Supporting Information

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Intranuclear Nanoribbons for Selective Killing of Osteosarcoma Cells

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Abstract: Here we show intranuclear nanoribbons formed upon dephosphorylation of leucine-rich L- or D-phosphopentapeptide catalyzed by alkaline phosphatase (ALP) to selectively kill osteosarcoma cells. Being dephosphorylated by ALP, the peptides firstly transformed into micelles and then convert into nanoribbons. The peptides/assemblies firstly aggregate on cell membrane, then enter cells via endocytosis, and finally accumulate in nuclei (mainly in nucleoli). Proteomics analysis suggests that the assemblies interact with histone proteins. The peptides kill osteosarcoma cells rapidly, and are nontoxic to normal cells. Moreover, the repeated stimulation of the osteosarcoma cells by the peptides sensitizes the cancer cells rather than inducing resistance. This work not only illustrates a novel mechanism for nucleus-targeting, but also may lead a new way to selectively kill osteosarcoma cells and overcome drug resistance.

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Experimental Procedures

Materials.
2-Cl-trityl chloride resin (1.0-1.2 mmol/g), HOBt, HBTU, Fmoc-OSu, and other Fmoc-amino acids were purchased from GL Biochem (Shanghai, China). Other chemical reagents and solvents were purchased from Fisher Scientific. Alkaline phosphatase was purchased from Biomatik (Cat. No. A1130, Alkaline Phosphatase [ALP], 30000 U/mL, in 50% Glycerol.), Fetal bovine serum (FBS) and penicillin-streptomycin from Gibco by Life Technologies.

General.
All precursors were purified with Agilent 1100 Series Liquid Chromatograph system, equipped with an XTerra C18 RP column and Variable Wavelength Detector. The LC-MS spectra were obtained with a Waters Acquity Ultra Performance LC with Waters MICROMASS detector, and ^{1}HNMR spectra on Varian Unity Inova 400. Circular dichroism (CD) spectra were obtained with a Jasco J-810 Spectropolarimeter.

Synthesis of compounds.
***Synthesis of NBD-β-Alanine*:** To the solution of β-Alanine (5 mmol, 1 g) and K_2CO_3 (15 mmol, 2 g) in H_2O (15 mL), the solution of NBD-Cl in MeOH (30 mL) was added dropwise under the protection of N_2. After reaction at room temperature for 3 h, the MeOH was removed by evaporation. After adding 70 mL H_2O, the pH was the solution was adjusted by 1 M HCl to ~ 3. The solution was extracted by diethyl ether (200 mL × 3), and the organic part was dried by Na_2SO_4, filtered and concentrated by evaporation. ^{1}H NMR of NBD-β-Alanine (400 MHz, CD_3OD-d_4) δ (ppm): 8.55 (m, 1H), 6.40 (d, 1H), 3.82 (s, 2H), 2.79 (t, 2H) (Figure S1). MS of NBD-β-Alanine: calc. $[M-H]^-$ = 251.04, obsvd. ESI-MS: M/Z = 250.95 (Figure S2).
***Synthesis of Fmoc-$_L$-Tyr(PO_3H_2)-OH and Fmoc-$_D$-Tyr(PO_3H_2)-OH*:** The mixture of P_2O_5 (35 mmol, 10 g), H_3PO_4 (133 mmol, 13 g) and H-$_L$-Tyr-OH (18 mmol, 3.22 g) was stirred for 24 h at 80ºC in N_2 atmosphere. After adding 30 mL H_2O and stirred for 30 min at 80ºC, the reaction mixture was cool to room temperature. The reaction mixture was added to butanol (650 mL) dropwise and recrystallized at 4ºC overnight, filtration provided H-$_L$-Tyr(PO_3H_2)-OH as white power. To the solution of H-$_L$-Tyr(PO_3H_2)-OH (2 mmol, 522 mg) in H_2O (20 mL), the solution of Fmoc-OSu (2.4 mmol, 808 mg) in MeCN (20 mL) was added. After adjusting pH to ~8 by triethylamine (TEA), the solution was stirred at room temperature for 2 h. After removal of MeCN by evaporation, 60 mL H_2O was added and the pH of the solution was adjusted to ~3 by 1 M HCl. After extraction by ethyl acetate (100 mL × 3), the organic part was washed by 1 M HCl (100 mL × 2) and brine (100 mL × 1). After being dried by Na_2SO_4, filtered and concentrated by evaporation, Fmoc-$_L$-Tyr(PO_3H_2)-OH was provided as white powder. By the same method, Fmoc-$_D$-Tyr(PO_3H_2)-OH was provided as white powder.
Synthesis of 1Lp, 1L, 1Dp, 1D, and 1DLp: **1Lp**, **1L**, **1Dp**, **1D** and **1DLp** were synthesized by solid phase peptide synthesis with 2-Cl-trityl chloride resin, Fmoc-$_L$-Tyr(PO_3H_2)-OH, Fmoc-$_D$-Tyr(PO_3H_2)-OH, Fmoc-$_L$-Tyr(tBu)-OH, Fmoc-$_D$-Tyr(tBu)-OH, Fmoc-$_L$-Leu-OH, Fmoc-$_D$-Leu-OH, NBD-β-Alanine, HOBt and HBTU. Purification with HPLC provided **1Lp**, **1L**, **1Dp**, **1D** and **1DLp** as yellow power. ^{1}H NMR of **1Lp** (400 MHz, DMSO-d_6) δ (ppm): 7.77 (d, 1H, J = 8 Hz), 7.14 (d, 2H, J = 8 Hz), 7.04 (d, 2H, J = 8 Hz), 6.45 (d, 1H, J = 8 Hz), 4.36 (m, 1H), 4.28 (m, 4H), 2.98 (m, 2H), 2.86 (m, 2H), 2.61 (m, 2H), 1.54 (m, 4H), 1.41 (m, 8H), 0.79 (m, 24H) (Figure S3). MS of **1Lp**: calc. $[M-H]^-$ = 946.41, obsvd. ESI-MS: M/Z = 946.63 (Figure S4). ^{1}H NMR of **1L** (400 MHz, DMSO-d_6) δ (ppm): 7.76 (d, 1H, J = 8 Hz), 6.97 (d, 2H, J = 8 Hz), 6.63 (d, 2H, J = 8 Hz), 6.45 (d, 1H, J = 8 Hz), 4.30 (m, 5H), 3.15 (m, 2H), 2.90 (m, 1H), 2.79 (m, 1H), 2.60 (m, 2H), 1.55 (m, 4H), 1.41 (m, 8H), 0.80 (m, 24H) (Figure S5). MS of **1L**: calc. $[M-H]^-$ = 866.44, obsvd. ESI-MS: M/Z = 866.52 (Figure S6). ^{1}H NMR of **1Dp** (400 MHz, DMSO-d_6) δ (ppm): 7.76 (d, 1H, J = 8 Hz), 7.13 (d, 2H, J = 8 Hz), 7.04 (d, 2H, J = 8 Hz), 6.45 (d, 1H, J = 8 Hz), 4.36 (m, 1H), 4.28 (m, 4H), 2.98 (m, 2H), 2.86 (m, 2H), 2.62 (m, 2H), 1.54 (m, 4H), 1.41 (m, 8H), 0.79 (m, 24H) (Figure S7). MS of **1Dp**: calc. $[M-H]^-$ = 946.41, obsvd. ESI-MS: M/Z = 946.57 (Figure S8). ^{1}H NMR of **1D** (400 MHz, DMSO-d_6) δ (ppm): 7.76 (d, 1H, J = 8 Hz), 6.97 (d, 2H, J = 8 Hz), 6.63 (d, 2H, J = 8 Hz), 6.45 (d, 1H, J = 8 Hz), 4.30 (m, 5H), 3.15 (m, 2H), 2.90 (m, 1H), 2.79 (m, 1H), 2.61 (m, 2H), 1.55 (m, 4H), 1.42 (m, 8H), 0.80 (m, 24H) (Figure S9). MS of **1D**: calc. $[M-H]^-$ = 866.44, obsvd. ESI-MS: M/Z = 866.59 (Figure S10). MS of **1DLp**: calc. $[M-H]^-$ = 946.41, obsvd. ESI-MS: M/Z = 947.75 (Figure S11).
Synthesis of 2Lp and 2Dp: Compounds **A** and **B** were synthesized by solid phase peptide synthesis with 2-Cl-trityl chloride resin, Fmoc-$_L$-Tyr(PO_3H_2)-OH, Fmoc-$_D$-Tyr(PO_3H_2)-OH, Fmoc-$_L$-Lys(Boc)-OH, Fmoc-$_D$-Lys(Boc)-OH, Fmoc-$_L$-Leu-OH, Fmoc-$_D$-Leu-OH, NBD-β-Alanine, HOBt and HBTU. Precipitation in ether provided **A** and **B** as yellow solid. **A** (0.5 mmol, 574 mg) was dissolved in trifluoroacetic acid (TFA, 5 mL) and stirred at room temperature for 1 h. After removed the TFA and dried in vacuum, the product was dissolved in 5 mL dry DMF. Biotin (0.6 mmol, 146 mg), HBTU (0.72 mmol, 273 mg) and DIEA (0.5 mL) were stirred in 2 mL dry DMF for 10 min, and then the mixture was added into the prior solution dropwise. After reaction at room temperature overnight, purification with HPLC provided **2Lp** as yellow power. By the same method, **2Dp** was provided as yellow power. MS of **2Lp**: calc. M = 1301.59, obsvd. ESI-MS: M/Z = 1301.86 (Figure S12). MS of **2Dp**: calc. M = 1301.59, obsvd. ESI-MS: M/Z = 1301.95 (Figure S13).

TEM sample preparation.
After placing 5 μL samples on 400 mesh copper grids coated with continuous thick carbon film (~35 nm) which was glowed discharged, we washed the grid with ddH_2O and UA (uranyl acetate). The sample loaded grid was stained with the UA. The residual UA was removed by filter paper and then dried in air. TEM images were obtained with FEI Morgagni 268 80 kV with a 1 k × 1 k AMT CCD camera.

Critical micelle concentration (CMC) measurement.

The CMCs were determined using pyrene as the fluorescent probe. Different concentrations of compounds were prepared in pyrene-saturate solutions. The fluorescence spectra of pyrene solutions with different concentration compounds were obtained. The intensity ratio of 378 nm/393 nm (I_{378}/I_{393}) was determined by a BioTek Synergy H1 Microplate Reader. Plot I_{378}/I_{393} against the concentrations of compounds. The concentration at the turning point is the CMC.

Cell culture.

SJSA-1 cell line, Saos2 cell line, HS-5 cell line, HEK293 cell line, HeLa cell line, HepG2 cell line, VCaP cell line and PC3 cell line, were purchased from American Type Culture Collection (ATCC, USA). A2780 cell line and A2780cis cell line were purchased from Sigma. RFP expressing SJSA-1 cell line was purchased from Angio-proteomie (cAP-0073RFP). The RFP expressing SJSA-1 cells were selected from the SJSA-1 cells transfected with RFP expressing lentiviruses resistant to Zeocin. OVSAHO cell line was given by Dr. Daniela Dinulescu group. SJSA-1 cells, RFP expressing SJSA-1 cells, OVSAHO cells, A2780 cells, and A2780cis cells were cultured in RPMI-1640 Medium supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin and 100 μg/mL streptomycin. Saos2 cells were cultured in McCoy's 5A Medium supplemented with 15% fetal bovine serum (FBS), 100 U/mL penicillin and 100 μg/mL streptomycin. HeLa cells, HepG2, and HEK293 cells were cultured in Eagle's Minimum Essential Medium (MEM) supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin and 100 μg/mL streptomycin. HS-5 cells, and VCaP cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin and 100 μg/mL streptomycin. All cells were maintained at 37°C in a humidified atmosphere of 5% CO_2.

Cell viability.

We determined the cytotoxicity against cells by using MTT assay. Cells were seeded in 96-well plates at 1×10^5 cells/well for 24 hours followed by culture medium removal and subsequently addition of culture medium containing treating reagent. After 1/2/3 hours, the culture medium with treating reagent was replaced by fresh culture medium and 10 μL MTT solution (5 mg/mL) was added to each well and incubated at 37°C for another 4 h. Then 100 μL of SDS-HCl solution was added to stop the reduction reaction and dissolve the formazan. The absorbance of each well at 595 nm was measured by a Beckman Coulter DTX880 Multimode Detector. The results were calculated as cell viability percentage relative to untreated cells. Data were obtained from three independent wells (n = 3).

Gene Transfection of HEK293 cell line.

The gene transfection in HEK293 cells was done according to a previously reported method[1]. Briefly, alkaline Phosphatase cDNA ORF Clone, Human, C-OFPSpark tag gene (Sino Biological Inc., Cat: HG10440-ACR) was incubated with polyethylenimine (PEI, MW = 25, 000) for 20 min. Then, the mixture was added to HEK293 cells incubated with serum-free culture medium for another overnight incubation. After the incubation, cells were detached from petri dish by trypsin, and then 1/3 of the cells were seeded backed to the petri dish. The cells were incubated in cell culture hood until further analysis.

Actin-RFP and tubulin-RFP transfection.

Invitrogen CellLight Actin-RFP, BacMam 2.0 and Invitrogen CellLight Tubulin-RFP, BacMam 2.0 were used as BacMam 2.0 reagents for actin-RFP and tubulin-RFP transfection. Cells in exponential growth phase were seeded in a confocal dish (3.5 cm) at 1.0×10^4 cells per dish and then incubated in incubator for 24 h. We removed culture medium, and added fresh medium containing 4 μM BacMam 2.0 reagent. The cells were incubated for 16 h at 37°C for further use.

Uptake test.

SJSA-1 Cells were seeded in 96-well plates at 3×10^5 cells/well for 6 hours followed by culture medium removal and subsequently addition of culture medium containing different concentration of **1Lp** or **1Dp** (immediately diluted from fresh prepared 10 mM stock solution) with or without endocytosis inhibitors. After 2 hours, washed the cells by HBS, added 100 μL DMSO, and then incubated the cells at 37°C for another 15 min. The fluorescence intensity of each well at 552 nm (λ_{ex} = 488 nm) was measured by a BioTek Synergy H1 Microplate Reader. The results were calculated as cell viability percentage relative to untreated cells. Data were obtained from three independent wells (n = 3).

Fractionation.

3 million SJSA-1 cells were seeded in 10 mL petri dishes, incubated 24 h for attachment, and then were treated with **1Lp** or **1Dp** (200 μM, 4 h). Following the treatment, cells were washed twice with PBS, harvested by trypsin treatment, and resuspended in PBS. After the cell number was counted, the cell suspension was centrifuged at 300g for 5 min, and the obtained pellet was resuspended in 10 ml of distilled water for 30 min to lyse the cells. Clumps of unbroken and ruptured cells were removed by centrifugation at 300 g for 5 min. Centrifugation at 600 g for 10 min provides **Sample N** and centrifugation at 15000 g for 5 min provides **Sample M**. The samples were dissolved in DMSO and the fluorescence intensity was measured at 552 nm (λ_{ex} = 488 nm) by a BioTek Synergy H1 Microplate Reader. Data were obtained from three independent wells (n = 3).

Confocal laser scanning microscopy (CLSM) imaging.

For live cell imaging, cells in exponential growth phase were seeded in a confocal dish (3.5 cm) at 1.0×10^5 cells per dish and then incubated in incubator for 24 h. We removed culture medium, and added fresh medium containing precursors for different time points. After removing the medium and washing the cells with live cell imaging solution (2 mL × 2), the cells were used for CLSM imaging.

For time-dependent live cell 2D imaging, cells in exponential growth phase were seeded in a confocal dish (3.5 cm) at 1.0×10^5 cells per dish and then incubated in incubator for 24 h. After removing culture medium, we treated the cells with 1 mL of Hoechst 33342 (1

μg/mL) for 10 minutes. After being washed with culture medium (2 mL × 2), the cells were incubated with fresh medium containing precursor in a Tokai Hit stage top incubator (STXF-WSKMX-SET) to be used for CLSM imaging.
For time-dependent live cell 3D imaging, cells in exponential growth phase were seeded in a confocal dish (3.5 cm) at 1.0×10^5 cells per dish and then incubated in incubator for 24 h. After removing culture medium, we treated the cells with 1 mL of Hoechst 33342 (1 μg/mL) for 10 minutes. After being washed with culture medium (2 mL × 2), the cells were used for CLSM imaging.
All the 2D CLSM images were obtained using Zeiss LSM 880 confocal microscopy at the lens of 63× with oil. All the 3D CLSM images were obtained using Zeiss LSM 880 confocal microscopy with airyscan at the lens of 63× with oil. The lasers used are 405 nm, 488 nm and 561 nm.

Proteomics.
1. Lysis and treatment: 13.5 million SJSA-1 cells were treated by 4 mL 400 μM **2Lp** for 2 h. After removing the treating solution, the cells were digested by trypsin and collected by centrifuge (2000 rpm, 3min). After washing by PBS (2 ×, 2000 rpm, 3 min), the cells were dispersed in 1 mL H_2O (1% protease inhibitor cocktail (Millipore Sigma Protease Inhibitor Cocktail Set III, EDTA-Free)) and lysed by freeze-thaw cycle (4 ×). The lysate sample was named as **Sample C**. 13.5 million SJSA-1 cells were digested by trypsin and collected by centrifuge (1000 rpm, 3 min), and then washed by PBS (2 ×, 1500 rpm, 3 min). The cells were dispersed in 960 μL H_2O (1% protease inhibitor cocktail) and lysed by freeze-thaw cycle (4 ×). Added 40 μL 10 mM **2Lp** (final concentration is 400 μM) into the lysate and incubated at 37°C for 2 h. The sample was named as **Sample L**.
2. Enrichment: The protein samples from the last step were enriched with the RayBio Biotin Magnetic Beads (Catalog #: 801-107). The protocol was described briefly as follow: a. Added 100 μL (0.5 mg) of beads to 1 mL of binding/wash buffer (TBS-0.05% Tween 20 detergent) in each tube to wash particles, and magnetically separated until the supernatant was clear and then removed the supernatant. Repeated to wash again. b. Resuspended the beads in 1350 μL binding buffer and mixed with 150 μL sample from the last step (**Sample C** or **Sample L**) to incubate in 37°C for 30 min. c. Magnetically separated, removed supernatant and washed with binding/wash buffer (0.5 mL × 2) to remove unbound proteins. d. The beads were mixed with 100 μL of elution buffer (8 M guanidine-HCl, pH 1.5) and incubated at room temperature for 10 minutes with occasional gentle mixing, and then collected the solution. Repeated and collected the solution. e. Dialyzed the samples with Spectrum™ Labs Spectra-Por™ dialysis membrane (MWCO = 3.5 kD) in water for 2 h to remove the salt. And the samples were lyophilized.
3. In-gel digestion: The lyophilized samples were dissolved in 1% SDS RIPA buffer (1% protease inhibitor cocktail) and treated in laemmli buffer at 95°C for 5-10 min. The samples were loaded to SDS-PAGE and ran under 90 V, until the samples were resolved about 1 cm in gel. The gels were cut into 1 × 1 to 2 × 2 mm pieces and digested with Thermo Scientific In-Gel Tryptic Digestion Kit. The sample were extracted with C18 using the StageTip protocol. The sample from Sample C is called **PC** (Proteomics Cell) and the sample from Sample L is called **PL** (Proteomics Lysate).
4. LC-MS analysis: The sample were separated by using a nanoElute nanoflow chromatograph with a Bruker 15 nanoLC column and then analyzed by a Bruker timsTOF pro mass spectrometry. The data is analyzed with the PEAKS proteomics software.

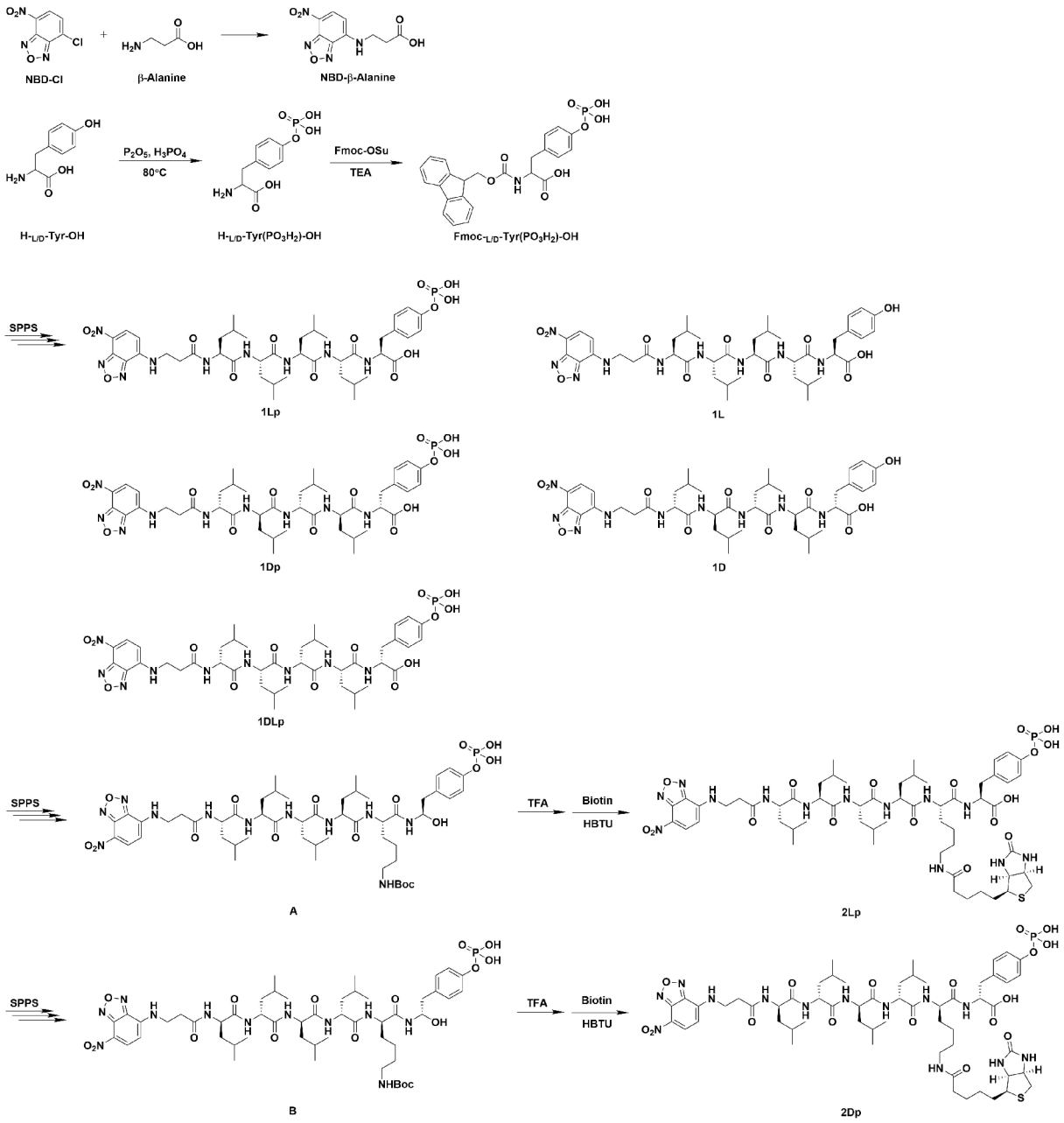
DNA sequestration.
Each test contained four groups: (1) DNA (50 μg/mL) + ALP (0.5 U/mL); (2) DNA (50 μg/mL) + **1Lp**/**1Dp** (200 μM) + ALP (0.5 U/mL); (3) DNA (50 μg/mL) + **1Lp**/**1Dp** (200 μM); (4) **1Lp**/**1Dp** (200 μM) + ALP (0.5 U/mL); (5) DNA (50 μg/mL). Group 5 is the control group, the remain ratio of DNA is 100%. All the samples were incubated at 37°C for 24 h and then centrifuged (10000 rpm, 10 min). The DNA concentrations of the supernatants were measured by using a FluoReporter Blue Fluorometric dsDNA Quantitation Kit (λ_{ex} = 360 nm, λ_{em} = 460 nm) as the concentrations of remain (unaggregated) DNA. The fluorescence intensity was measured by a BioTek Synergy H1 Microplate Reader. Data were obtained from three independent wells (n = 3).

Co-culture.
0.8 million RFP expressing SJSA-1 and 0.8 million HS-5 were co-cultured overnight to adhere. The cell mixtures were treated with **1Lp** or **1Dp** for 4 h. After refreshing the culture media, the cells were cultured overnight and then seeded into CLSM dish. After overnight culture to adhere, the cells were stained with Hoechst, and counted by using CLSM.

Drug resistance test.
SJSA-1 cells were divided into three culture dishes. **Group 1**: The SJSA-1 cells were incubated with **1Lp** by gradually increasing the concentration from 20 μM to 101 μM for 5 weeks. **Group 2**: The SJSA-1 cells were incubated with **1Dp** by gradually increasing the concentration from 20 μM to 69 μM for 5 weeks. **Group 3**: SJSA-1 cells without any treatment of any compounds. We seeded stimulated cells in from **Group 1** and unstimulated SJSA-1 cells (**Group 3**) in 96-well plates at 1×10^5 cells/well for 24 hours, and then we treated the cells with **1Lp** for 24 h. We measured the cell viability by MTT assay. We seeded stimulated cells in from **Group 2** and unstimulated SJSA-1 cells (**Group 3**) in 96-well plates at 1×10^5 cells/well for 24 hours, and then we treated the cells with **1Dp** for 24 h. We measured the cell viability by MTT assay.

Statistical analysis.
Data were presented as means ± standard deviation (s.d.). The sample number (n) indicates the number of independent biological samples in each experiment. All the means, standard deviation and statistical differences were calculated with Microsoft Excel.

Supplementary Figures



Scheme S1. Synthetic route of **1Lp, 1L**, **1-Dp**, **1-D**, **1-DLp**, **2Lp** and **2Dp**.

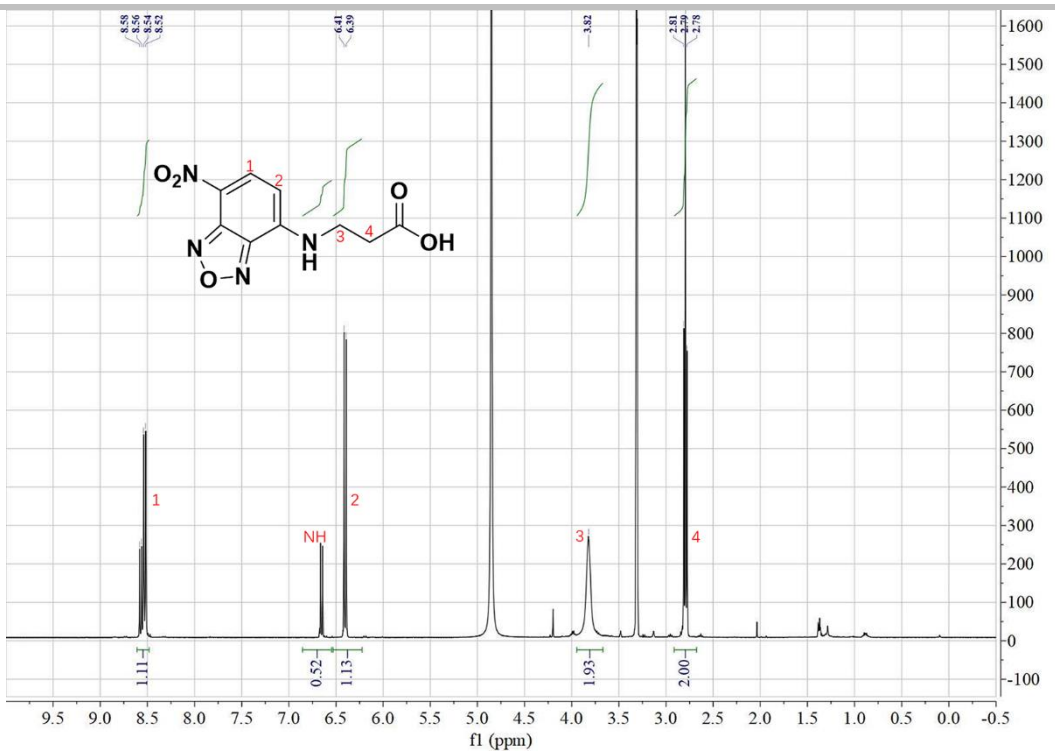


Figure S1. ^{1}H NMR spectrum of NBD-β-Alanine.

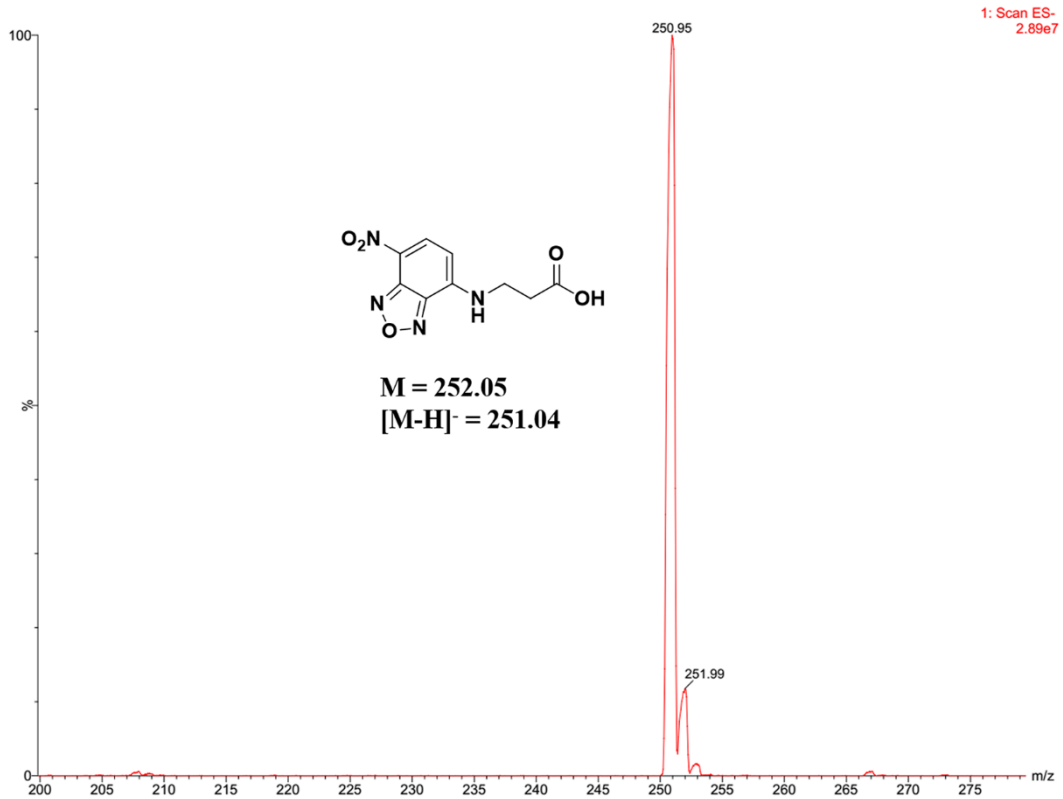


Figure S2. Mass spectrum of NBD-β-Alanine (M/Z = 250.95).

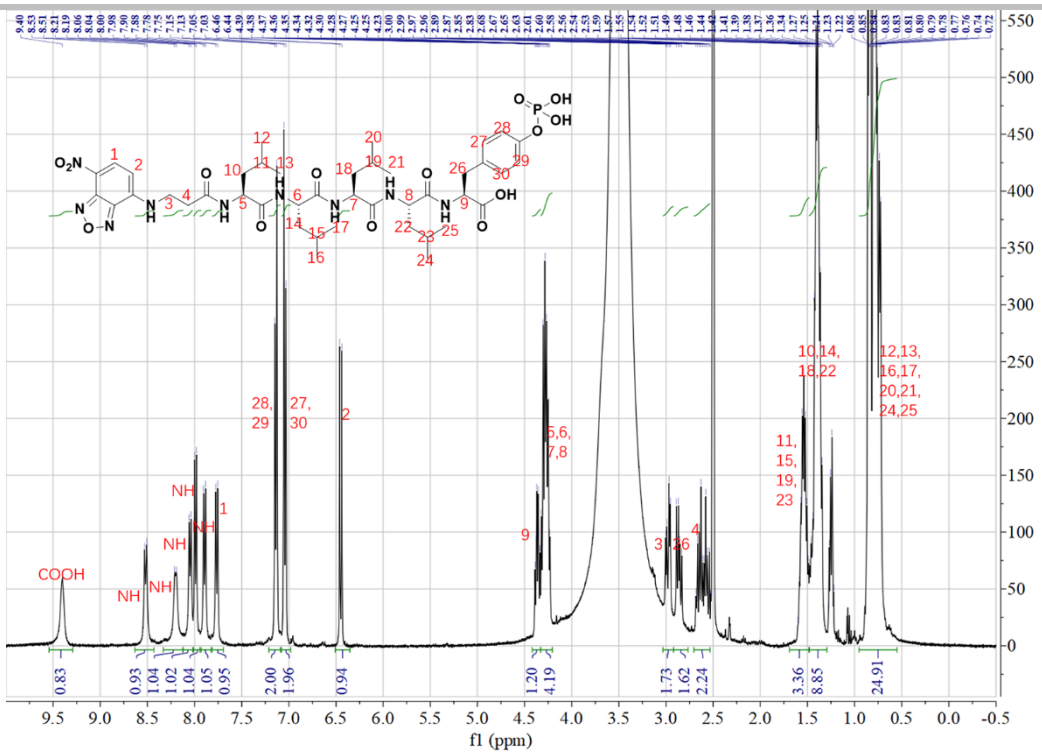


Figure S3. ^{1}H NMR spectrum of **1Lp**.

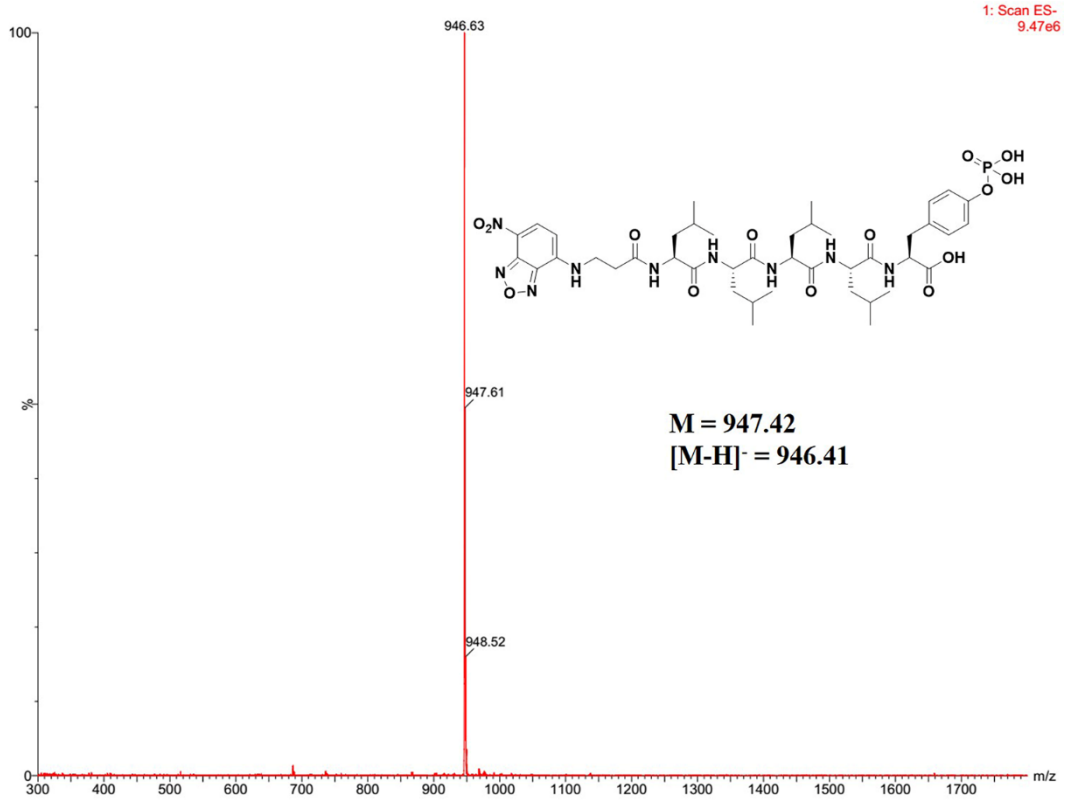


Figure S4. Mass spectrum of **1Lp** (M/Z = 946.63).

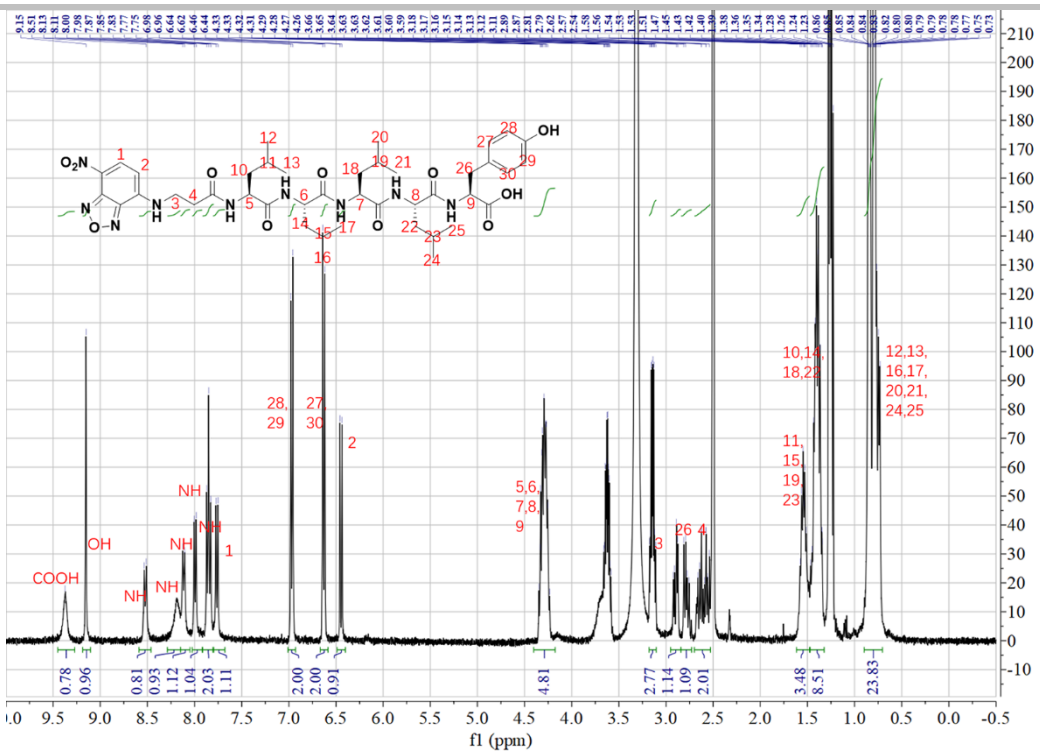


Figure S5. ^{1}H NMR spectrum of **1L**.

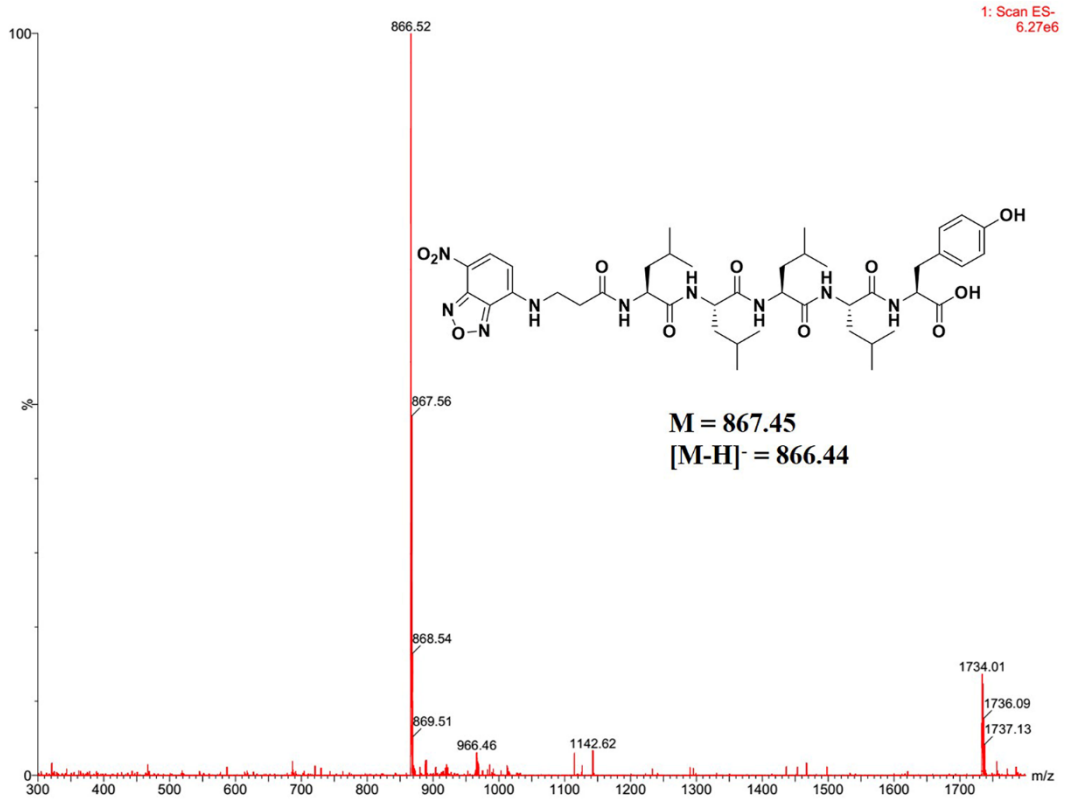


Figure S6. Mass spectrum of **1L** (M/Z = 866.52).

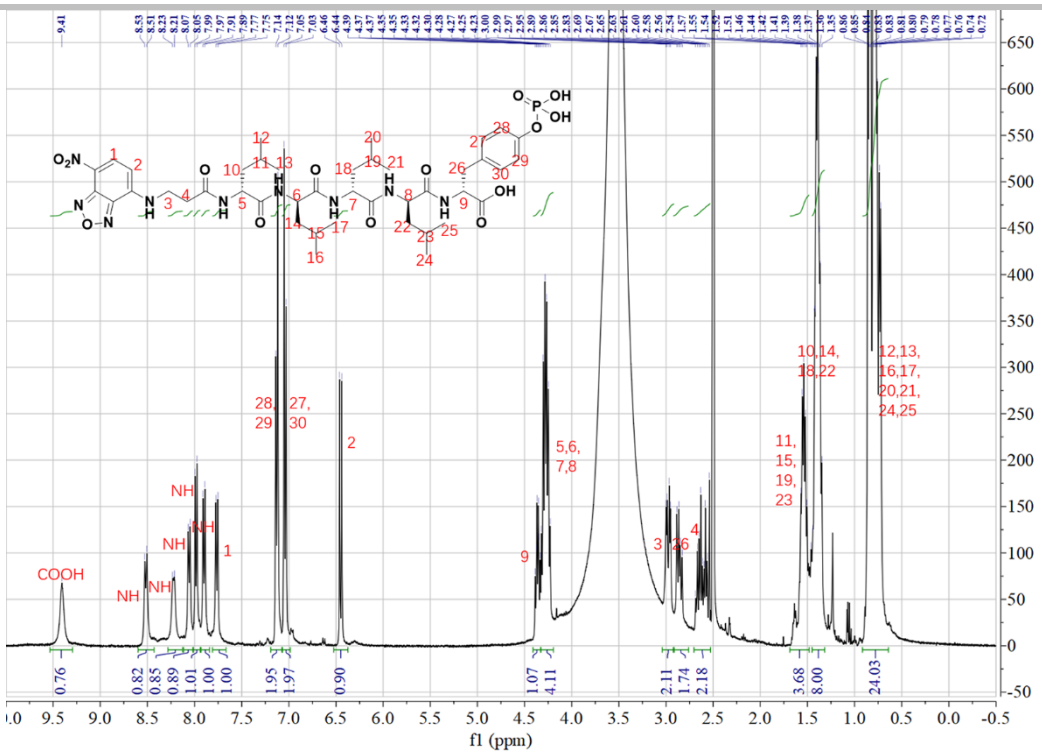


Figure S7. ^{1}H NMR spectrum of **1Dp**.

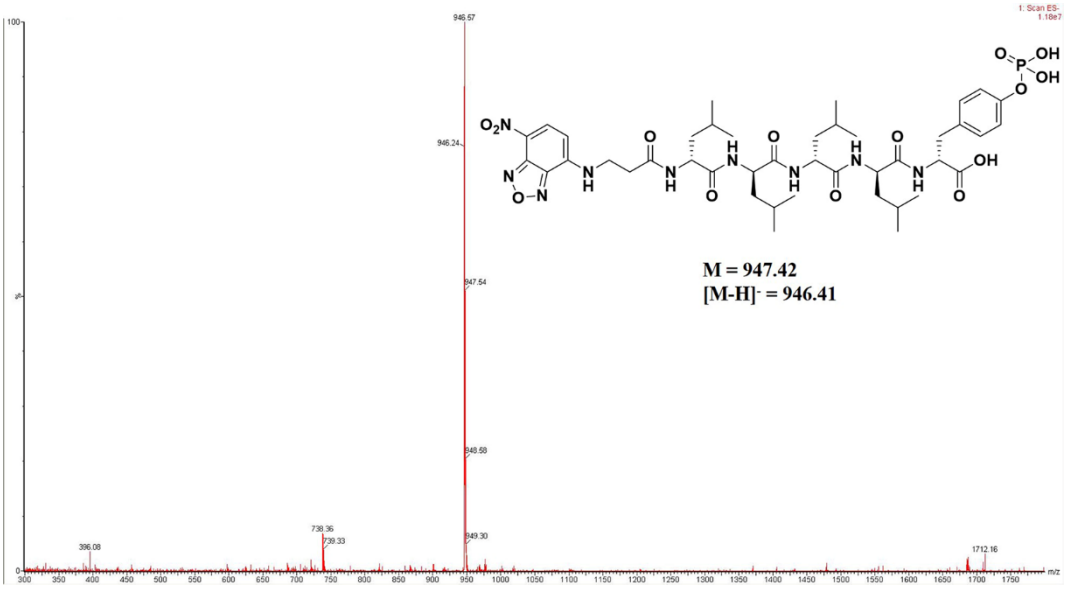


Figure S8. Mass spectrum of **1Dp** (M/Z = 946.57).

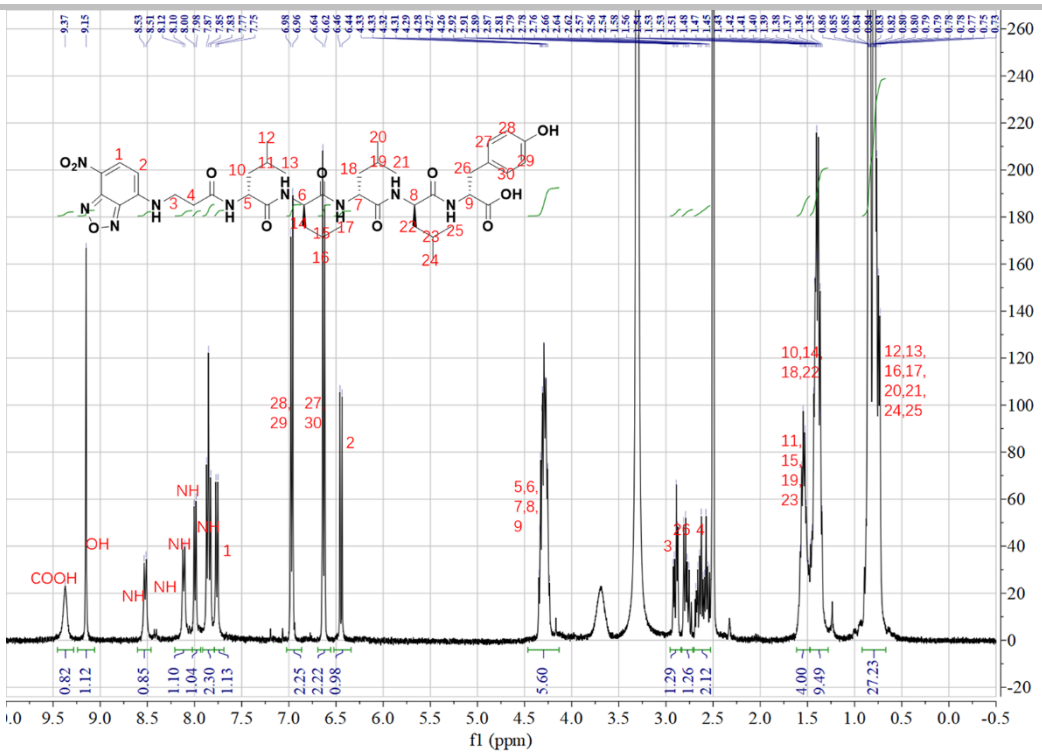


Figure S9. ^{1}H NMR spectrum of **1D**.

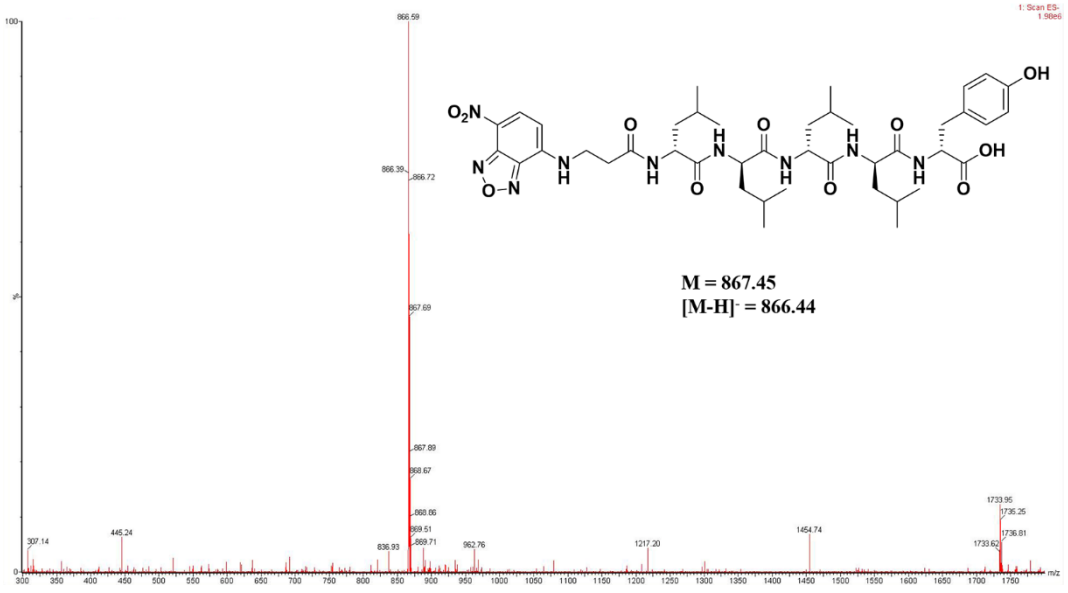


Figure S10. Mass spectrum of **1D** (M/Z = 866.59).

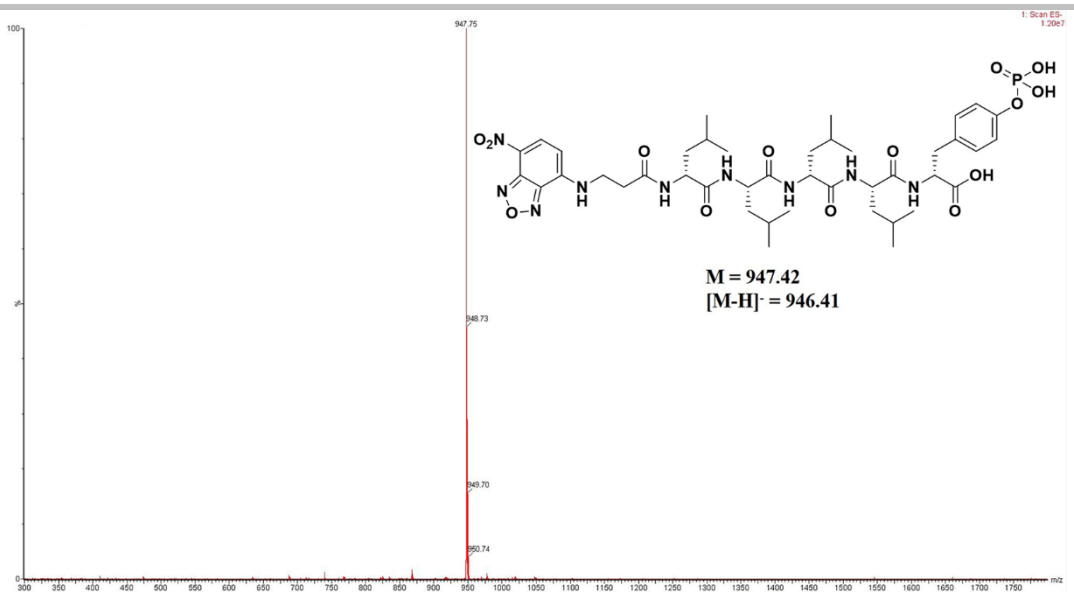


Figure S11. Mass spectrum of **1DLp** (M/Z = 947.75).

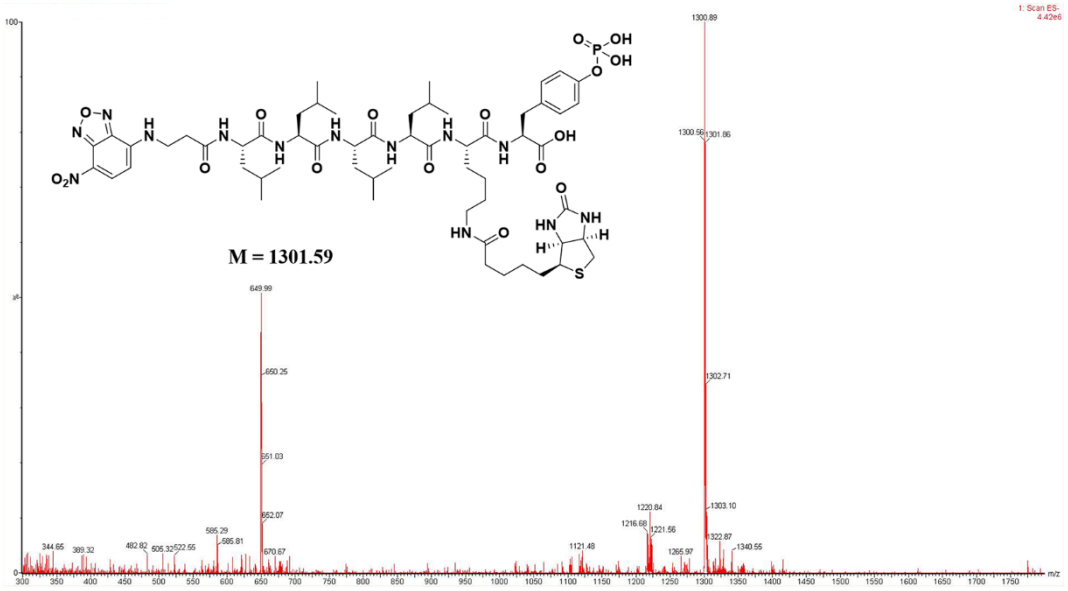


Figure S12. Mass spectrum of **2Lp** (M/Z = 1301.86).

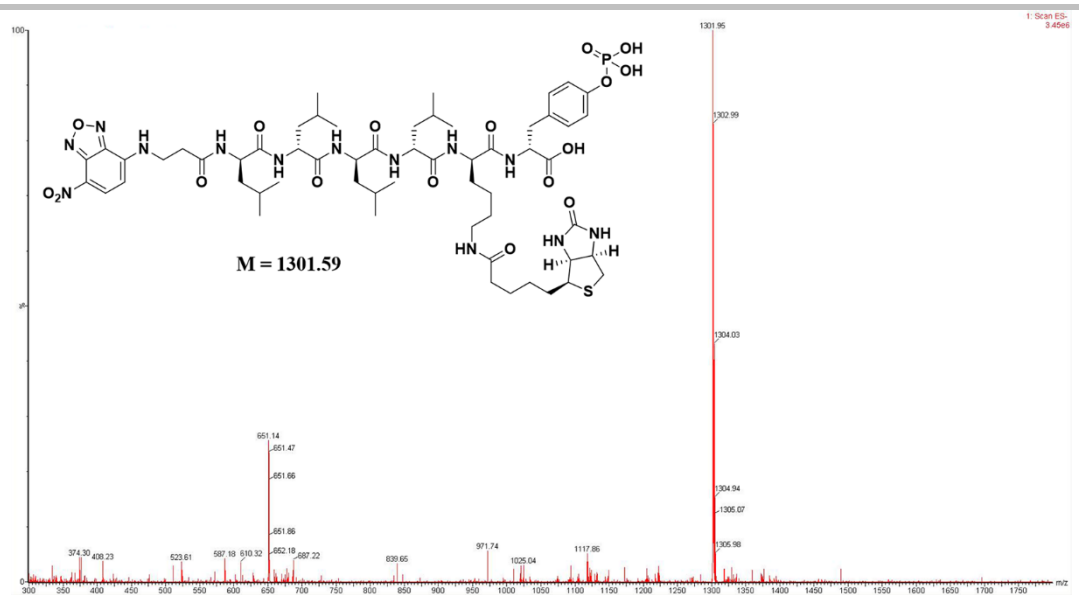


Figure S13. Mass spectrum of **2Dp** (M/Z = 1301.95).

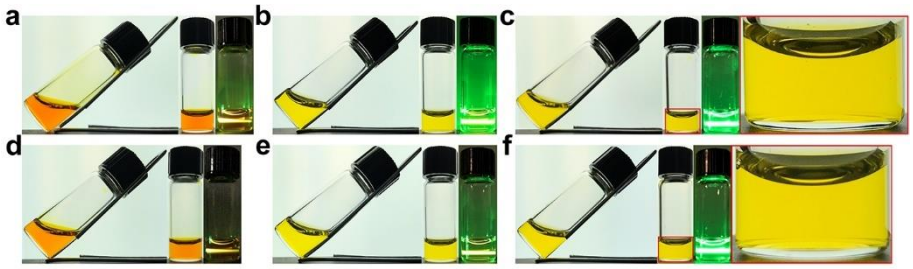


Figure S14. Optical photos of a) 10 mM **1Lp**, b) 200 μM **1Lp**, c) 200 μM **1Lp** after dephosphorylation by ALP (0.5 U/mL) for 24 h. Optical photos of d) 10 mM **1Dp**, e) 200 μM **1Dp**, f) 200 μM **1Dp** after dephosphorylation by ALP (0.5 U/mL) for 24 h.

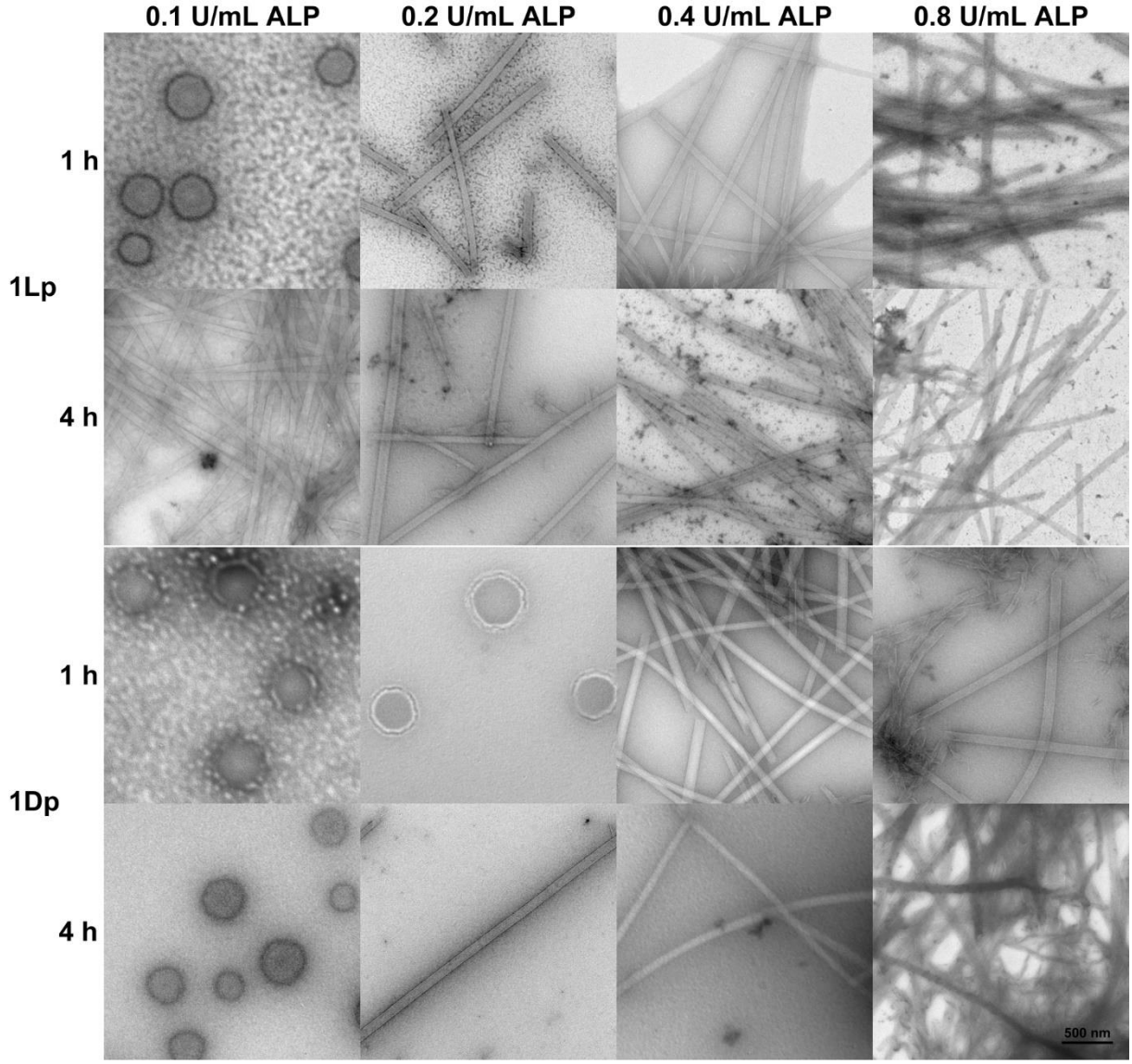


Figure S15. Transmission electron microscopes (TEM) images of **1Lp** and **1Dp** (200 μM, PBS) after dephosphorylation by ALP (0.1, 0.2, 0.4, and 0.8 U/mL) for 1 h and 4 h.

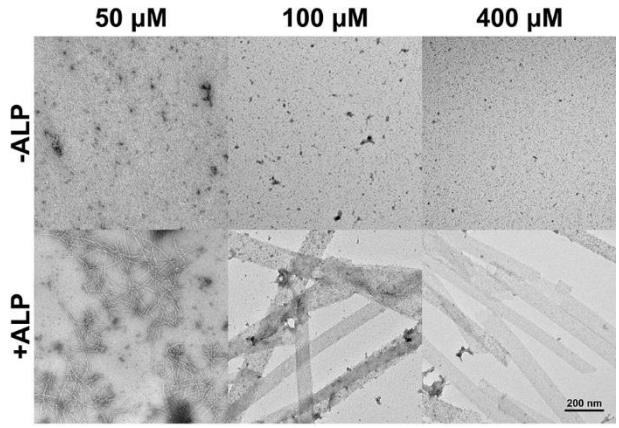


Figure S16. Transmission electron microscopes (TEM) images of **1Dp** (50 μM, 100 μM, and 400 μM, PBS) before and after dephosphorylation by ALP (0.5 U/mL) for 24 h.

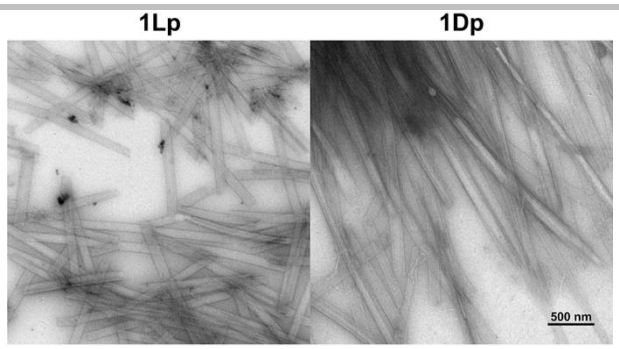


Figure S17. Transmission electron microscopes (TEM) images of **1Lp** and **1Dp** (200 μM, PBS) after dephosphorylation by ALP (0.1 U/mL) for 24 h.

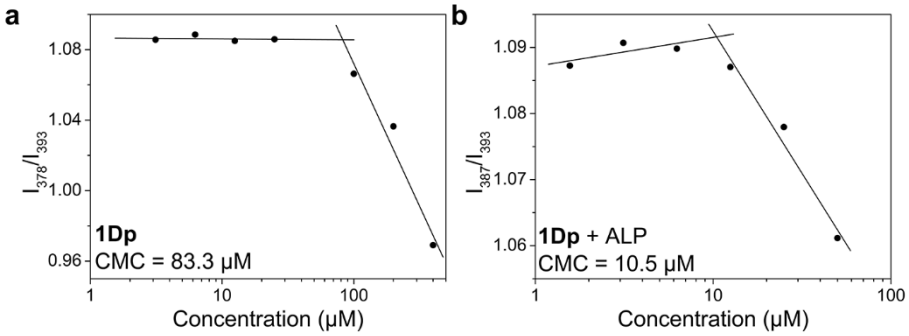


Figure S18. Critical micelle concentration (CMC) of a) **1Dp** (PBS) and b) **1Dp** + ALP (PBS, 37°C, 24 h).

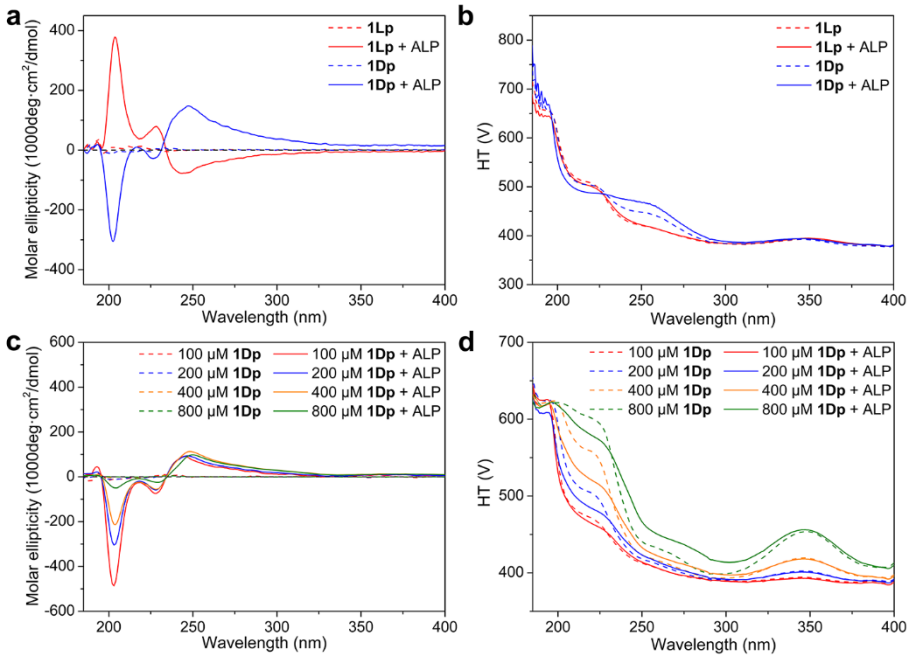


Figure S19. a) Circular dichroism (CD) spectra of **1Lp** and **1Dp** (200 μM, PBS) before and after dephosphorylation by 0.5 U/mL of ALP for 24 h. b) HT voltages of a). c) Circular dichroism (CD) spectra of **1Dp** (PBS) at different concentrations before and after dephosphorylation by 0.5 U/mL of ALP for 24 h. d) HT voltages of c).

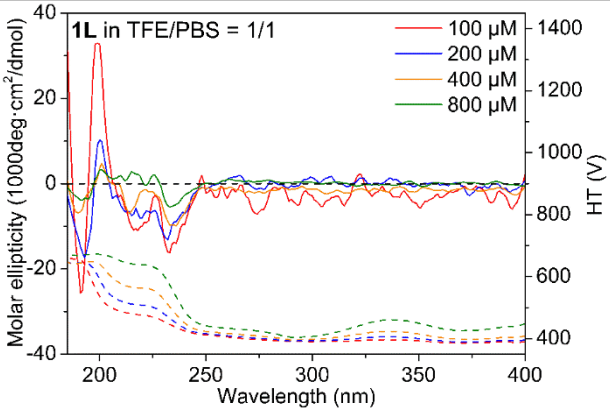


Figure S20. Circular dichroism (CD) spectra (solid line) and HT voltages (dash line) of **1L** in TFE/PBS = 1/1.

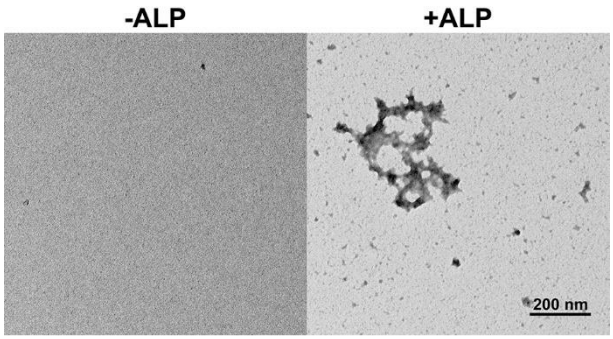


Figure S21. Transmission electron microscopes (TEM) images of **1DLp** (200 μM, PBS) before and after dephosphorylation by ALP (0.5 U/mL) for 24 h.

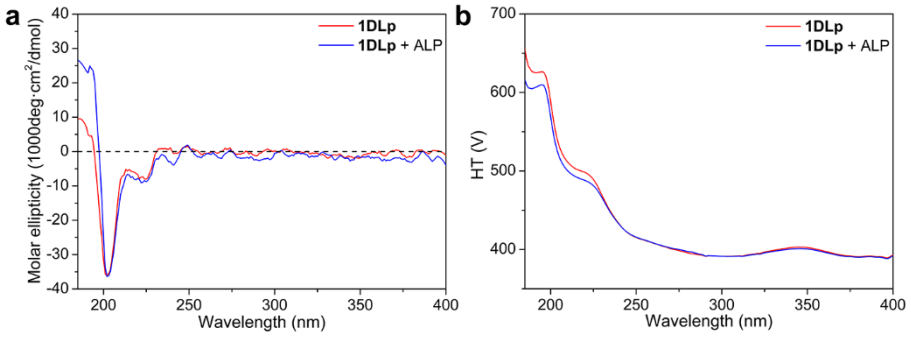


Figure S22. a) Circular dichroism (CD) spectra of **1DLp** (200 μM, PBS) before and after dephosphorylation by 0.5 U/mL of ALP for 24 h. b) HT voltages of a).

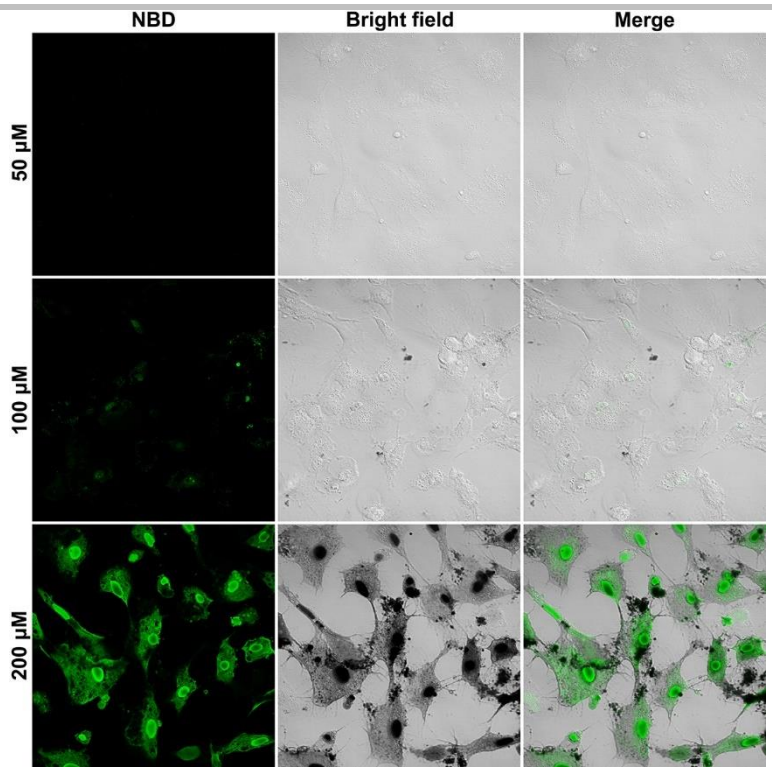


Figure S23. Confocal laser scanning microscopy (CLSM) images of SJSA-1 cells after being treated by **1Lp** (50 μM, 100 μM, or 200 μM) for 4 h.

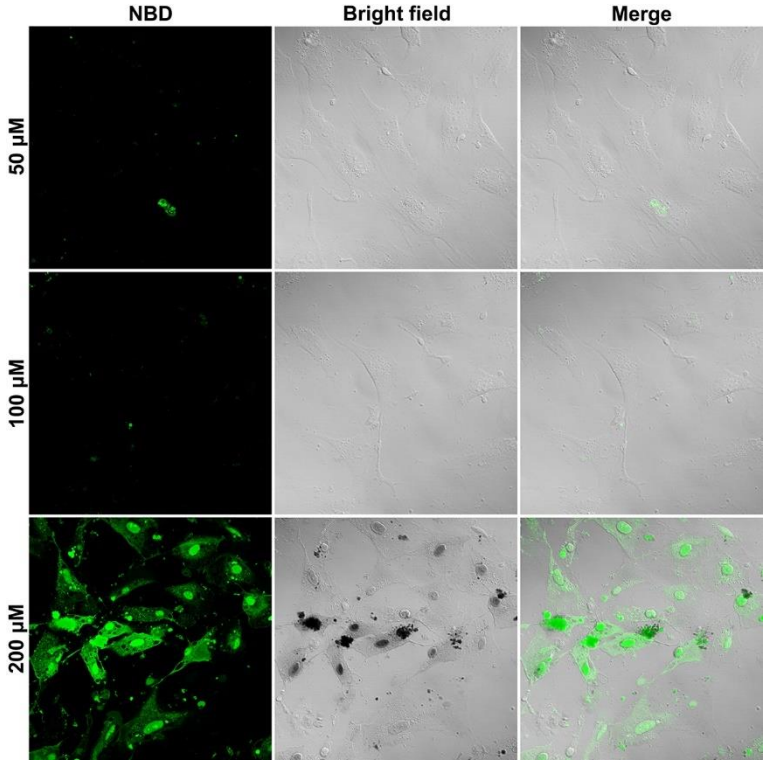


Figure S24. Confocal laser scanning microscopy (CLSM) images of SJSA-1 cells after being treated by **1Dp** (50 μM, 100 μM, or 200 μM) for 4 h.

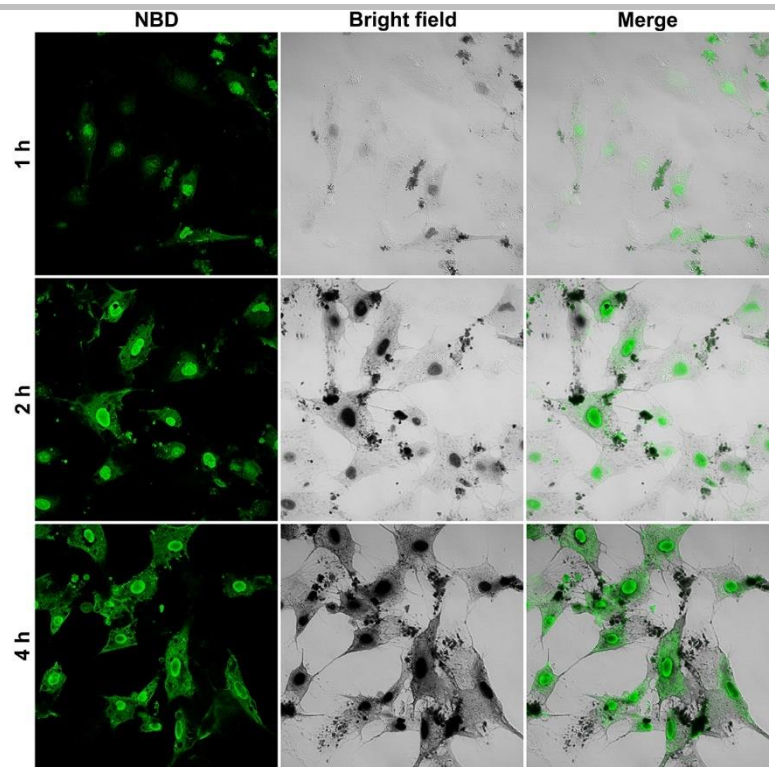


Figure S25. Confocal laser scanning microscopy (CLSM) images of SJSA-1 cells after being treated by **1Lp** (200 μM) for 1 h, 2 h, or 4 h.

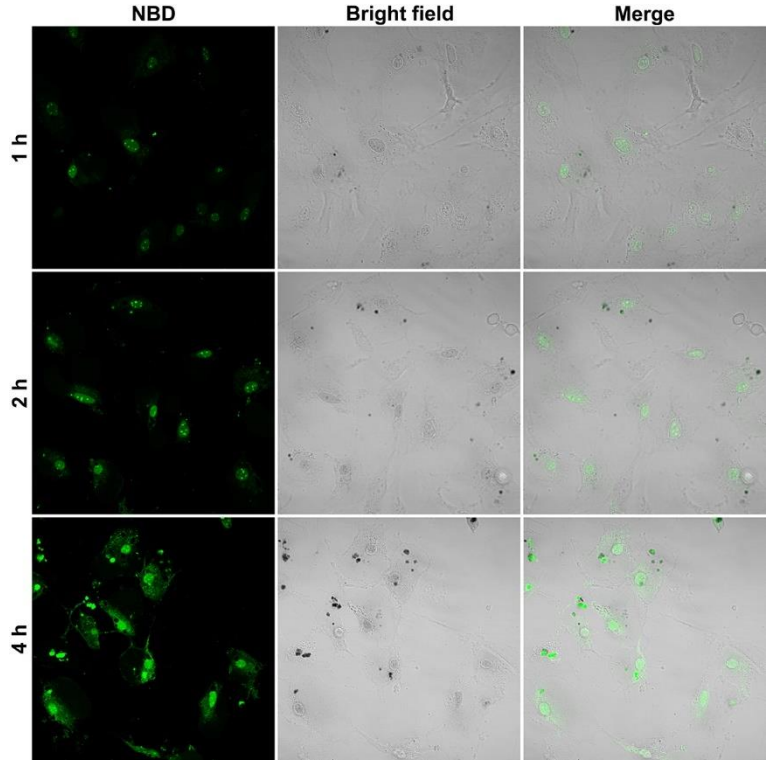


Figure S26. Confocal laser scanning microscopy (CLSM) images of SJSA-1 cells after being treated by **1Dp** (200 μM) for 1 h, 2 h, or 4 h.

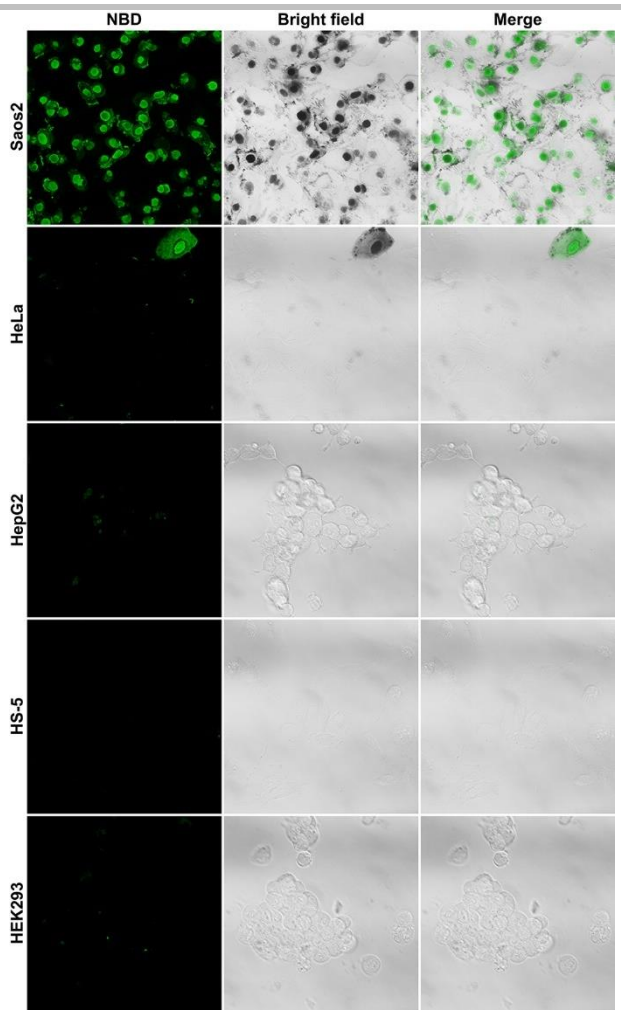


Figure S27. Confocal laser scanning microscopy (CLSM) images of Saos2, HeLa, HepG2, HS-5, or HEK293 cells after being treated by **1Lp** (200 μM) for 4 h.

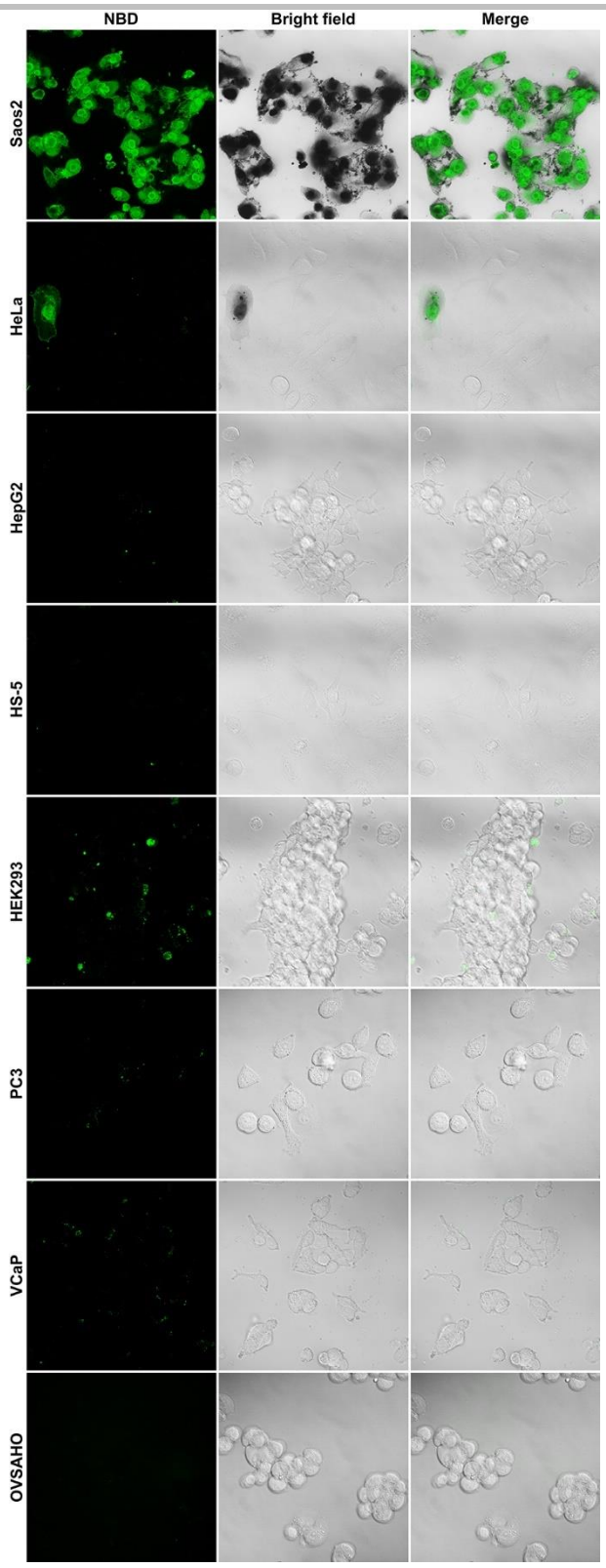


Figure S28. Confocal laser scanning microscopy (CLSM) images of Saos2, HeLa, HepG2, HS-5, HEK293, PC3, VCaP, and OVSAHO cells after being treated by **1Dp** (200 μM) for 4 h.

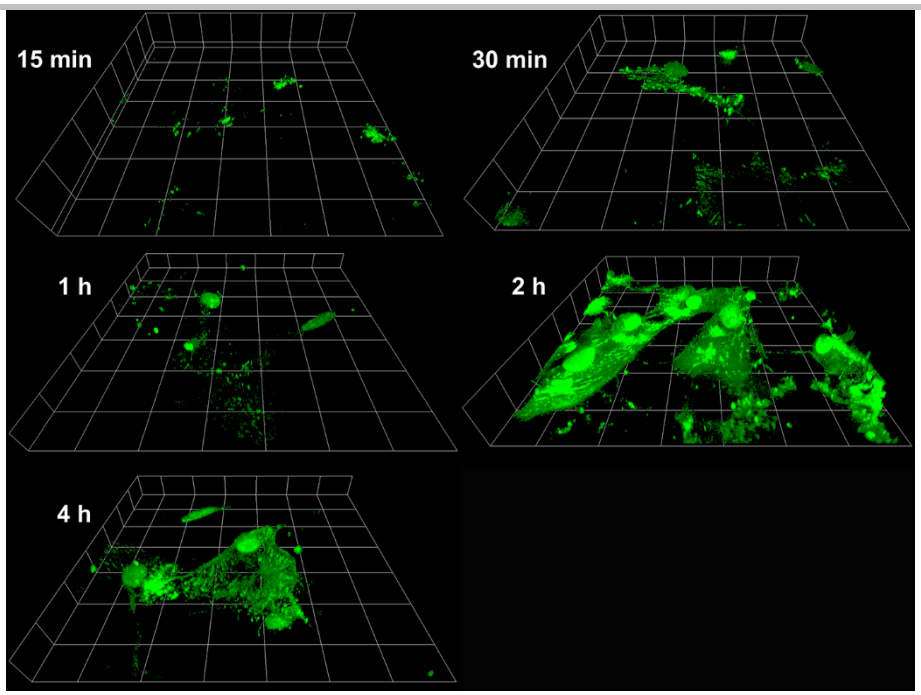


Figure S29. 3D Confocal laser scanning microscopy (CLSM) images of SJSA-1 cells after being treated by **1Lp** (200 μM) for 15 min, 30 min, 1 h, 2 h, or 4 h.

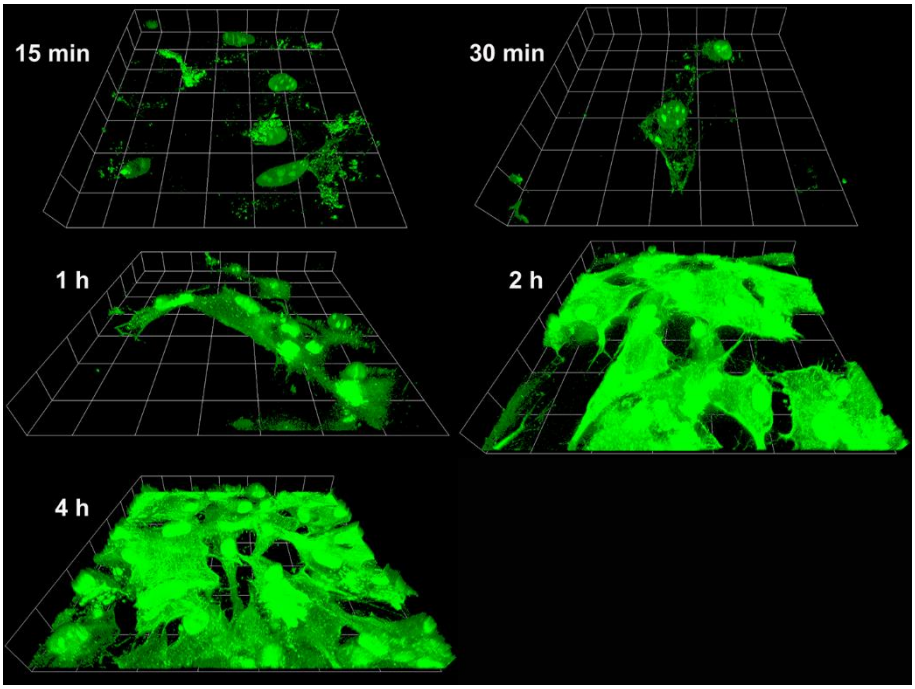


Figure S30. 3D Confocal laser scanning microscopy (CLSM) images of SJSA-1 cells after being treated by **1Dp** (200 μM) for 15 min, 30 min, 1 h, 2 h, or 4 h.

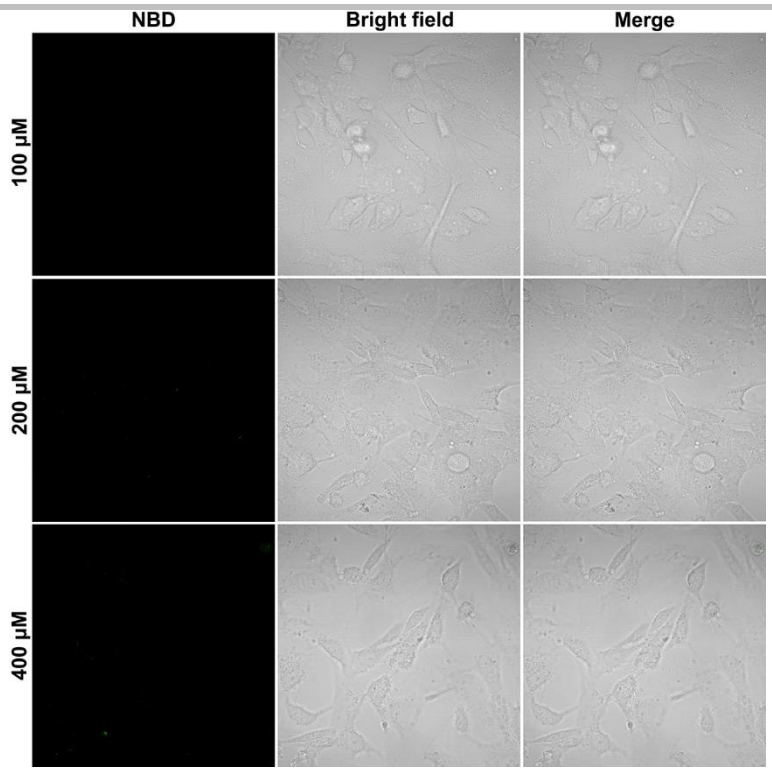


Figure S31. Confocal laser scanning microscopy (CLSM) images of SJSA-1 cells after being treated by **1DLp** (100 μM, 200 μM, or 400 μM) for 4 h.

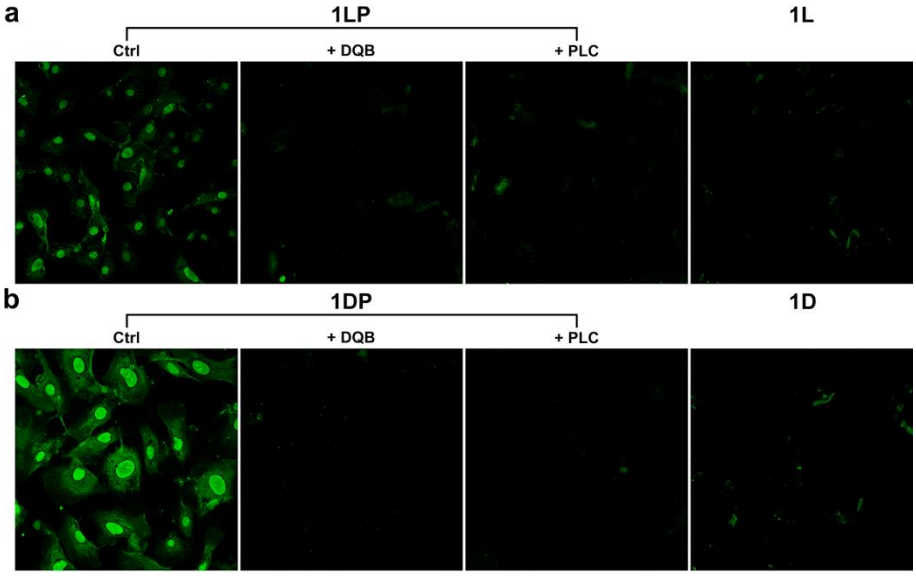


Figure S32. a) Confocal laser scanning microscopy (CLSM) images of SJSA-1 cells after being treated by **1Lp** (200 μM) in absence (Ctrl) or presence of DQB (5 μM) or PLC (0.2 U/mL, pretreat for 24 h), or **1L** for 4 h. b) CLSM images of SJSA-1 cells after being treated by **1Dp** (200 μM) in absence (Ctrl) or presence of DQB (5 μM) or PLC (0.2 U/mL, pretreat for 24 h), or **1D** for 4 h.

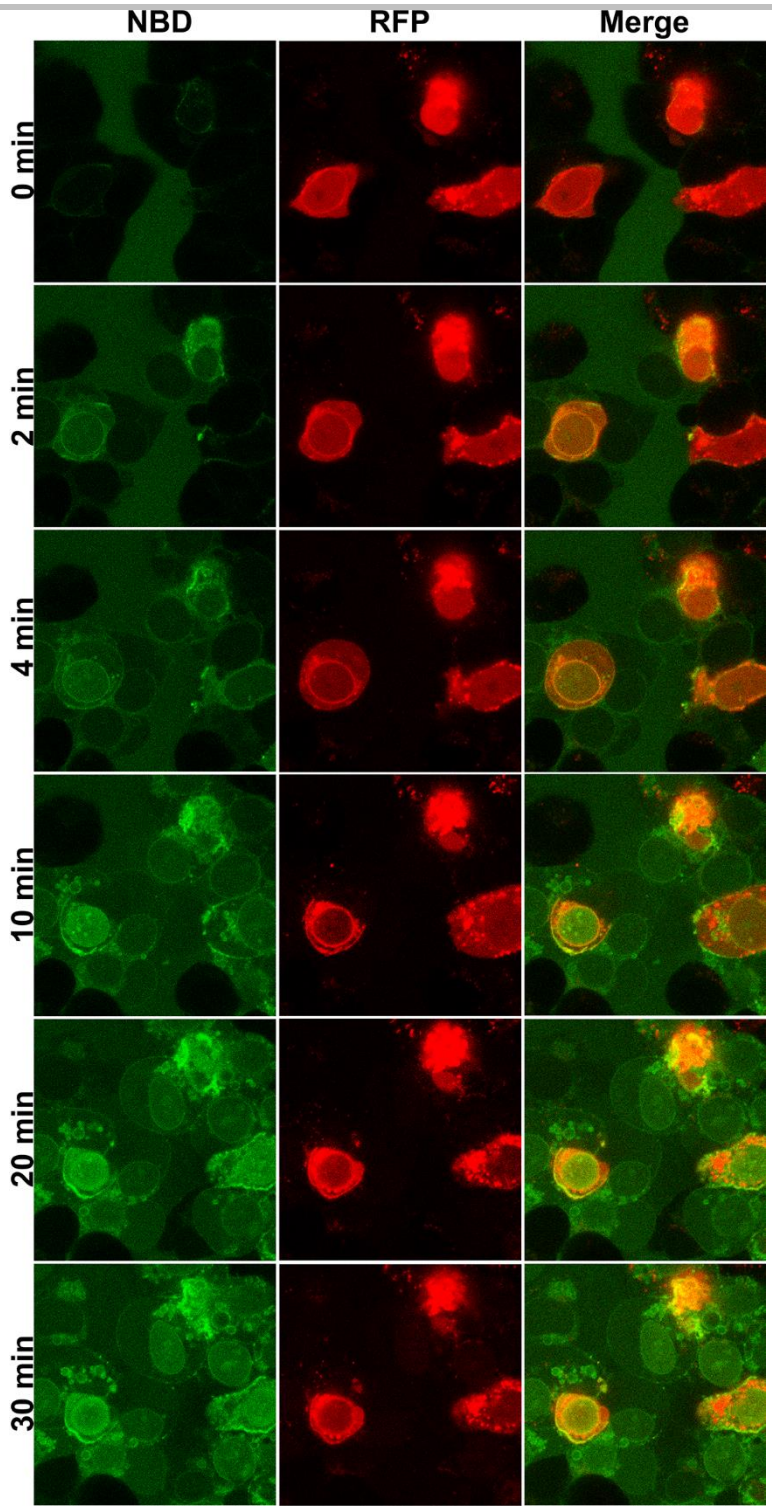


Figure S33. Time-dependent CLSM images of TNAP-RFP transfected HEK293 cells treated by **1Lp** (200 μM) (Movie 9).

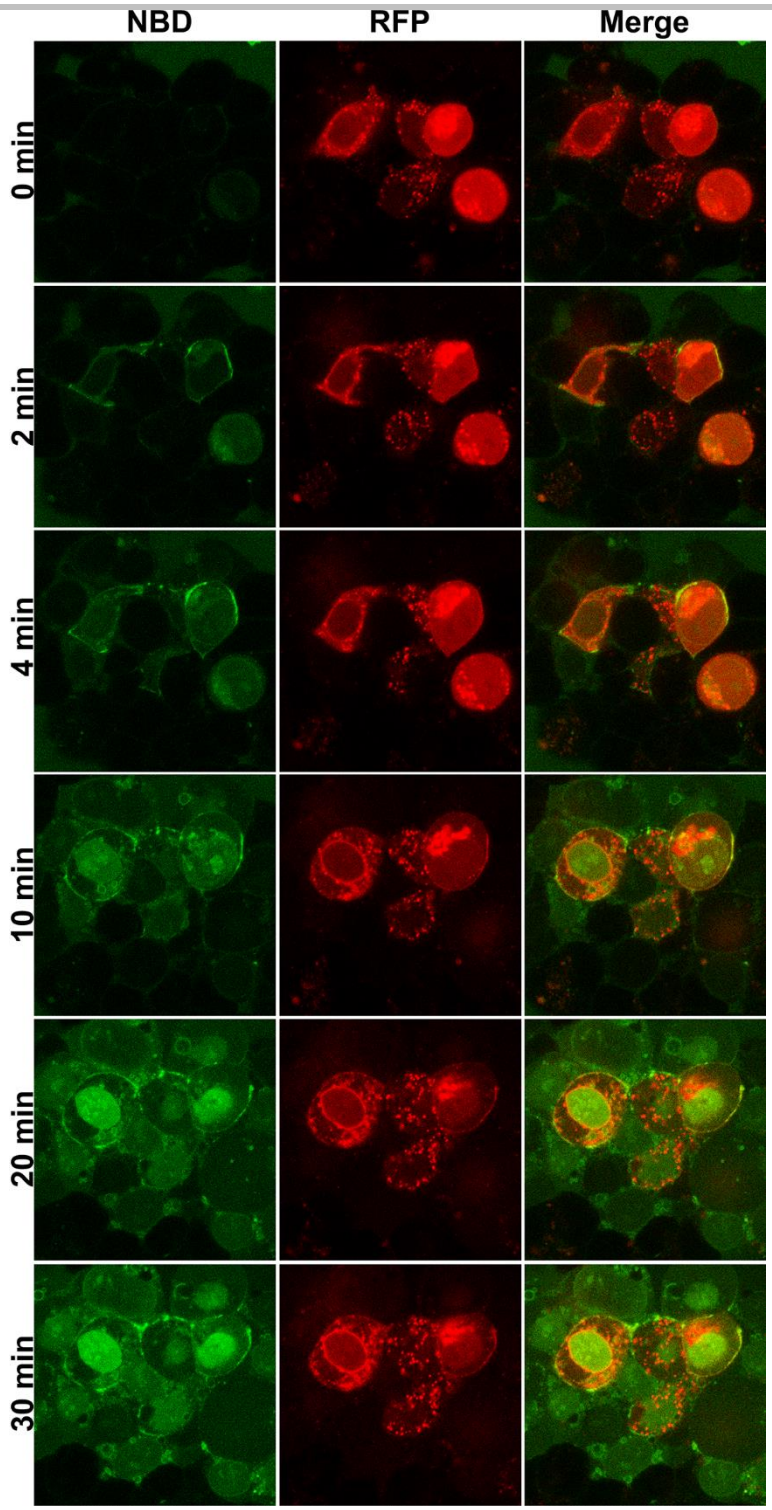


Figure S34. Time-dependent CLSM images of TNAP-RFP transfected HEK293 cells treated by **1Dp** (200 μM) (Movie 10).

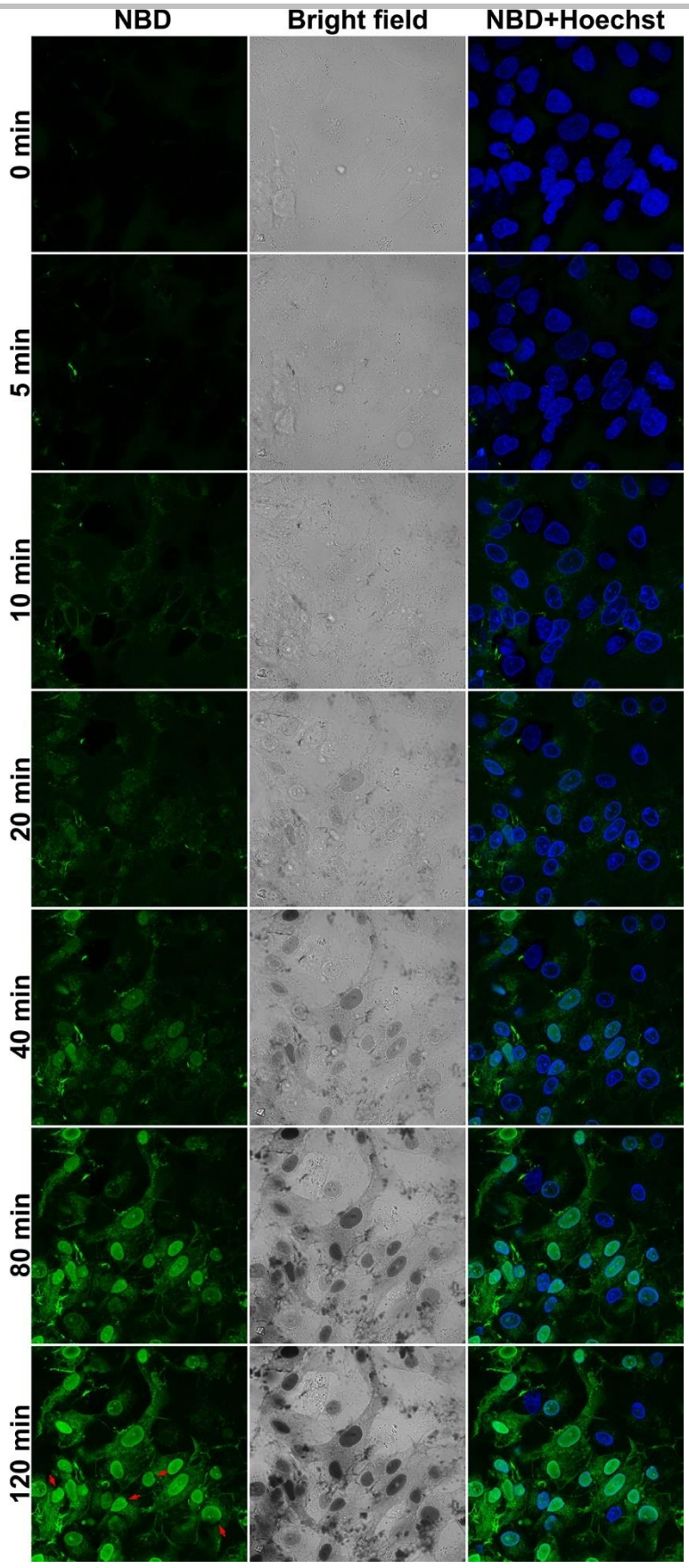


Figure S35. Time-dependent CLSM images of SJSA-1 after incubating with **1Lp** (200 μM) (Movie 1). The red arrows show the disruption of nucleus membrane.

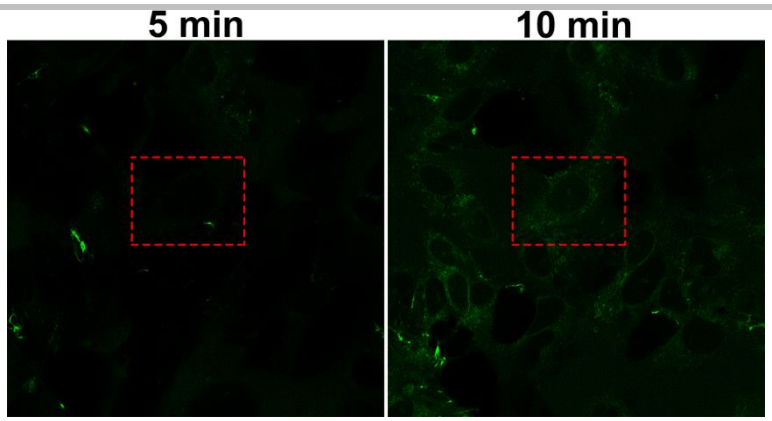


Figure S36. CLSM images of SJSA-1 cells after incubating with **1Lp** (200 μM) for 5 min and 10 min (Movie 1). The red dash squares show the exampled cell. The cell exhibits fluorescence in the cytoplasm after 5 min incubation and in the cell nucleus after 10 min incubation.

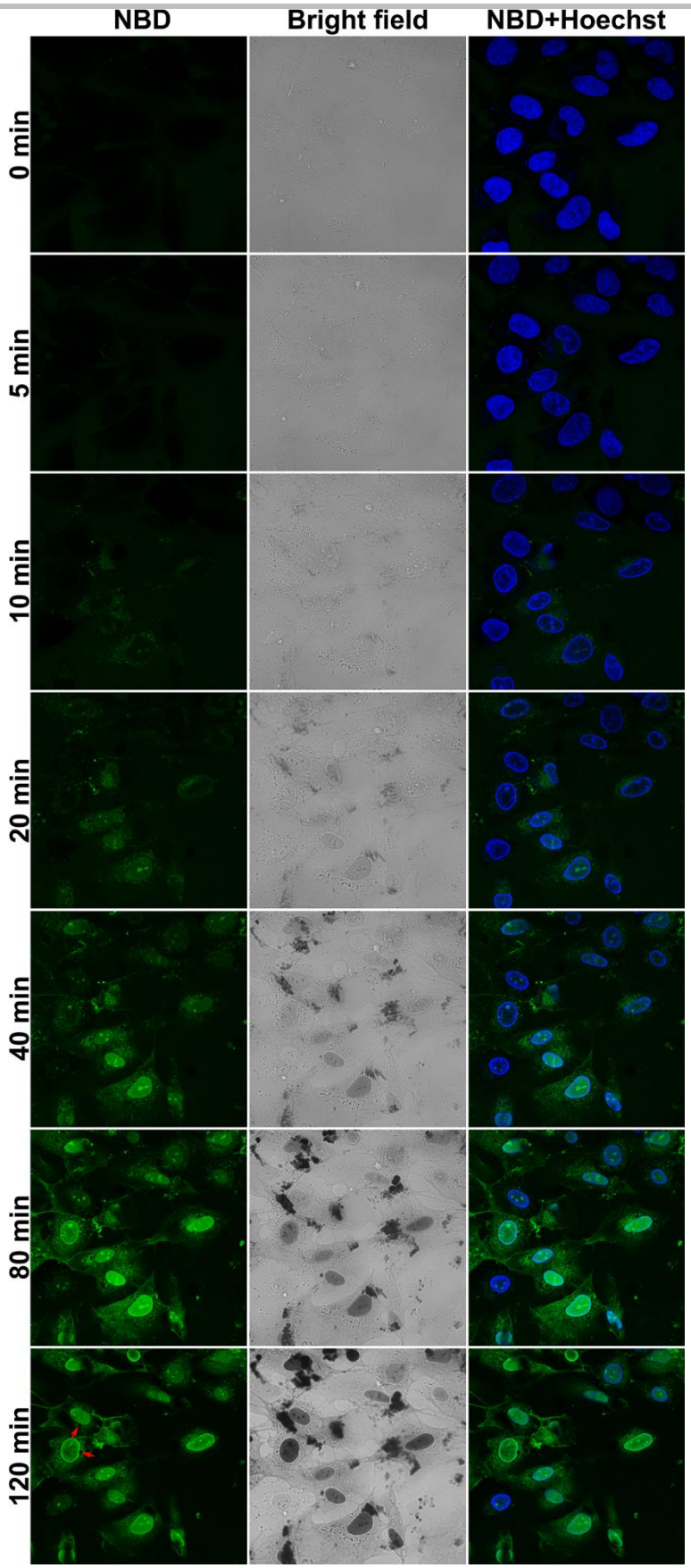


Figure S37. Time-dependent CLSM images of SJSA-1 after incubating with **1Dp** (200 μM) (Movie 2). The red arrows show the disruption of nucleus membrane.

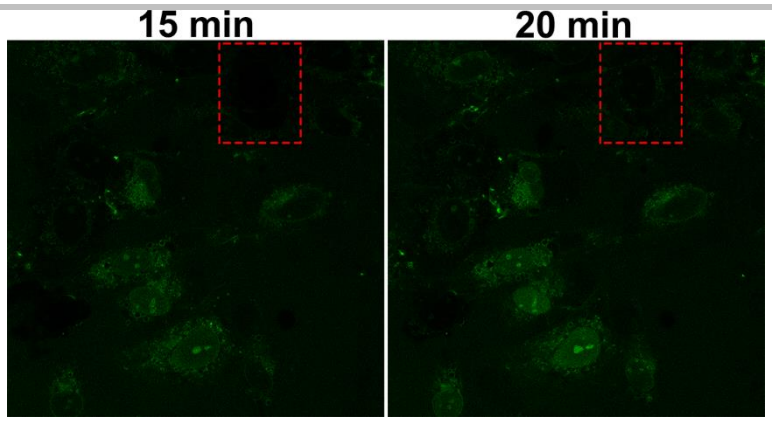


Figure S38. CLSM images of SJSA-1 cells after incubating with **1Dp** (200 μM) for 15 min and 20 min (Movie 2). The red dash squares show the exampled cell. The cell exhibits fluorescence in the cytoplasm after 15 min incubation and in the cell nucleus after 20 min incubation.

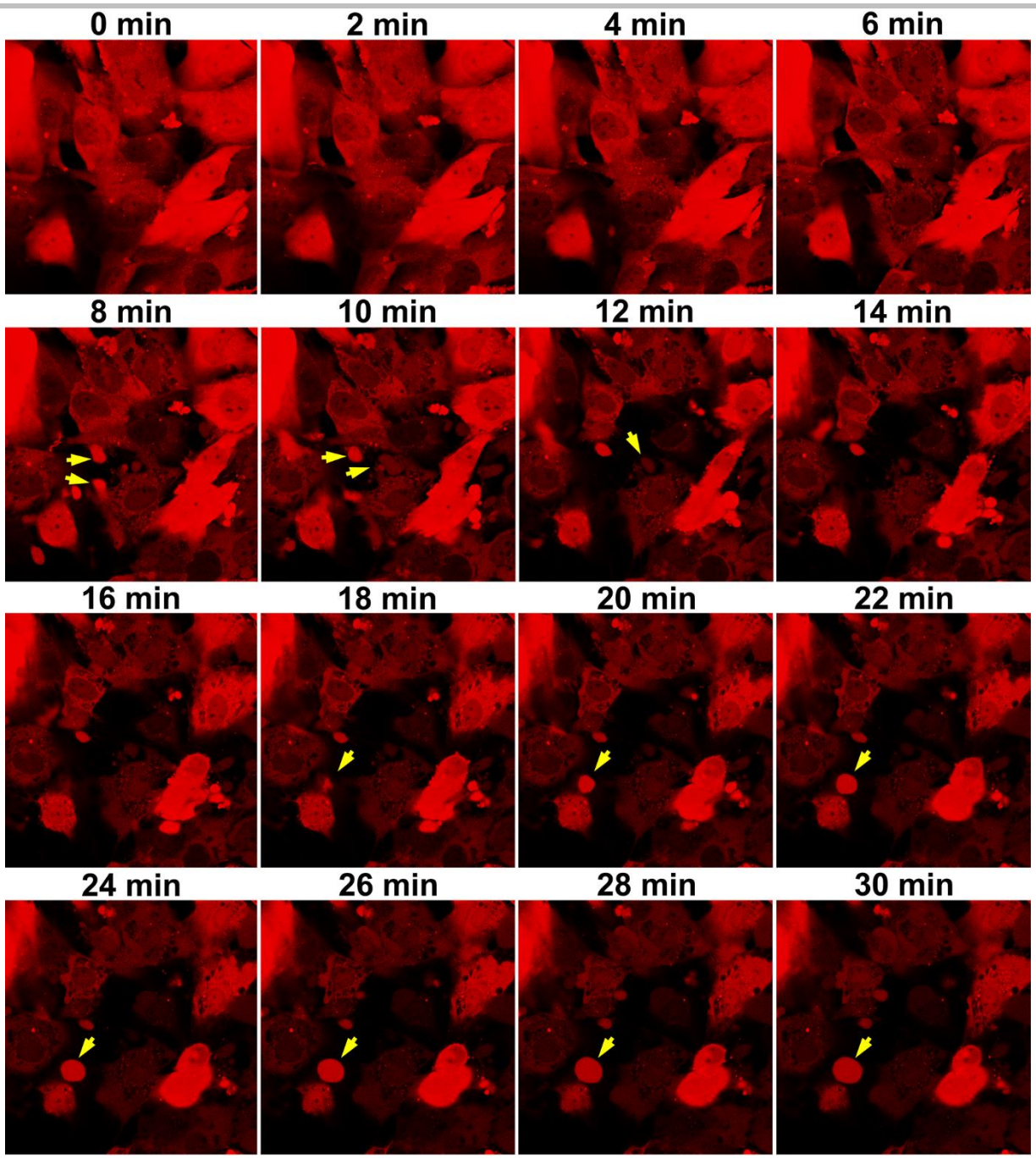


Figure S39. CLSM images of RFP-expressing SJSA-1 after incubating with **1Lp** (200 μM) for different time (Movie 7). The yellow arrows show the disruption of cell membranes and release of cytoplasm.

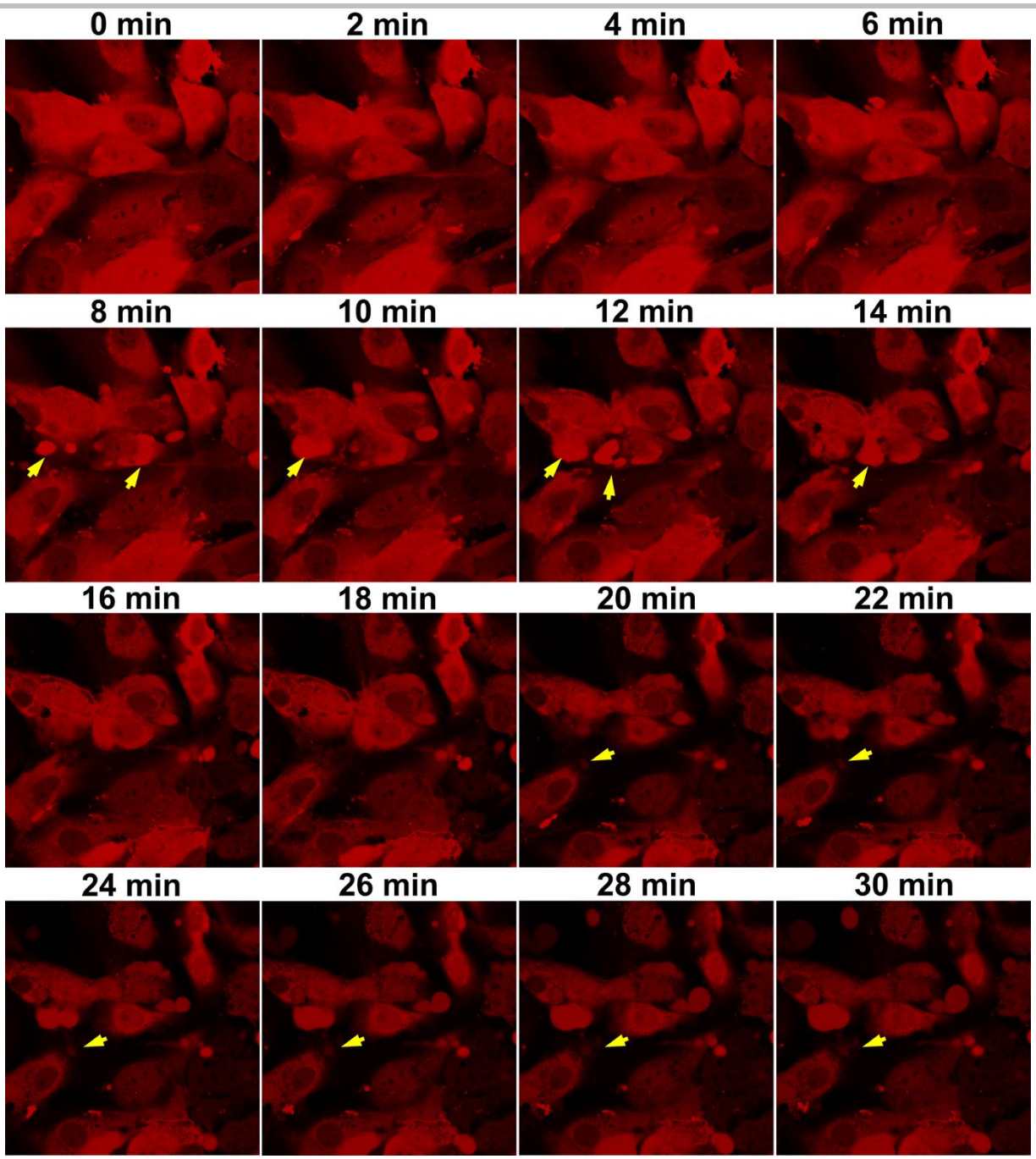


Figure S40. CLSM images of RFP-expressing SJSA-1 after incubating with **1Dp** (200 μM) for different time (Movie 8). The yellow arrows show the disruption of cell membranes and release of cytoplasm.

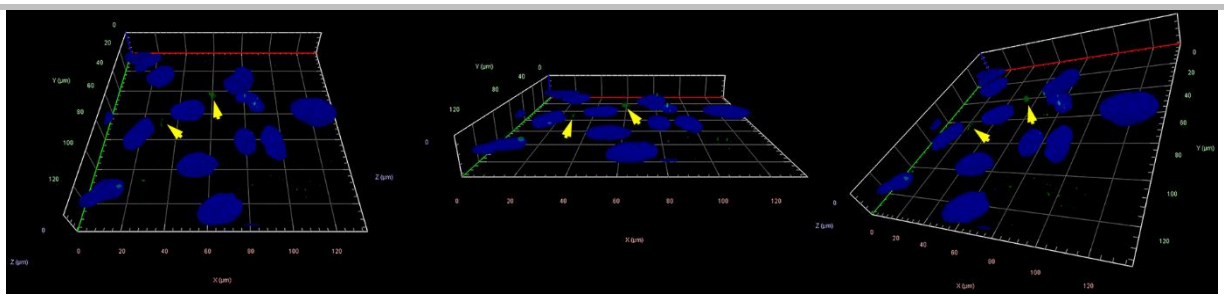


Figure S41. 3D CLSM images of SJSA-1 after incubated with **1Lp** (200 μM) for 3 min (Movie 3). The yellow arrows show the NBD fluorescence on the contact points of cells.

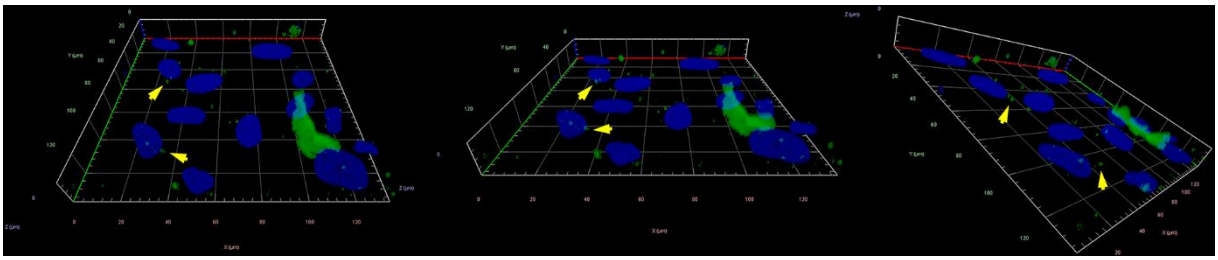


Figure S42. 3D CLSM images of SJSA-1 after incubated with **1Dp** (200 μM) for 3 min (Movie 4). The yellow arrows show the NBD fluorescence on the contact points of cells.

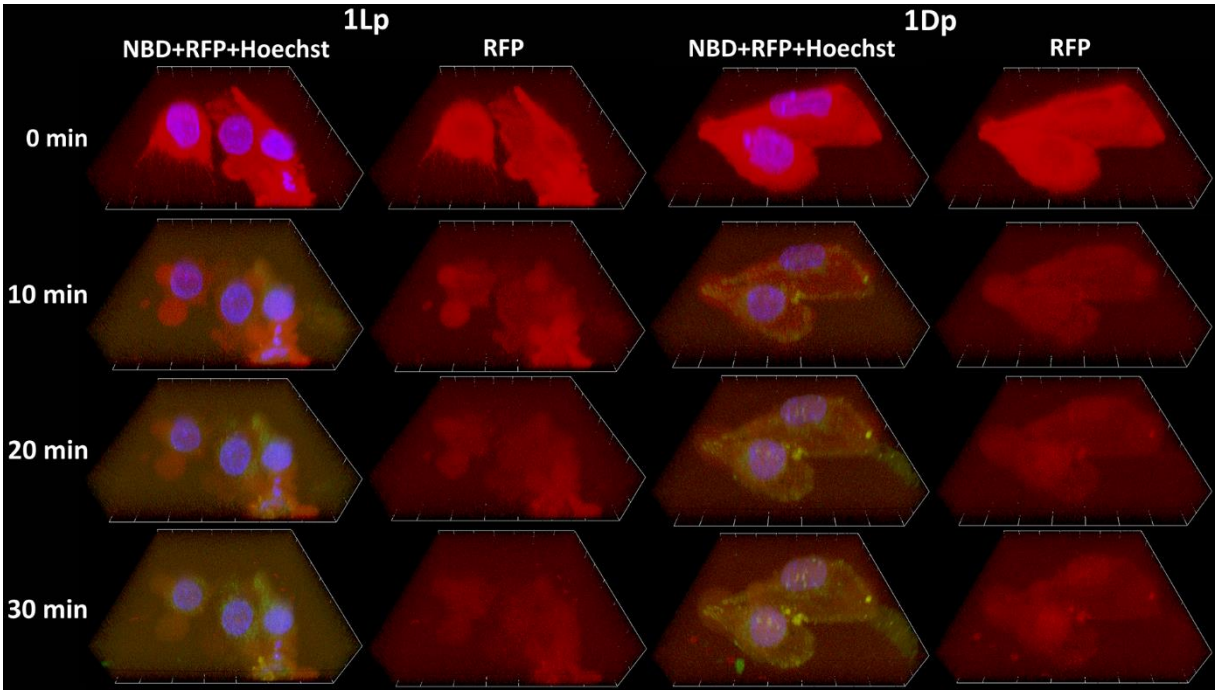


Figure S43. The Z-stack of CLSM images of tubulin-RFP transfected Saos2 cells after incubating with **1Lp** or **1Dp** for 0, 10, 20, and 30 min.

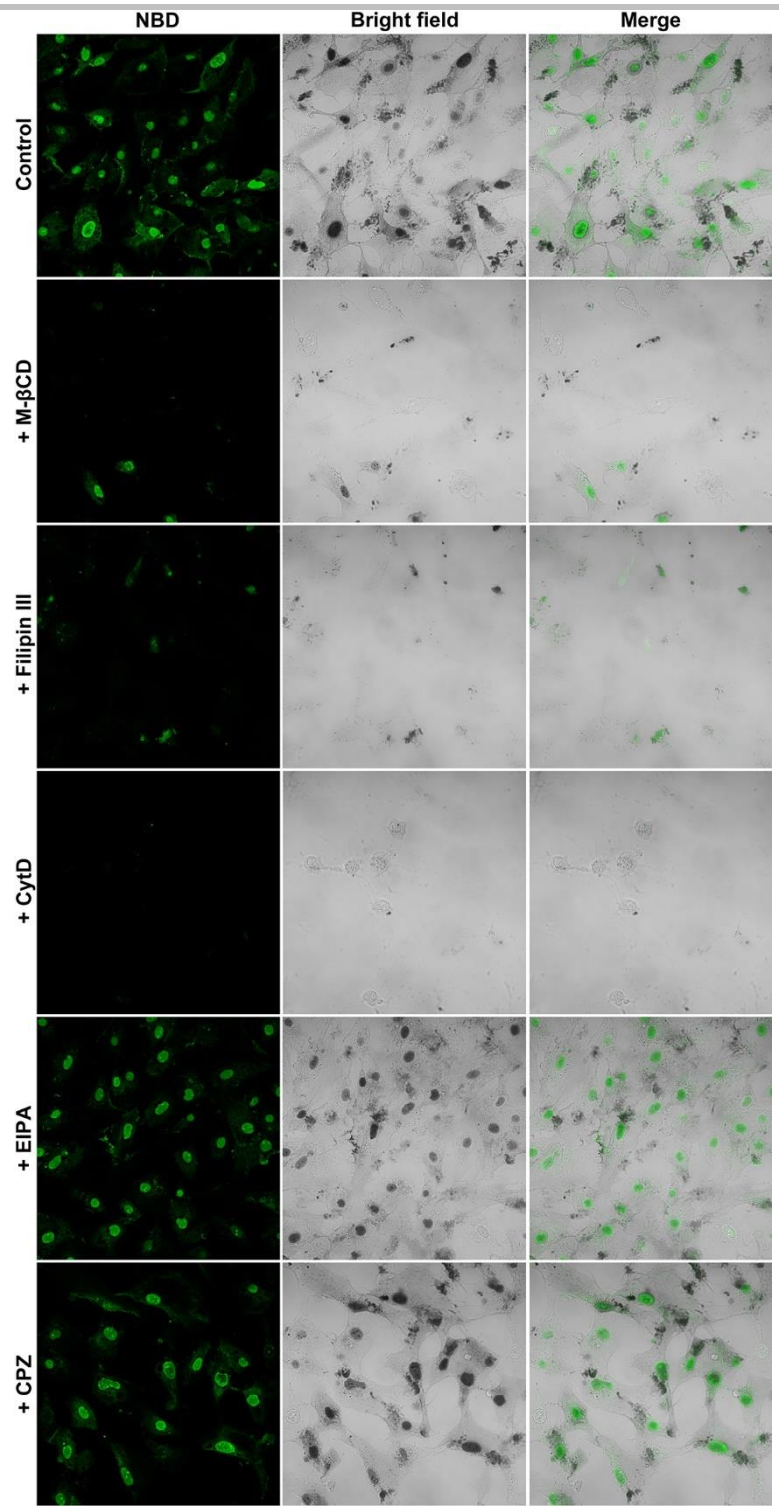


Figure S44. Confocal laser scanning microscopy (CLSM) images of SJSA-1 cells after being treated by **1Lp** (200 μM) for 4 h in absence (Control) or presence of inhibitors M-βCD (2 mM), Filipin III (2 μg/mL), CytD (2.5 μg/mL), EIPA (1 μM), or CPZ (30 μM).

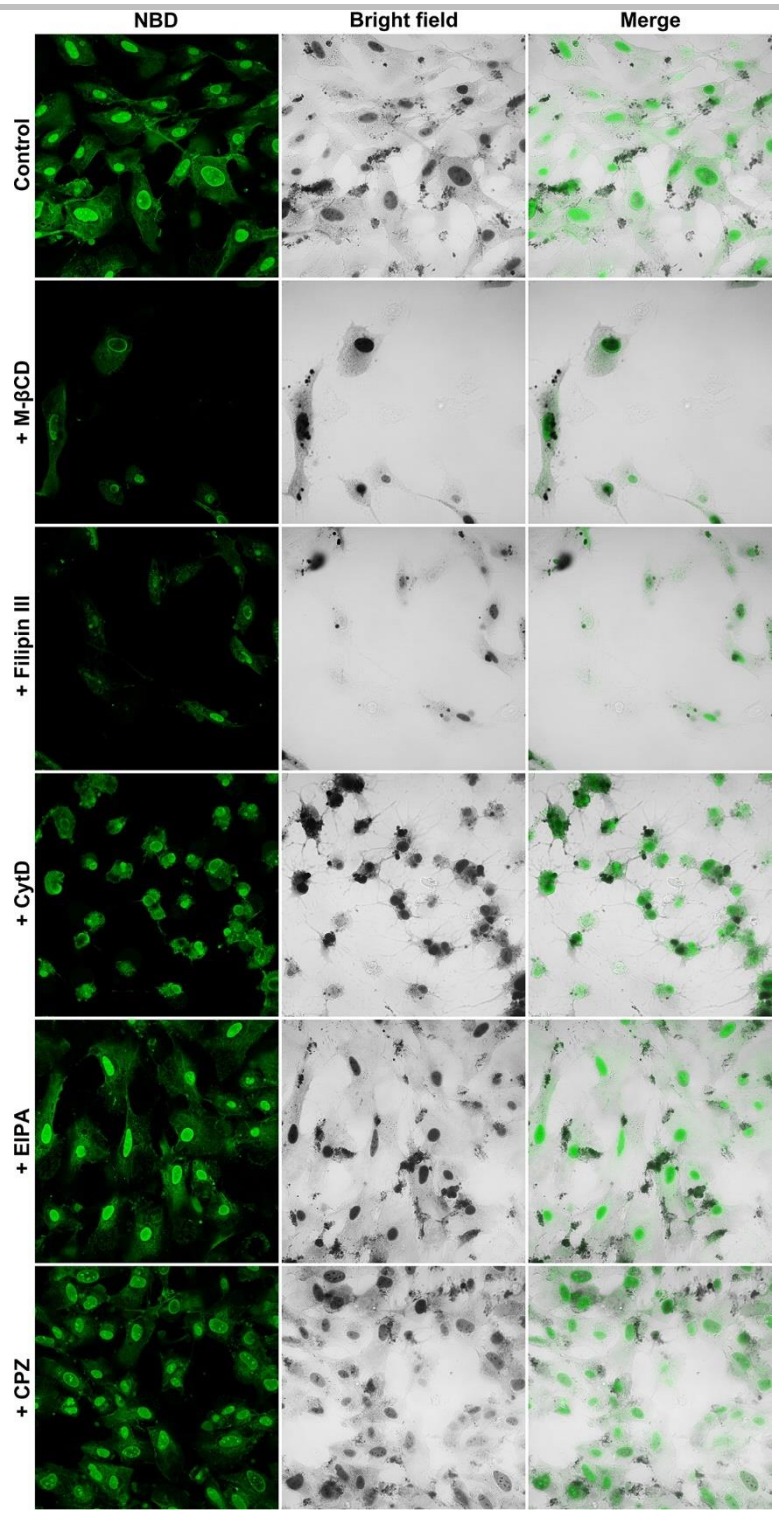


Figure S45. Confocal laser scanning microscopy (CLSM) images of SJSA-1 cells after being treated by **1Dp** (200 μM) for 4 h in absence (Control) or presence of inhibitors M-βCD (2 mM), Filipin III (2 μg/mL), CytD (2.5 μg/mL), EIPA (1 μM), or CPZ (30 μM).

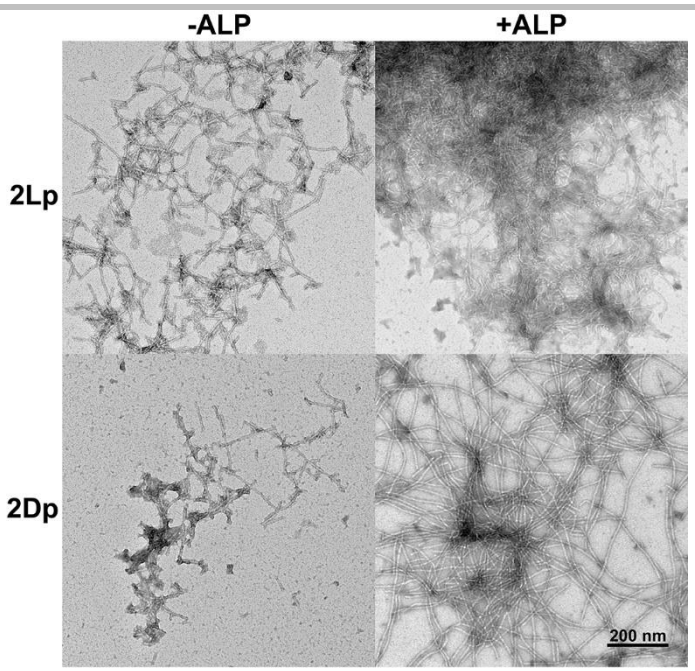


Figure S46. Transmission electron microscopes (TEM) images of **2Lp** and **2Dp** (400 μM, PBS) before and after dephosphorylation by ALP (0.5 U/mL) for 24 h.

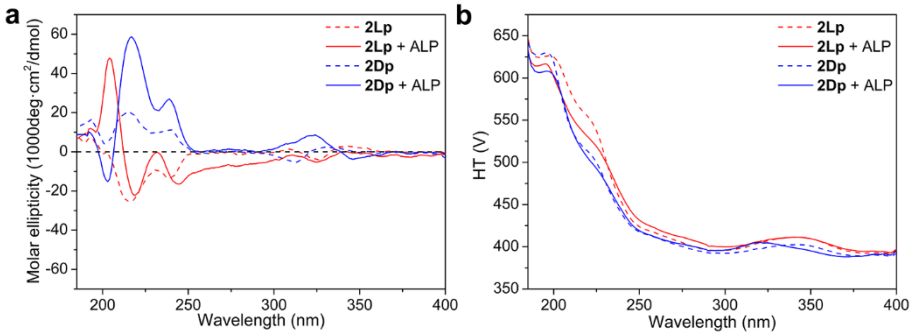


Figure S47. a) Circular dichroism (CD) spectra of **2Lp** and **2Dp** (400 μM, PBS) before and after dephosphorylation by 0.5 U/mL of ALP for 24 h. b) HT voltages of a).

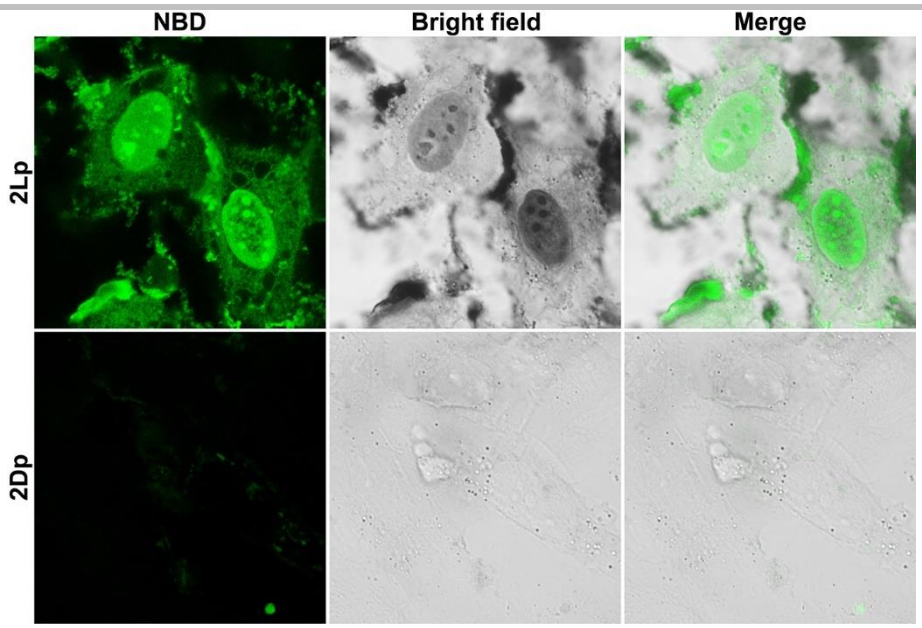


Figure S48. Confocal laser scanning microscopy (CLSM) images of SJSA-1 cells after being treated by **2Lp** or **2Dp** (400 μM) for 2 h.

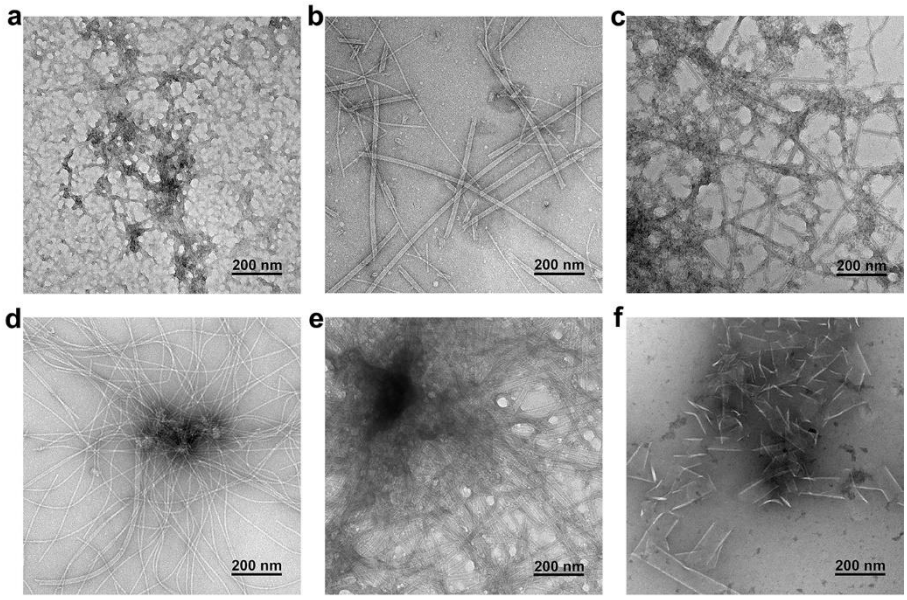


Figure S49. Transmission electron microscopes (TEM) images of a) H4C1 (1 mg/mL, PBS), b) **1Lp** (10 mM, PBS), c) **1Dp** (10 mM, PBS), d) **1Lp** (5 mM, PBS) + H4C1 (0.5 mg/mL, PBS), e) **1Dp** (5 mM, PBS) + H4C1 (0.5 mg/mL, PBS), f) **1Dp** (5 mM, PBS) + H4C1 (0.5 mg/mL, PBS) + ALP (1 U/mL, 24 h).

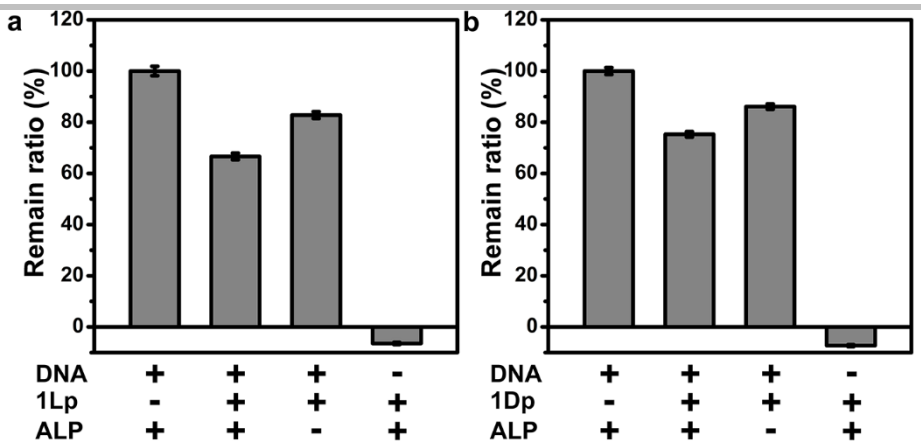


Figure S50. Remain ratio of DNA (the original concentration is 50 μg/mL) in solution after incubating with a) **1Lp** or b) **1Dp** (200 μM) and ALP (0.5 U/mL) for 24 h.

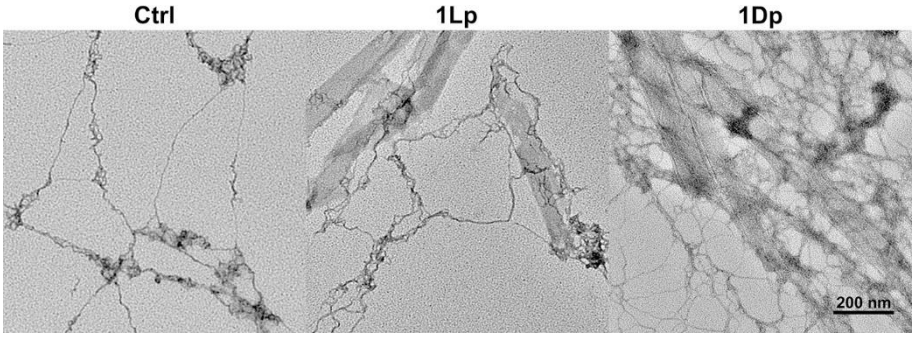


Figure S51. Transmission electron microscopes (TEM) images of DNA (50 μg/mL) after incubating with **1Lp** or **1Dp** (200 μM) and ALP (0.5 U/mL) for 24 h.

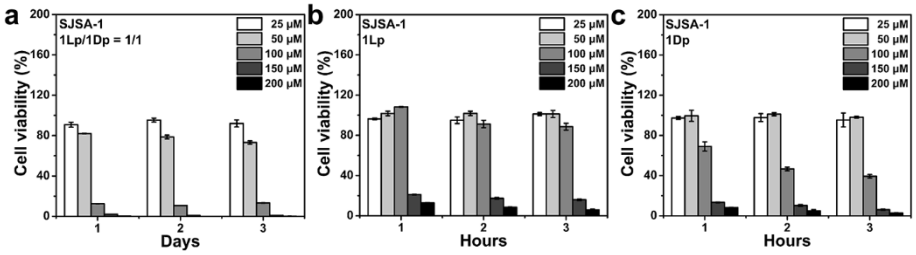


Figure S52. a) Cell viability of SJSA-1 cells after incubating with the mixture of **1Lp** and **1Dp** ([**1Lp**] = [**1Dp**], the concentration is [**1Lp**] + [**1Dp**]) for 24, 48 or 72 h. Cell viability of SJSA-1 cells after incubating with b) **1Lp** or c) **1Dp** for 1, 2, or 3 h.

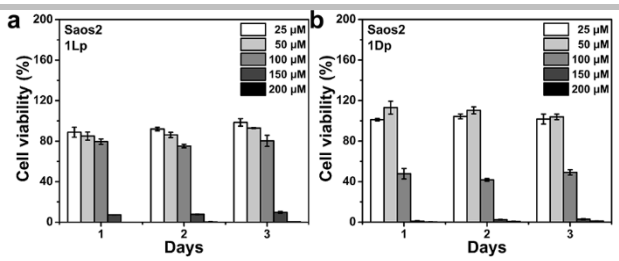


Figure S53. Cell viability of Saos2 cells after incubating with a) **1Lp** or b) **1Dp** for 24, 48, or 72 h.

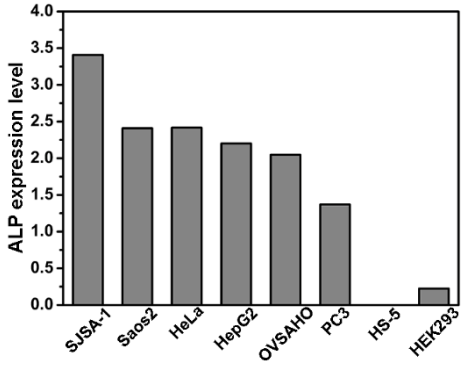


Figure S54. ALP expression levels of different cell lines[2].

Supplementary Tables

Table S1. The gene name and the protein coverage in Sample **PC**

Protein Coverage (%)	Gene Name	Protein Coverage (%)	Gene Name	Protein Coverage (%)	Gene Name	Protein Coverage (%)	Gene Name
62	H4C1	51	UBB	48	KRT1	48	KRT9
46	KRT2	44	RPS27A	43	ANXA2	40	GAPDH
39	KRT10	39	PFN1	39	UBC	38	ACAA2
38	H3C1	38	KRT16	37	HSD17B10	37	KRT14
37	PSMA6	34	CFL1	34	PKM	33	EEF1A1
33	EEF1A1P5	32	PDIA3	31	HSPD1	31	VDAC1
30	DECR1	30	HSPA5	29	ETFA	29	NACA
29	VDAC2	28	ECHS1	28	FN1	28	MDH2
28	PSMB5	28	RPL12	27	CKAP4	27	PHB
27	RPS3	26	ATP5F1B	26	RAN	25	ALDOA
25	CCT8	25	HSPA8	25	KRT5	24	ATP5F1A
24	CCT4	24	ENO1	24	RACK1	24	RPS2
23	SDCBP	22	ACTN1	22	CCT7	22	DSTN
22	PPIA	22	RPS4X	22	RPSA	22	TCP1
22	TMED7-TICAM2	21	EIF4A1	21	PHB2	21	PPIB
21	RPL18	21	RPLP0	20	CCT3	20	FLNA
20	GNB1	20	ILF2	20	PSMB4	20	SFXN1
20	STOM	19	ACTN4	19	ECH1	19	H1-5
19	TMED7	19	UQCRC2	19	VCP	18	ATP1A1
18	CAP1	18	MYH9	18	RPN1	18	RPS3A
18	SLC25A3	18	VAT1	18	VIM	17	FH
17	GLUD1	17	HSP90AB1	17	HSPA9	17	LDHA
17	PDIA6	17	RPL13A	17	RPL7A	16	DLD
16	EEF2	16	NXF1	16	VCL	15	EIF2S3
15	GOT2	15	HADHA	15	LDHB	15	MYOF
15	RARS1	15	RCC2	15	RPN2	15	SMU1
14	ANXA1	14	AP2M1	14	FSCN1	14	GANAB
14	ITGB1	14	LTA4H	14	THBS1	13	CCT6A
13	COL12A1	13	HSP90B1	13	HSPG2	13	MYO1C
13	TFRC	13	TOMM40	12	ACTR2	12	ACTR3
12	EIF3L	12	HNRNPU	12	VDAC3	11	ACADVL
11	AGPS	11	ALB	11	AP2A1	11	ATP2A2
11	FASN	11	FLNB	11	HARS1	11	HSPA4
11	LRPPRC	11	PFKM	11	PFKP	10	APMAP
10	DDX17	10	PIP4K2C	10	TKT	9	AP2A2
9	HSD17B4	9	ITGAV	9	PDIA4	9	SHMT2
8	CCT2	8	PLOD1	8	PLOD3	8	SDHA
7	ACO2	7	AP2B1	7	CAT	7	CLTC
7	COL1A1	7	COL6A3	7	CPT2	7	EFTUD2
7	EPB41L2	7	ILF3	7	MCM3	7	MTHFD1
7	NNT	7	PRKDC	7	SEC23A	6	ACLY
6	AHSG	6	ATP2B4	6	CD248	6	COL1A2
6	COPB2	6	DYNC1H1	6	HGFAC	6	NIBAN2
6	NRP1	6	PLOD2	6	SNRNP200	6	TLN1
6	UGGT1	5	IGF2R	5	PXDN	5	SLC3A2
4	FBN1	4	ITGA3	4	ITIH2	4	PLEC
3	C3	3	FAT1	3	PLG		

Table S2. The gene name and the protein coverage in Sample **PL**

Protein Coverage (%)	Gene Name	Protein Coverage (%)	Gene Name	Protein Coverage (%)	Gene Name	Protein Coverage (%)	Gene Name
46	VIM	40	CFL1	38	ENO1	25	HSPA8
25	TUBB	23	GAPDH	21	KRT2	20	ANXA2
18	KRT1	15	KRT10	14	ANXA1	14	KRT5
13	MARCKS	13	PDIA3	13	PGK1	12	KRT9
10	FKBP4	9	ANXA2P2	9	PDIA4	7	MSN
3	FLNA	2	FLNB				

Supplementary Movies: Description of the Supplementary Movies

Movie 1: 2D time-lapse CLSM of SJSA-1 cells being incubated with **1Lp** (200uM)
Movie 2: 2D time-lapse CLSM of SJSA-1 cells being incubated with **1Dp** (200uM)
Movie 3: 3D time-lapse CLSM of SJSA-1 cells being incubated with **1Lp** (200uM)
Movie 4: 3D time-lapse CLSM of SJSA-1 cells being incubated with **1Dp** (200uM)
Movie 5: 2D time-lapse CLSM of Saos2 cells being incubated with **1Lp** (200uM)
Movie 6: 2D time-lapse CLSM of Saos2 cells being incubated with **1Dp** (200uM)
Movie 7: 2D time-lapse CLSM of RFP expressing SJSA-1 cells being incubated with **1Lp** (200uM)
Movie 8: 2D time-lapse CLSM of RFP expressing SJSA-1 cells being incubated with **1Dp** (200uM)
Movie 9: 2D time-lapse CLSM of TNAP-RFP transfected HEK293 cells being incubated with **1Lp** (200uM)
Movie 10: 2D time-lapse CLSM of TNAP-RFP transfected HEK293 cells being incubated with **1Dp** (200uM)
Movie 11: 3D time-lapse CLSM of actin-RFP transfected SJSA-1 cells being incubated with **1Lp** (200uM)
Movie 12: 3D time-lapse CLSM of actin-RFP transfected SJSA-1 cells being incubated with **1Dp** (200uM)
Movie 13: 3D time-lapse CLSM of actin-RFP transfected Saos2 cells being incubated with **1Lp** (200uM)
Movie 14: 3D time-lapse CLSM of actin-RFP transfected Saos2 cells being incubated with **1Dp** (200uM)
Movie 15: 3D time-lapse CLSM side views of SJSA-1 cells and Saos2 cells as controls

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Author Contributions

S. L. and B. X. conceived and designed the experiments and wrote the manuscript. S. L. performed most of the experiments, analyzed data, prepared figures. Q. Z. helped the synthesis. H. H. helped with the transfection of HEK293 cells. M. Y. helped with up-taking and fractionation study. W. T. helped with the 3D CLSM imaging. J. G. helped with TEM imaging.