, 3H), 4.13 (s, 1H), 3.95 (s, 1H), 3.69 (s, 1H), 3.59 (s, 1H), 3.37 (s, 4H), 3.13 – 2.94 (m, 6H), 2.83 (dd, J = 12.5, 5.1 Hz, 1H), 2.76 (d, J = 16.6 Hz, 1H), 2.59 (d, J = 12.4 Hz, 1H), 2.47 (dd, J = 10.5, 4.9 Hz, 1H), 2.34 (d, J = 13.6 Hz, 1H), 2.12 – 2.01 (m, 4H), 1.95 (s, 2H), 1.84 (q, J = 11.5, 9.8 Hz, 2H), 1.71 (d, J = 17.3 Hz, 6H), 1.60 (d, J = 6.1 Hz, 2H), 1.54 – 1.40 (m, 6H), 1.37 – 1.22 (m, 3H).*^{13}C* NMR (151 MHz, DMSO-d_6) δ 171.97, 165.91, 164.33, 162.66, 158.28, 158.06, 142.69, 130.03, 129.40, 118.88, 63.01, 61.02, 60.27, 59.20, 55.34, 54.92, 54.67, 52.76, 49.23, 48.32, 48.15, 42.61, 38.02, 37.71, 35.21, 34.55, 33.60, 29.87, 28.82, 28.15, 27.99, 25.49, 25.30, 24.73, 23.55, 23.08, 18.60, 18.06. HRMS: calculated, $C_{40}H_{58}N_6O_{10}S$ $[M+H]^+$: 807.4266; found 807.4350.

(f) 2-Amino-ethoxy-matrine (3).

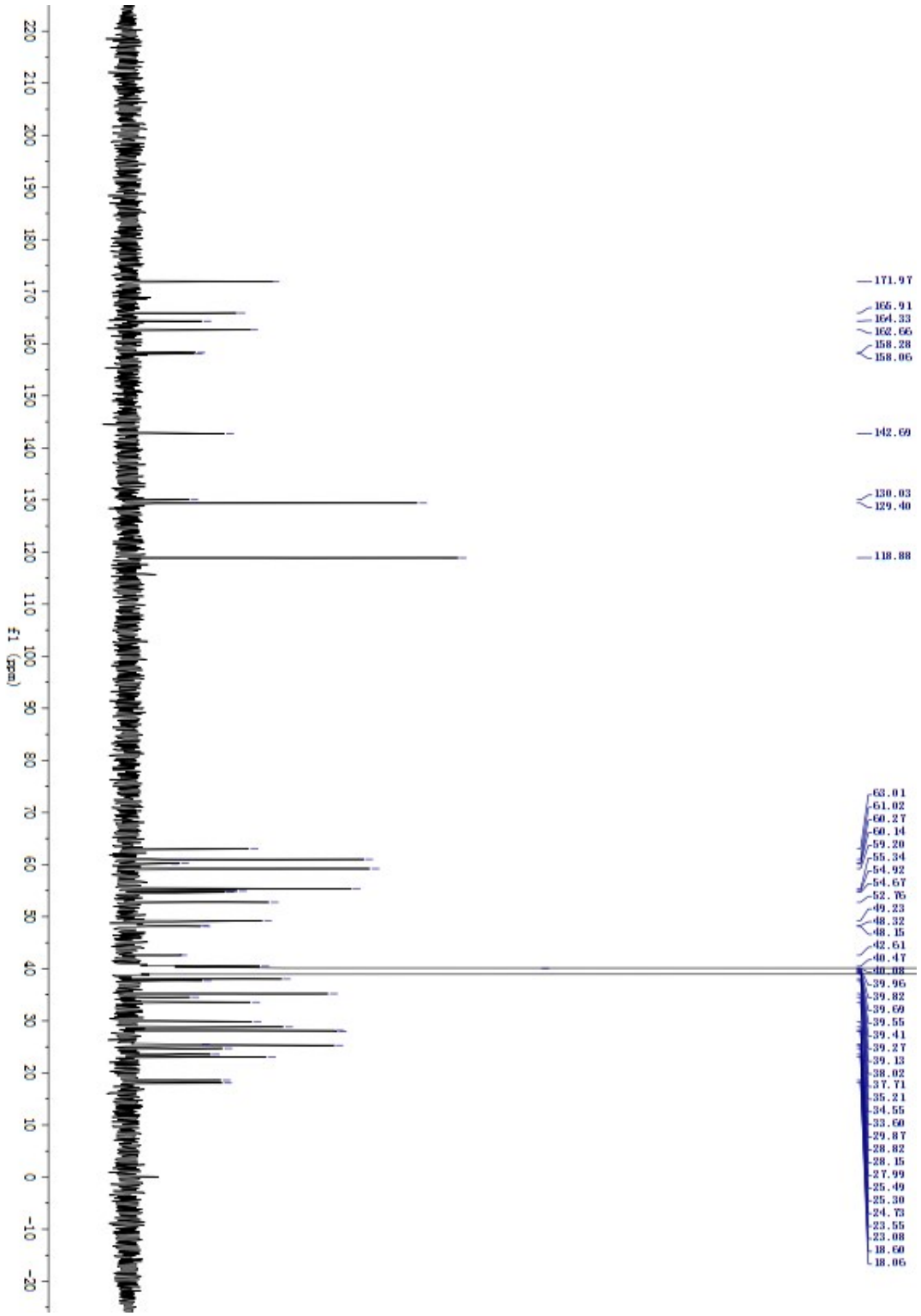
Probe **3** was prepared following a reported procedure.[S3] In brief, sodium metal (0.93 g, 40.6 mmol) was added to 100 mL dry ethanolamine. The solution was refluxed under N_2 protection overnight and then cooled to room temperature. Sophocarpine (8.0 g, 32.5 mmol; ChemBest, Shanghai, China) was dissolved in 5 mL ethanolamine and the mixture was slowly added to the solution above. Then the reaction was maintained at 60 °C for 30 hours with gentle stirring. The reaction was quenched with 15 mL of water, dried over by anhydrous magnesium sulfate, and then concentrated under vacuum. The product **3** was purified by column chromatography as a white solid (yield: 71%). ^{1}H-NMR (600 MHz, DMSO-d_6): δ 4.46 (s, 1H), 4.10 (dd, J = 12.5, 4.3 Hz, 1H), 3.79 (dt, J = 8.6, 3.1 Hz, 1H), 3.42 (t, J = 5.9 Hz, 2H), 2.92 (q, J = 3.1 Hz, 1H), 2.87 (t, J = 12.6 Hz, 1H), 2.77-2.65 (m, 2H), 2.60-2.50 (m, 2H), 2.34 (dd, J = 16.7, 4.3 Hz, 1H), 2.08 (ddd, J = 16.8, 5.2, 1.9 Hz, 1H), 2.00 (d, J = 2.9 Hz, 1H), 1.93-1.79 (m, 4H), 1.75-1.38 (m, 8H), 1.38-1.24 (m, 3H). HRMS: calculated, $C_{17}H_{29}N_3O^2$ $[M+H]^+$: 308.4258, found 308.4257.

4. NMR Spectra of Compound 1-3.

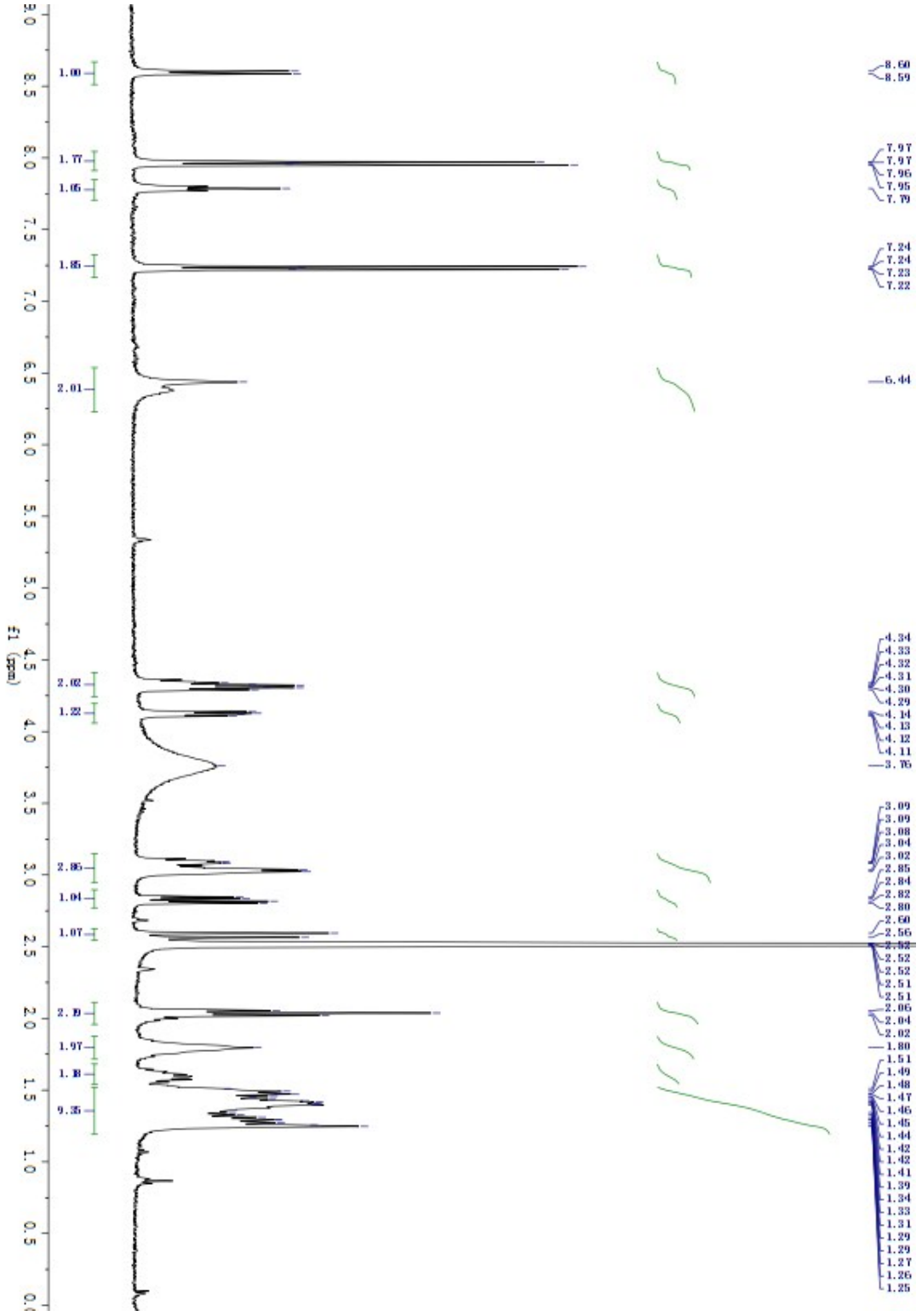
a) ^{1}H-NMR, probe 1:



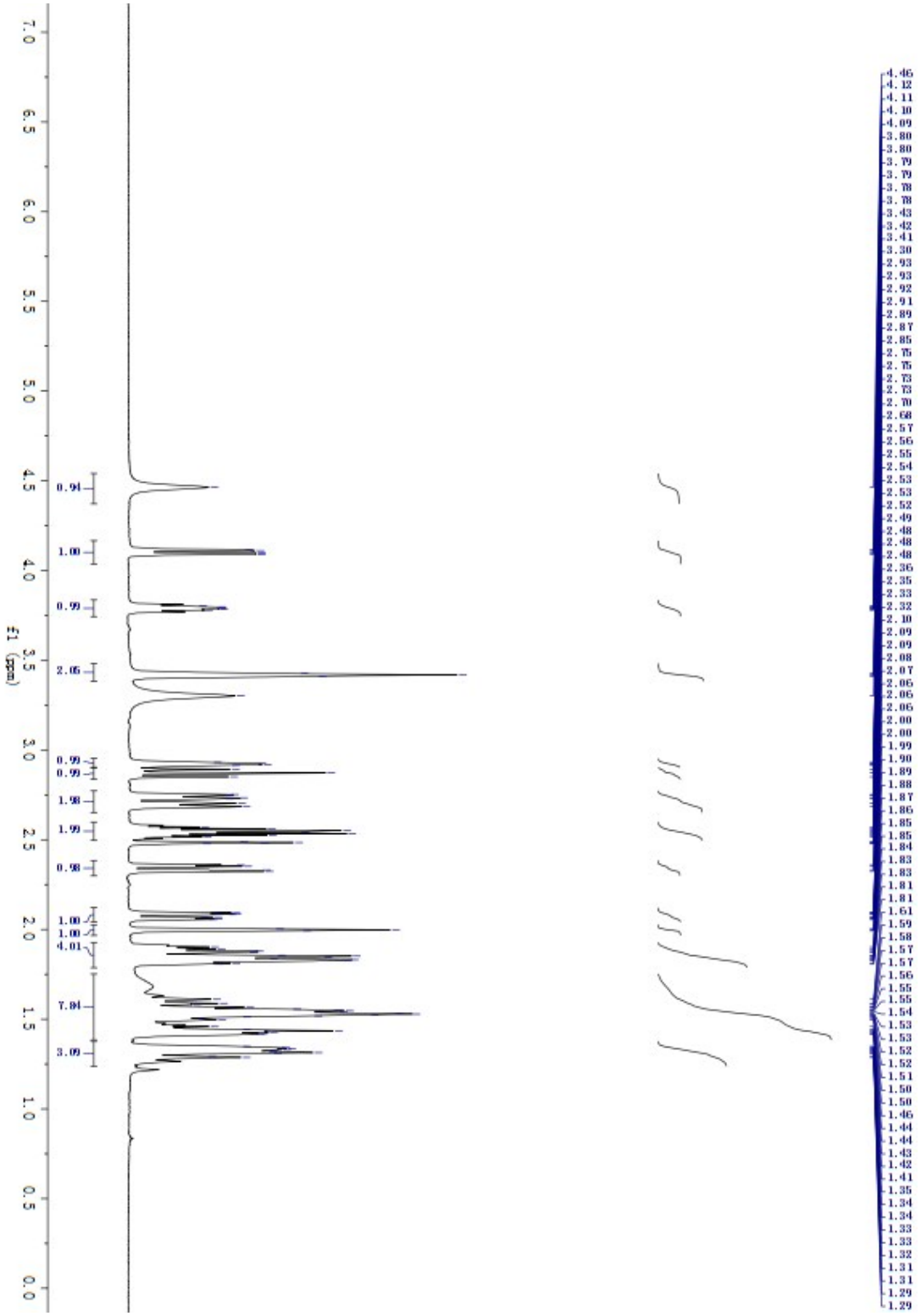
b) **^{13}C-NMR, probe 1:**



c) **^{1}H-NMR, probe 2:**



d) ^{1}H-NMR, compound 3:



5. Wound-Healing Assay.

Hep3B cells were seeded in six-well plates and cultured until confluent. A sterile pipette tip was used to make a straight scratch would, and then cells were washed with 500 μL PBS twice to remove cell debris. Cells were cultured with DMEM medium (2 mL each well without FBS, 37 °C, 5% CO_2) and treated with matrine, probe **1**, or analogue **3** (10 μM in water). In control experiments, only the same volume of water was added. In experiments with antibody addition, the cells were added with respective antibody (2 μg/mL) before compound addition. Cell images were captured at different time points after scratch as indicated in the main text with a microscope. Cell migration rate was measured based on the distance of the edges of scratch using the Image J software (Rawak, Germany), following the manufacturer's instructions. Relative migration rate was quantified against the water only control experiments.

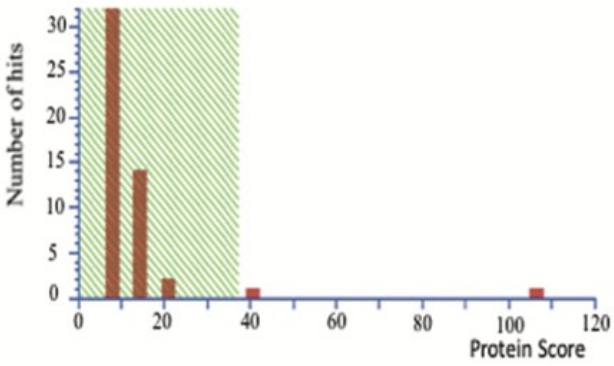
6. Affinity Pull-down Experiments.

Hep3B cells were lysed (3 mg) and incubated with either probe **1** or probe **2** (10 μM in water) at 4 °C for 12 hours. The mixture was irradiated at 365 nm for 1 minute, and then 20 μL streptavidin beads (Thermo Fisher, USA) were added. The suspension was vortexed at 4 °C for another 4 hours. In order to thoroughly remove non-specifically binding proteins, stringent washing buffer (1% SDS) was used. The beads were washed with 1% SDS for 10 times and PBS for 3 times. 1x loading buffer was added to the beads and then boiled at 95 °C for 5 minutes to elute streptavidin-captured proteins. Eluted samples were directly analyzed by 12% SDS-PAGE.

7. Identification of Annexin A2 and Moesin.

After eluted protein samples were separated by SDS-PAGE, gels were stained with commercial silver-stain kit (Beyotime, China). The two specific protein bands shown in Figure 2a of the main text were excised and submitted directly for protein identification. Protein identification was performed by Shanghai Sangon Biotech (Shanghai, China) using matrix-assisted laser desorption/ionization time-of-flight/time-of-flight mass spectrometry (MALDI-TOF/TOF-MS).

a)



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annexin A2 isoform 2 [Homo sapiens]
Check to include this hit in error tolerant search

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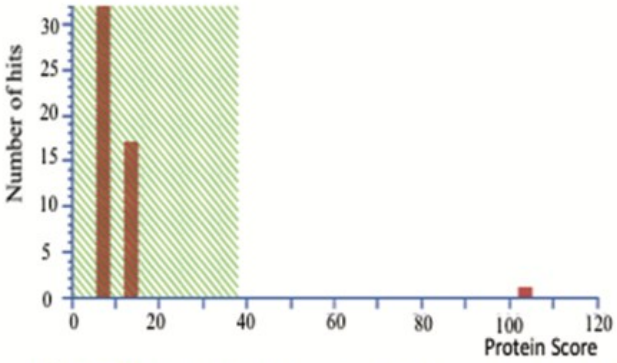
83	1460.7762	1459.7689	1459.6653	71.0	0	4	1.7e+02	2	U	K.SYSPYDMLESIR.K
92	1542.9443	1541.9370	1541.8413	62.1	0	20	1.9	1	U	K.GVDEVTIVNILTNR.S
144	1909.0146	1908.0073	1907.8748	69.4	0	24	0.85	1	U	R.AEDGSVIDYELIDQDAR.D

b)



gi|14719519 **Mass:** 40962 **Score:** 104 **Matches:** 4(1) **Sequences:** 4(1) **emPAI:** 0.14
Chain A, Structure Of Isolated Ferm Domain And First Long Helix Of Moesin
Check to include this hit in error tolerant search

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23	1104.6191	1103.6118	1103.5764	32.1	0	21	3.6	1	U	K.IGFPWSEIR.N
34	1182.6249	1181.6176	1181.5869	26.0	0	37	0.081	1	U	K.APDFVFYAPR.L
41	1233.6515	1232.6442	1232.6050	31.8	0	4	2.7e+02	3	U	R.IQVWHEEHR.G
144	2082.0552	2081.0479	2080.9953	25.3	0	42	0.016	1	U	K.FYPEDVSEELIQDITQR.L

Figure S2: MALDI-TOF/TOF-MS analysis results of the two protein bands shown in Figure 2a of the main text. a) The lower band was identified as Annexin A2; b) the upper band was identified as moesin.

8. SPR Analysis.

SPR analysis was performed on a Biacore TM T200 instrument (GE Healthcare). Proteins were immobilized on a CM5 chip via EDC/NHS-mediated crosslinking reaction. Small molecule ligands were diluted in PBS at concentrations ranging from 0.5 μM to 32 μM. The analysis was then performed according to the protocol provided by GE Healthcare.[3] In each analysis, the middle concentration was duplicated at the end of the wash run to confirm the stability of the sensor surface. The parameters of SPR were set as follows: flow rate, 30 μL/min; contact time, 60 s, disassociation time, 300 s. Affinity curve fitting was performed with the Biacore T200 software using a steady-state affinity model (1:1) to calculate disassociation constant (K_d). Detailed SPR measurement data are shown below in Table S1. Moesin did not show detectable affinity with either matrine or analogue 3; the SPR sensorgrams are shown below in Figure S3.

Table S1: SPR measurement data of ANXA2 and moesin with small molecules.

protein	ligand	K_d (µM)	R_{max}	offset	Chi^2
ANXA2	matrine	12.83	32.87	-1.819	0.0622
ANXA2	**3**	23.34	42.88	-0.293	0.576
moesin	matrine	-	-	-	-
moesin	**3**	-	-	-	-

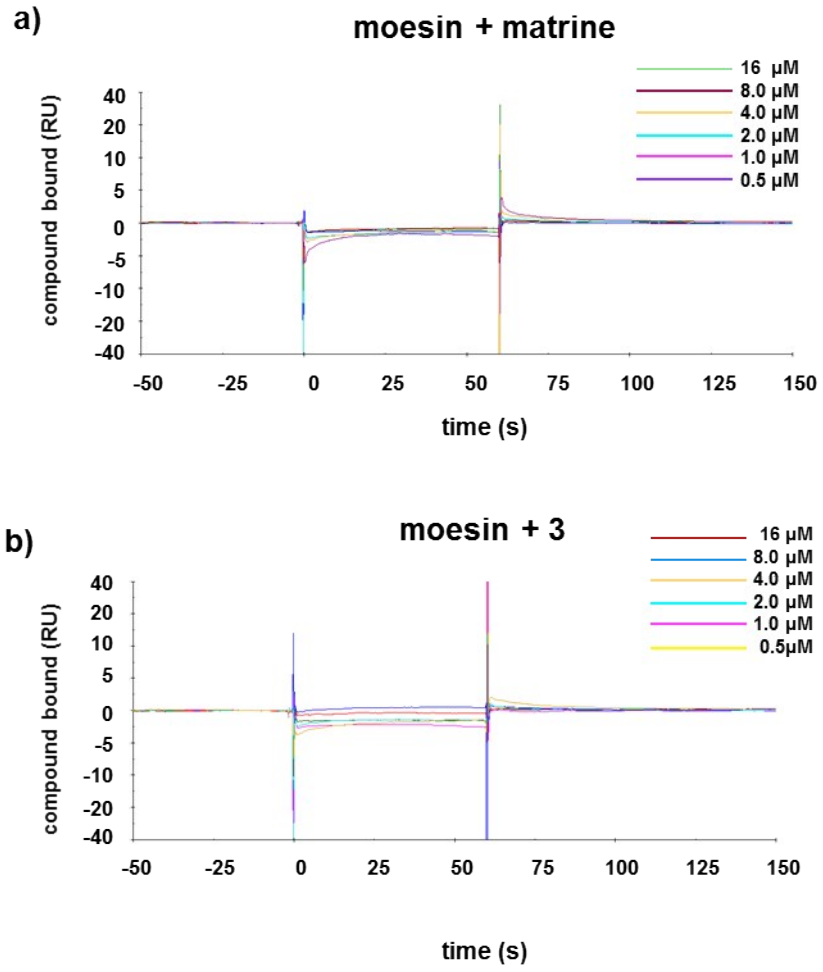

Figure S3: SPR sensorgrams of moesin with matrine and analogue **3**.

9. RNAi, Plasmid Transfection, Real-time PCR and Western Blot

The shDNA (short hairpin DNA) encoding siRNAs (small interfering RNA) targeting ANXA2 transcripts (GeneBank: NM_001002858) and the control shDNA were both synthesized by GeneChem (China); they were used to construct the plasmid (Pgv248/ANXA2-shRNA) and the control plasmid, respectively, based on a published report.[4]

Cells were seeded in multiple 6-well plates to 80% confluence. For each well, 1.6 μg of plasmid was transfected with lipofectamine 2000 in serum-free medium according to the manufacturer's manual (GeneChem, Shanghai).

- **Validation of knocking-down ANXA2 gene transcription by real-time PCR:** After 6 hours, the medium was replaced by a fresh batch and continued to culture for 24 hours. RNA was isolated using the UNiQ-10 column total RNA purification kit (Sangon Biotech) and reverse transcribed using the AMV first-strand cDNA synthesis kit (Sangon Biotech). Quantitative real-time reverse transcription-PCR (qRT-PCR) was performed with fluorescent dye SYBR on a StepOne™ real-time PCR system (Applied Biosystems) following the manufacturer's instructions. PCR condition: one cycle at 94 °C for 4 min, 40 cycles at 94 °C for 30 s, 60 °C at 30 s, and then 72 °C for 30 s. Each reaction was performed in triplicate.

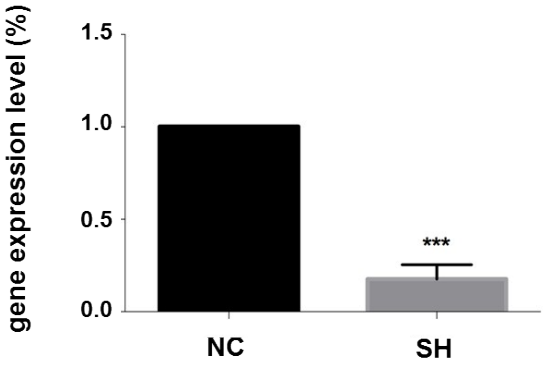

Figure S4: ANXA2 gene transcription levels after transfection by the ANXA2-targeting plasmid (SH) and the control plasmid (NC).

- **Validation of knocking-down ANXA2 protein expression by Western blot:** Fresh cell lysates were prepared as described above. Protein concentrations were determined with Bradford assay. Cell lysates (20 μg) were then dissolved in SDS sample buffer and resolved by SDS-PAGE gel (12%). After

electrophoresis, proteins were transferred onto a nitrocellulose and the blotted following the typical blocking, washing and visualization protocol.

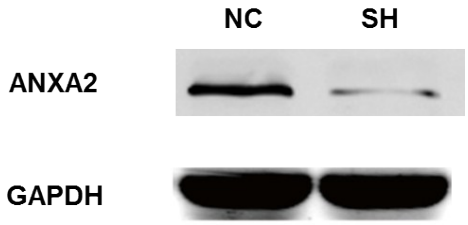

Figure S5: ANXA2 protein expression level was significantly knocked-down after transfection with the ANXA2-targeting plasmid (SH), in comparison with the control experiment (NC).

10. Cell Images of Wound-Healing Assay for Figure 3c and 3d.

(a) For Figure 3c: in the presence of an ANXA2 antibody or a control-IgG antibody.

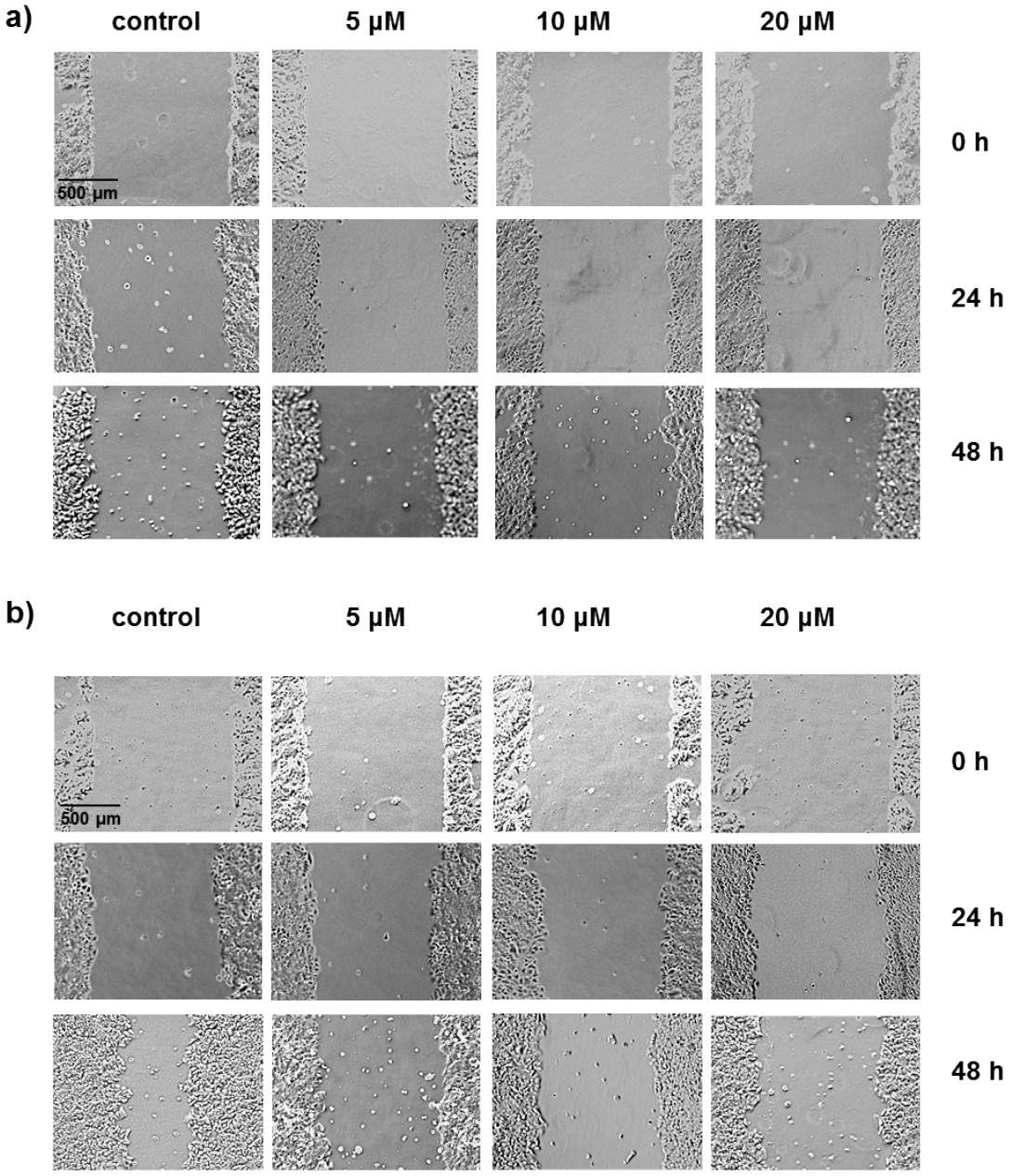

Figure S6: Images of wound-healing assay with a) an anti-ANXA2 antibody (2 μg/mL; Santa Cruz) or b) a control antibody (IgG, 2 μg/mL; Santa Cruz). Cells were treated with different concentrations of matrine (as marked on top of the images). Images were taken at 0 h, 24 h, and 48 h after scratch.

(b) For Figure 3d: cells treated with an ANXA2-targeting plasmid (SH) or a control plasmid (NC).

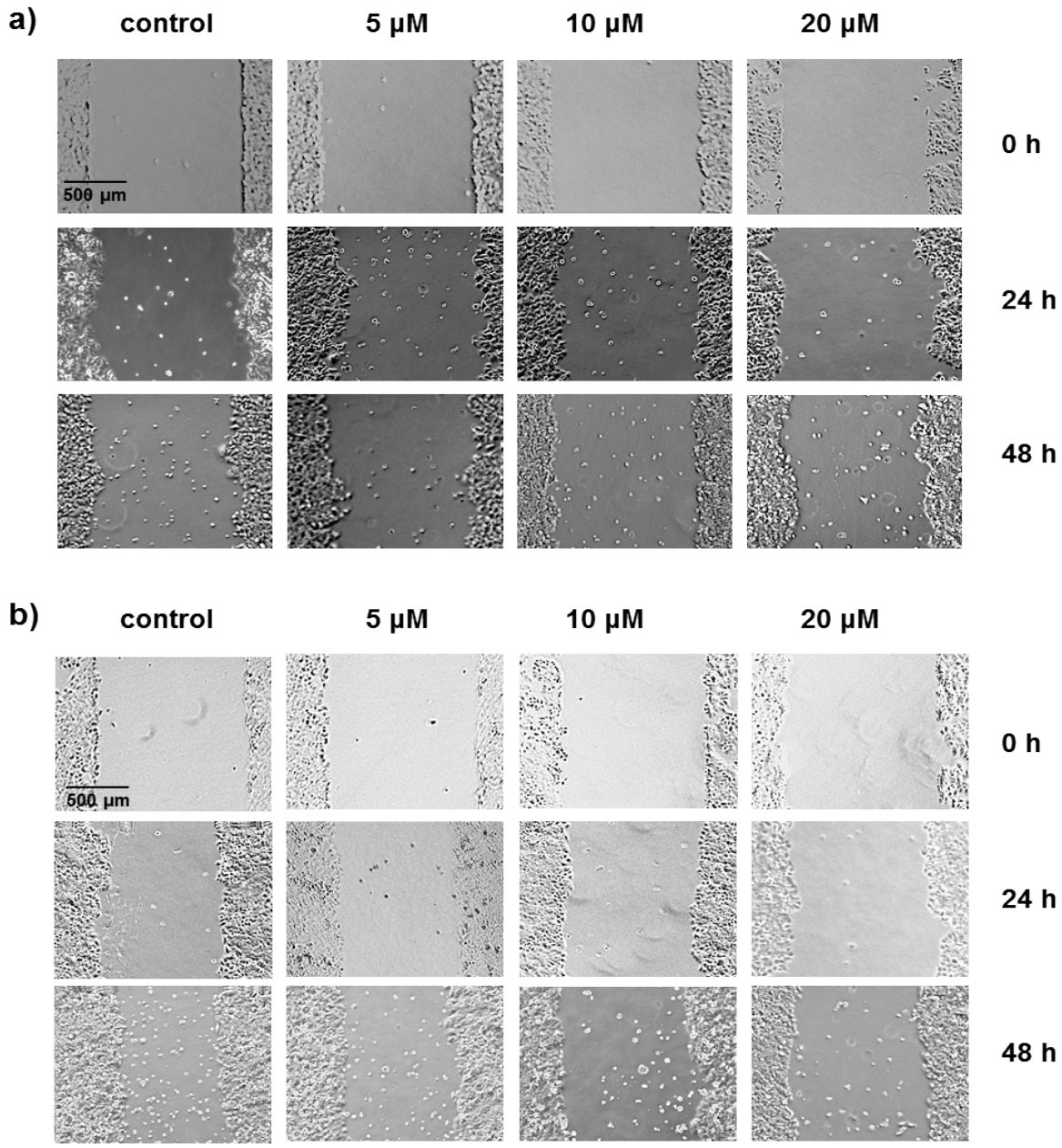

Figure S7: Images of wound-healing assay: a) cells treated with an ANXA2-targeting plasmid; b) cells treated with a control plasmid. Cells were treated with different concentrations of matrine (as marked on top of the images) after plasmid transfection. Images were taken at 0 h, 24 h, and 48 h after scratch.

11. Plasminogen and Plasmin Measurement.

Hep3B cells were treated with different concentrations of matrine for 24 hours, and then cell culture supernatants were collected and spun at 2000 × g for 10 min at 4 °C. The levels of plasminogen and the total concentrations of plasminogen and plasmin were measured with an ELISA kit (Abcam, USA) following the manufacturer's protocol.

12. Statistical analysis.

Data are shown as the mean ± s.e. for each group. Statistical significance was determined by Student's t-test and was set at $P < 0.05$.

References:

1. Su, Y.; Pan, S.; Li, Z.; Li, L.; Wu, X.; Hao, P.; Sze, S. K.; Yao, S. Q., Multiplex imaging and cellular target identification of kinase inhibitors via an affinity-based proteome profiling approach. *Sci. Rep.* **2015,** *5*, 7724.
2. Shi, H.; Cheng, X.; Sze, S. K.; Yao, S. Q., Proteome profiling reveals potential cellular targets of staurosporine using a clickable cell-permeable probe. *Chem. Comm.* **2011,** *47*, 11306.
3. P. Wang, Z.-w. Tao, X.-h. Zheng, Y. Zhang and L.-N. Ma, *Huaxue Shiji*, 2012, **34**, 208-210.