Supplementary Materials

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Materials and Methods

Cell culture: All cell lines were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). The HeLa cells were propagated in MEM supplemented with 10% fetal bovine serum (FBS) and antibiotics in a fully humidified incubator containing 5% CO_2 at 37°C. The Ect1/E6E7 cells were propagated in Keratinocyte-Serum Free medium supplemented with 0.1 ng/ml human recombinant EGF, 0.05 mg/ml bovine pituitary extract, and additional calcium chloride 44.1 mg/L in a fully humidified incubator containing 5% CO_2 at 37°C. The MES-SA cells were propagated in McCoy's 5A supplemented with 10% fetal bovine serum (FBS) and antibiotics in a fully humidified incubator containing 5% CO_2 at 37°C. The MES-SA/dx5 cells were propagated in McCoy's 5A supplemented with 10% fetal bovine serum (FBS), antibiotics and 10 nM taxol in a fully humidified incubator containing 5% CO_2 at 37°C.

Pericellular hydrogelation: Dissolve D-**1** at 8.09 mg/mL in ddH_2O to get stock solution of 11.2 mM, adjust pH to 7.4 by 1N NaOH. 2×10^5 of the cells in exponential growth phase were seeded in 35mm Petri dish with 1 mL complete culture medium. After overnight incubation, the medium was replaced by 1 mL of medium containing D-**1** (diluted from the stock solution immediately before use). After incubation at 37°C for 2 h, the Petri dishes were taken out from incubator and tilted on bench to visualize the pericellular hydrogel. In the co-incubation of phosphatase inhibitors, the cells were first washed with medium containing Pierce phosphatases inhibitor cocktail (Thermo) (contains sodium fluoride, sodium orthovanadate, sodium pyrophosphate and beta-glycerophosphate) for 3 time. Then 1 mL of medium containing both the inhibitor cocktail and D-**1** was applied to cells.

Conditioned medium: 2×10^5 of cells in exponential growth phase were seeded in 35mm Petri dish with 1 mL complete culture medium. After overnight incubation, 1 mL of new medium was replaced and incubated for 24h at 5% CO_2, 37°C. The medium is removed and centrifuged at 10,000 rpm for 5 min. The suspension were placed into a 1.5mL eppendorf tube and stocked at -20°C in aliquot. The stock conditioned medium was thawed and warm to 37°C in water bath immediately before use.

TEM: Carbon coated copper grids were glow-discharged to increase their hydrophilicity before use. After obtain pericellular hydrogel/nanofibrils by incubating D-**1** at 280 μM with HeLa cells for 2 h, the medium was removed to expose the pericellular hydrogel. The carbon coated side of the grid was gently pressed onto the pericellular hydrogel for 1s, and then the sample-loaded surface was washed by touching a drop of ddH_2O. The edge of the grid was immediately leaned to touch a filter paper for three times to remove water from the grid. The grid was stained by letting the grid touch a drop of 2.0 % (w/v) uranyl acetate with the sample-loaded surface. Excessive stain solution was removed by gently touching the grid with a drop of ddH_2O then dried by touching the edge of the grid with a filter paper for 3 times. The grid was air dried for a few minutes and was then examined immediately.

SEM: Cells in exponential growth phase were seeded in glass bottomed 35 mm Petri dish at 2×10^4 cell/well. The cells were allowed for attachment for 24 h at 37 ºC, 5% CO_2. The culture medium was removed, and new culture medium containing D-**1** was added. After 12 h of incubation, cells were washed with PBS buffer for 3 times and the solution was removed by touch a filter paper to the edge of the dish. Holding the edge of the plastic dish by a long tweezers, the dish was immersed into liquid N_2 for 5s (the glass crack during the freezing) and quickly transferred into a freeze dryer. After drying overnight, the dish was removed from the freeze dryer. The glass bottom was carefully detached from the dish by tweezers, and placed in a sputter coater for coating of gold (2 nm thick). The coated glass was imaged immediately.

Confocal microscopy: Cells in exponential growth phase were seeded in glass bottomed culture chamber at 2×10^4 cell/well. The cells were allowed for attachment for 24 h at 37 ºC, 5% CO_2. The culture medium was removed, and new culture medium containing D-**1** at 280 μM was added. After 12 h of incubation, cells were washed with PBS buffer for 3 times and stained with 0.1 mg / mL Congo red and 0.6 μM DAPI in PBS buffer for 30 min at 37 ºC in dark. The cells were rinsed three times in PBS, and then kept in the PBS buffer for imaging.

Cell migration assay: Cell migration was evaluated by the wound healing assay using the CytoSelect Wound Healing Assay Kit (Cell Biolabs). Wound healing inserts with width at 0.9 mm were put into 24-well plates. 0.8 mL of HeLa cells at 2×10^5 cell/mL were added to either side of the insert and incubated overnight to form a confluent layer. Then the inserts were carefully removed to expose the gap. After removing the culture medium, new medium containing the molecules were added to cells. Then the cells were incubated at 37 ºC, 5% CO_2 for 18 h. Images of gap before and after addition of the molecules were captured using a microscope at a magnification of ×40. Cell migration was quantified by measuring the change of gap distance.

Cell adhesion assay: Cells in exponential growth phase were detached by 0.25% trypsin –EDTA. After neutralizing trypsin by addition of complete culture medium, the cells were collected by centrifuge (1000 rpm, 3 min). The cell pellet was suspended by addition of culture medium to a concentration of 4×10^5 cell/mL. 1 mL of the cell suspension was mixed with 1 mL of 2X D-**1** or D-**2** containing culture media. The resulting solutions were added into 96 well plate at 100 μL/well. After desired time of incubation at 37 ºC, 5% CO_2, the solution was gently removed, 100 μL of culture medium and 10 μL of 5 mg/mL MTT were added to each well. After incubate at dark for 4 h, 100 μL of 10% SDS with 0.01M HCl was added to each well to stop the reduction reaction and to dissolve the purple. After incubation of the cells at 37 ºC for overnight, the viability is measured. Data represent the mean ± standard deviation of three independent experiments.

Phosphatase activity assay: Phosphatase activity was evaluated by the alkaline phosphatase assay using the alkaline phosphatase assay kit (Abcam) following the supplied protocol. In brief, 50 μL of 5 mM *p*NPP solution in assay buffer was mixed with 80 μL of the sample solutions in 96 well plate. The mixed solution was incubated at 25 ºC, protect from light. 20 μL of stop solution was added to each test and the OD at 405 nm of the solution was measured in a microplate reader.

Cell viability assay: Cells in exponential growth phase were seeded in a 96 well plate at a concentration of 2×10^4 cell/well. The cells were allowed to attach to the wells for 24 h at 37 ºC, 5% CO_2. The culture medium was removed and 100 μL culture medium containing compounds (immediately diluted from fresh prepared stock solution of 10 mM) at gradient concentrations (0 μM as the control) was placed into each well. After culturing at 37 ºC, 5% CO_2 for 48 h, each well was added by 10 μL of 5 mg/mL MTT ((3-(4, 5-DimethylthiazoL-2-yl)-2, 5-diphenyltetrazolium bromide), and the plated cells were incubate at dark for 4 h. 100 μL 10% SDS with 0.01M HCl was added to each well to stop the reduction reaction and to dissolve the purple. After incubation of the cells at 37 ºC for overnight, the OD at 595 nm of the solution was measured in a microplate reader. Data represent the mean ± standard deviation of three independent experiments.

Apoptosis pathway assay: The apoptosis pathway was evaluated by the PathScan® apoptosis multi-target sandwich ELISA kit (Cellsignal) following the supplied protocol. Cells in exponential growth phase were seeded in a 6 cm Petri dish at a concentration of 10×10^5 cell/dish (5 dishes). The cells were allowed to attach to the wells for 24 h at 37 ºC, 5% CO_2. The culture medium was removed and 5mL medium containing D-**1** (immediately diluted from fresh prepared stock solution of 11.2 mM) at concentration of 280 μM was placed into each dish. At 0, 6, 12, 24, 32 h, remove the medium and wash the cells by cold PBS buffer for 3 times. 0.9 mL lysis buffer was used to lyse cells in each dish, and 0.9

mL of diluents buffer was used to dilute each of the cell lysate. The lysate was incubated in the provided 96 well plate at 4 ºC for 24 h.

Table S1. Conversion and degradation of D-**1** and L-**1**. Composition of the pericellular hydrogel/nanofibrils and the suspension medium of HeLa cells after incubation with D-**1** or L-**1** at 560 μM at 37 ˚C for 12 h.

Precursor	**Pericellular hydrogel** (μM)	**Suspension medium** (μM)	
D-**1**	D-**2** 2053.9	D-**2** 502.5	
L-**1**	[a]N/A	L-**2** 176.6	L-**3** 270.4

[a]N/A: no pericellular hydrogel/nanofibrils detected.

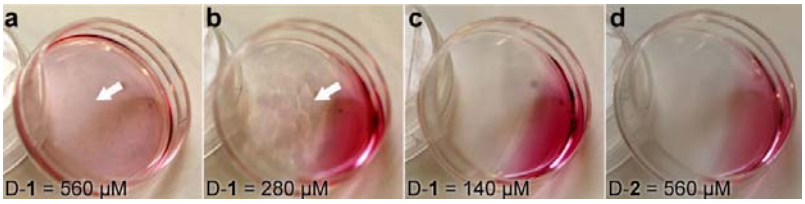


Figure S1. Formation of the hydrogel of D-**1** in HeLa cell conditioned medium. Optical images of D-**1** at a) 560 µM of, b) 280 µM, c) 140 µM, or d) D-**2** at 560 µM incubated the conditioned medium of HeLa cells for 48 h.

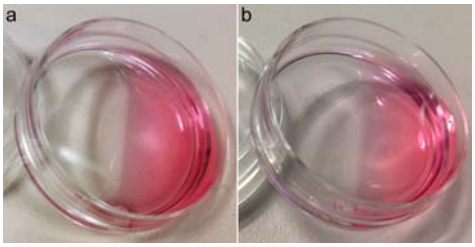


Figure S2. High gradient of phosphatases in pericellular space is essential for the formation of pericellular hydrogel. a) Optical images of HeLa cells with 560 µM of D-**1** and phosphatases inhibitors (Pierce™: sodium fluoride, sodium orthovanadate, sodium pyrophosphate and beta-glycerophosphate)) for 2h. b) Optical images of HeLa cells with 560 µM of D-**1** and ALP (0.1 U/mL) for 2 h.

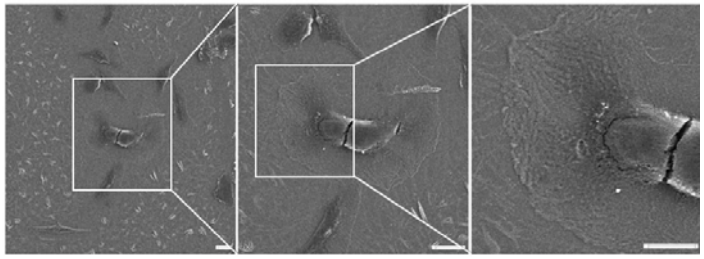


Figure S3. SEM images of freeze dried HeLa cells treated with D-**1** at 280 µM for 2h. Scale bar = 10 mm.

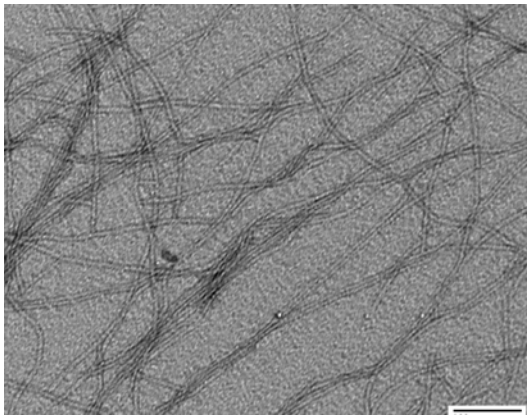


Figure S4. TEM image of hydrogel formed by ALP induced enzymatic gelation of D-**1** in PBS buffer. The hydrogel was formed by incubating 0.6 U/mL of ALP with D-**1** at 0.2 wt% for 6 h. Scale bar = 100nm.

Figure S5. Optical image of Ect1/E6E7 cells incubated with D-**1** at 560 μM for 48h.

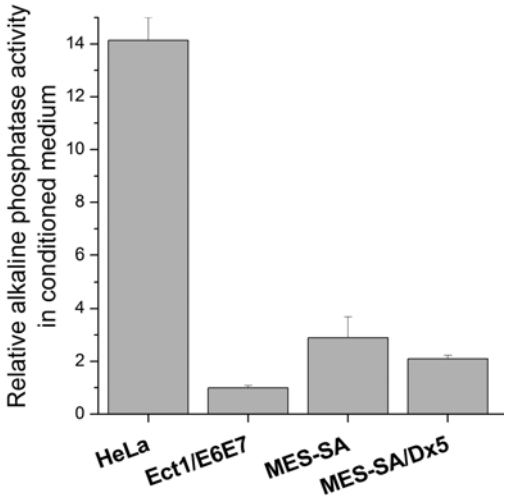


Figure S6. Relative activities of phosphatases in the conditioned media of HeLa, Ect1/E6E7, MES-SA and MES-SA/Dx5 cells.

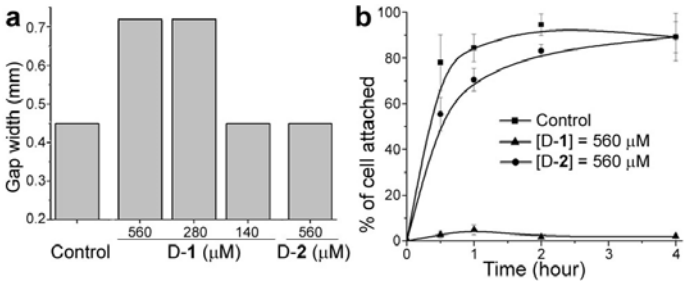


Figure S7. Inhibitory effect of D-**1** to HeLa cells. a) Migration of the HeLa cells after incubation with D-**1** or D-**2**, or just the culture medium as the control for 18 h. Original gap with width = 0.90 mm. b) The plot of time versus the percentage of cell attached to Petri dish after suspended HeLa cells being incubated with 560 μM of D-**1** or D-**2**, or just culture medium as control.

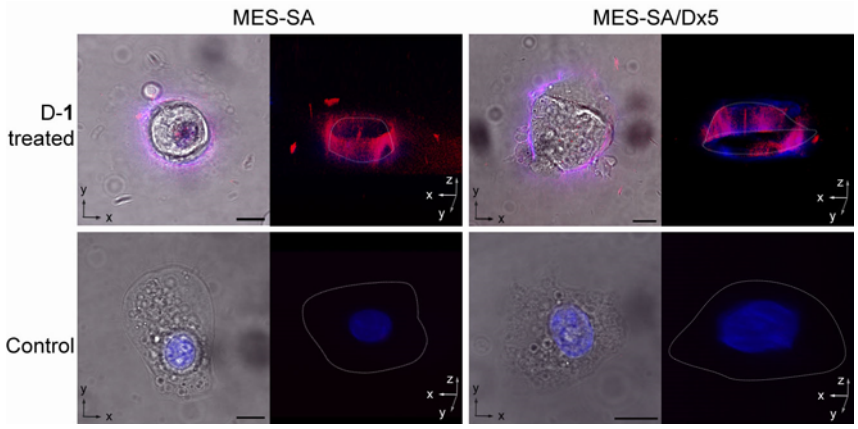


Figure S8. Overlaid images and 3D stacked z-scan images of Congo red and DAPI stained MES-SA and MES-SA/Dx5 treated by D-**1** at 280 μM or just medium as control for 12 h. Scale bar = 10 μm. White dots outline the cells.

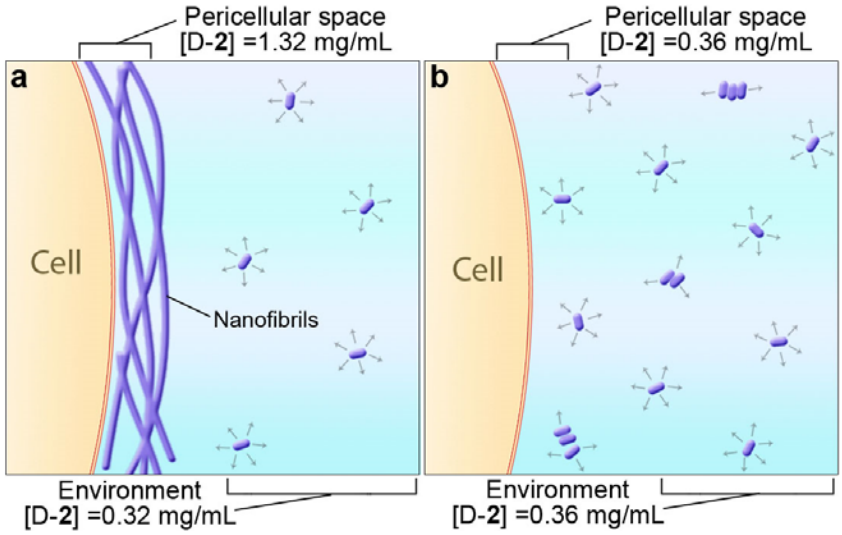


Figure S9. Distribution of D-**2** around the cells. a) Distribution of D-**2** around the HeLa cells due to pericellular dephosphorylation of D-**1**. b) Distribution of D-**2** around the HeLa cells via direct addition of D-**2**. The gray arrows indicate the direction of motion of the D-**2** molecules.

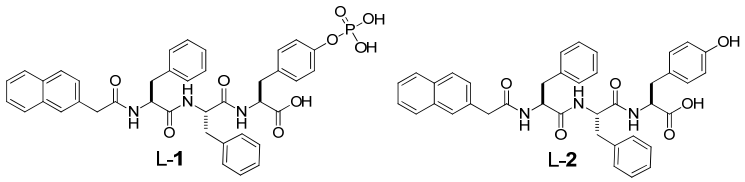


Figure S10. Chemical structures of L-**1** and L-**2**, the enantiomers of D-**1** and D-**2**.

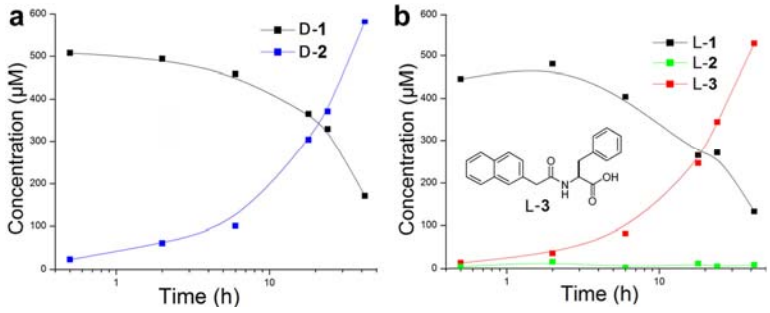


Figure S11. Formation of pericellular hydrogel/nanofibrils is dependent to the bio-stability of the molecules. a) Time dependent conversion curve of D-**1** by complete culture medium containing secretory enzymes from HeLa cells. b) Time dependent degradation curve of L-**1** by complete culture medium containing secretory enzymes from HeLa cells.

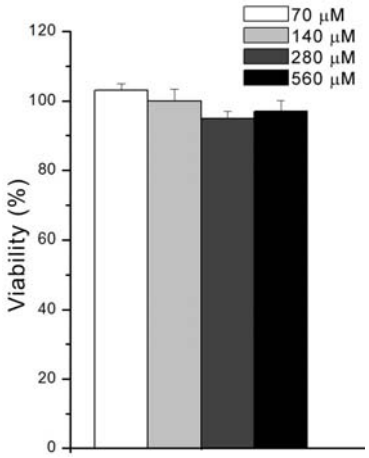
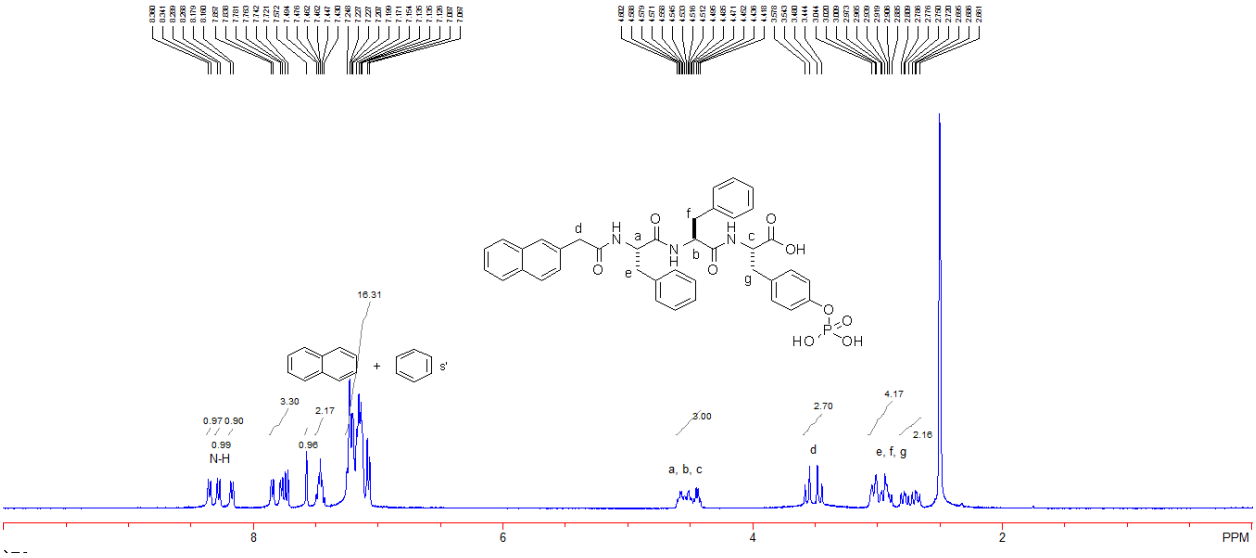
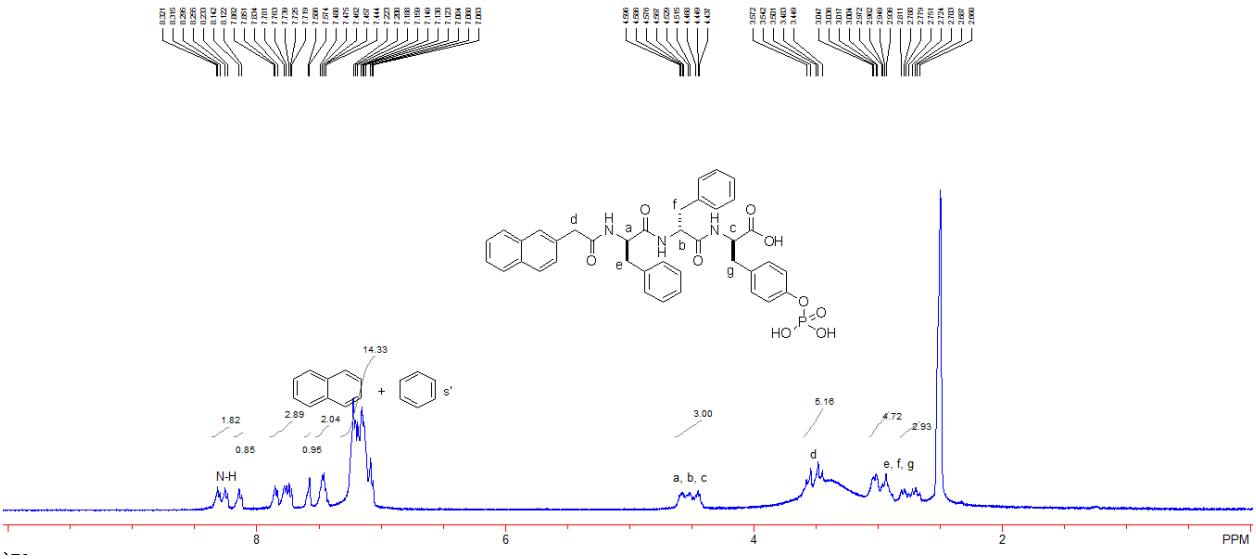


Figure S12. 48 h cytotoxicity of HeLa cells with removal of pericellular hydrogel/nanonets at 4 h. After incubation with D-**1** at the indicated concentrations for 4 h, the medium were removed, and the cells were washed with PBS for 3 time. Then the cells were incubated with fresh medium for 48 h.



^{1}H NMR of L-**1**



^{1}H NMR of D-**1**

Figure S13. 1H NMR of L-**1** and D-**1**.