Supporting Information for

Enzymatically Forming Intranuclear Peptide Assemblies for Selectively Killing Human Induced Pluripotent Stem Cells (iPSCs)

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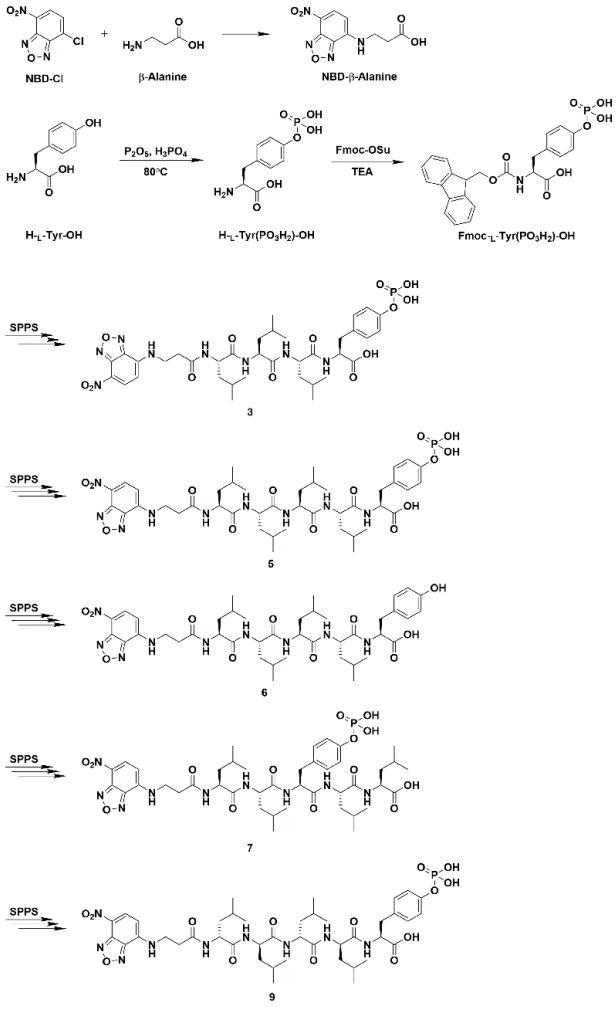
Content

S1. Materials and instruments

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. 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. UV-Vis spectra were obtained with a Varian Cary 50 Bio UV-Visible Spectrophotometer.

S2. Synthesis and characterization of the precursors



Scheme S1. Synthetic route of **3**, **5**, **6**, **7** and **9**.

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). MS: calc. $[M-H]^-$ = 251.04, obsvd. ESI-MS: M/Z = 250.95.

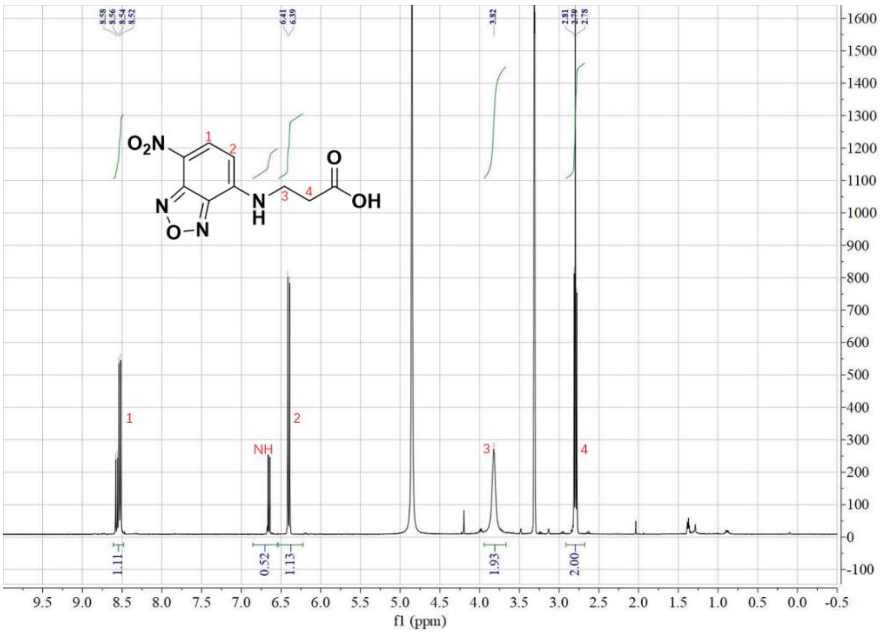


Figure S1. ^{1}H NMR spectrum of NBD-β-alanine.

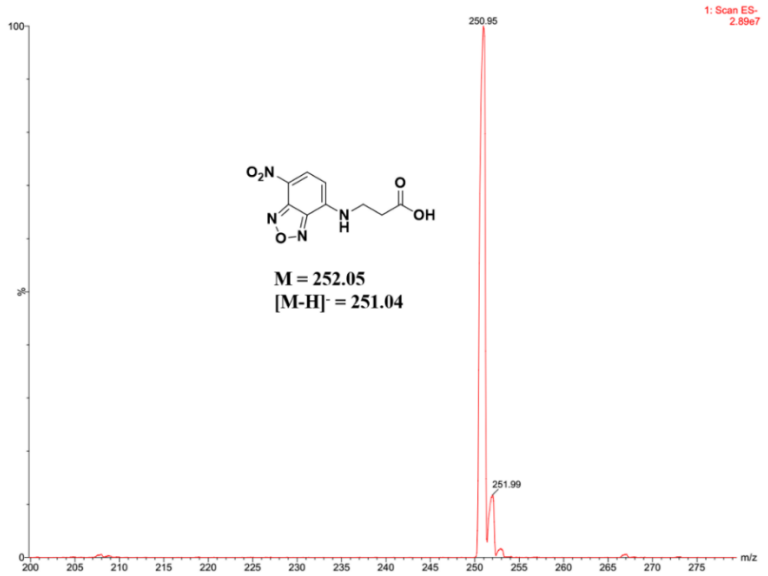


Figure S2. Mass spectrum of NBD-β-Alanine (M/Z = 250.95).

Synthesis of Fmoc-$_L$-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.

Synthesis of 3, 5, 6, 7 and 9

3, **5**, **7** and **9** were synthesized by solid phase peptide synthesis with 2-Cl-trityl chloride resin, Fmoc-$_L$-Tyr(PO_3H_2)-OH, Fmoc-$_L$-Tyr(tBu)-OH, Fmoc-$_L$-Leu-OH, Fmoc-$_D$-Leu-OH, NBD-β-alanine, HOBt and HBTU. Purification with HPLC provided **3**, **5**, **6**, **7** and **9** as yellow power.

MS of **3**: calc. $[M-H]^-$ = 833.32, obsvd. ESI-MS: M/Z = 833.61.

^{1}H NMR of **5** (400 MHz, DMSO-d_6) δ (ppm): 7.77 (d, 1H, J = 12 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, 3H), 1.41 (m, 8H), 1.25 (m, 1H), 0.79 (m, 24H). MS of **5**: calc. $[M-H]^-$ = 946.41, obsvd. ESI-MS: M/Z = 946.63.

MS of **6**: calc. $[M-H]^-$ = 866.44, obsvd. ESI-MS: M/Z = 866.52.

^{1}H NMR of **7** (400 MHz, DMSO-d_6) δ (ppm): 7.72 (d, 1H, J = 12 Hz), 7.08 (d, 2H, J = 8 Hz), 7.02 (d, 2H, J = 8 Hz), 6.44 (d, 1H, J = 12 Hz), 4.36 (m, 1H), 4.28 (m, 4H), 2.98 (m, 2H), 2.80 (m, 2H), 2.62 (m, 2H), 1.52 (m, 2H), 1.39 (m, 8H), 1.24 (m, 2H), 0.79 (m, 24H). MS of **7**: calc. $[M-H]^-$ = 946.41, obsvd. ESI-MS: M/Z = 946.60.

^{1}H NMR of **9** (400 MHz, DMSO-d_6) δ (ppm): 7.75 (m, 1H), 7.12 (d, 2H, J = 8 Hz), 7.00 (d, 2H, J = 8 Hz), 6.44 (d, 1H, J = 8 Hz), 4.46 (m, 1H), 4.23 (m, 4H), 2.98 (m, 2H), 2.75 (m, 2H), 2.61 (m, 2H), 1.48 (m, 12H), 0.81 (m, 24H). MS of **9**: calc. $[M-H]^-$ = 946.41, obsvd. ESI-MS: M/Z = 946.53.

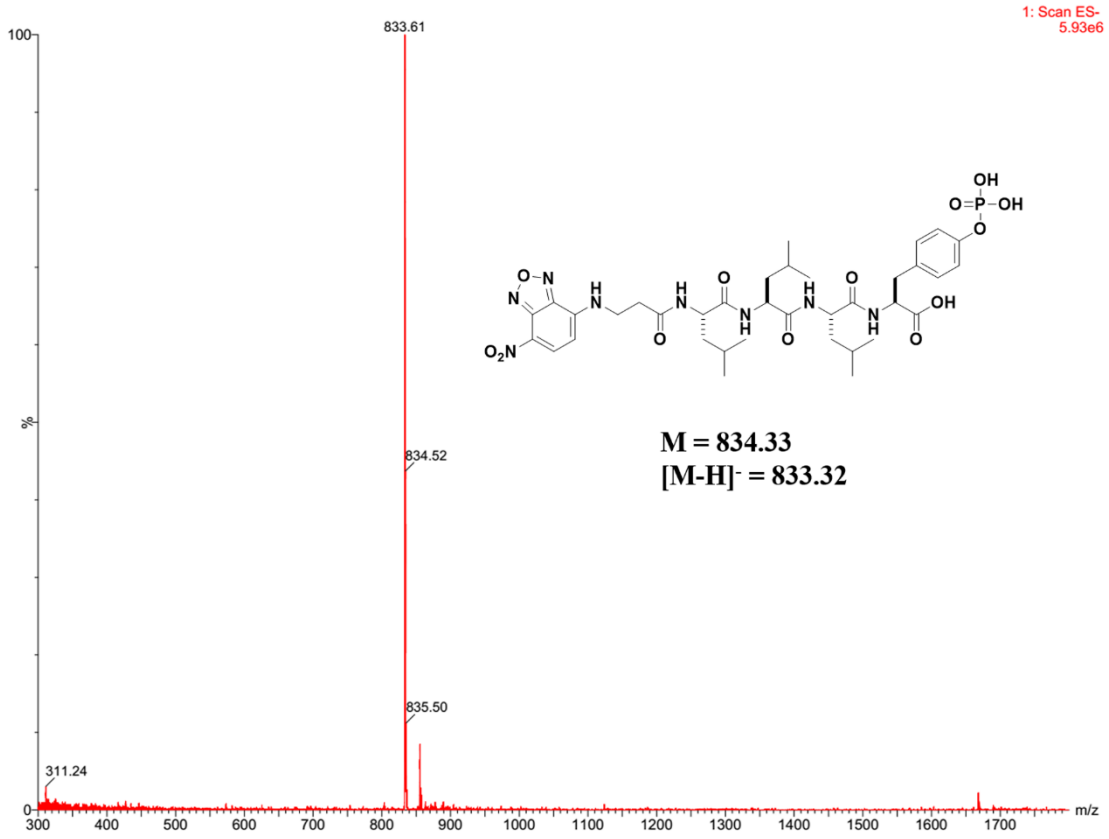


Figure S3. Mass spectrum of **3** (M/Z = 833.61).

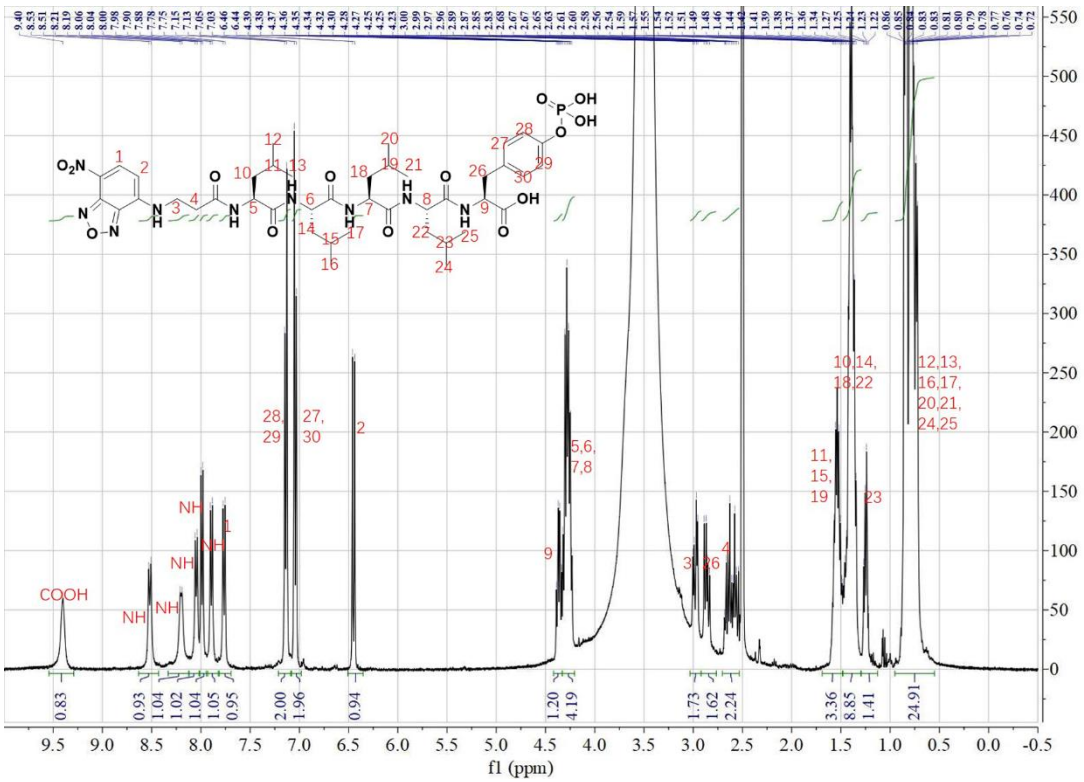


Figure S4. [1]H NMR spectrum of **5**.

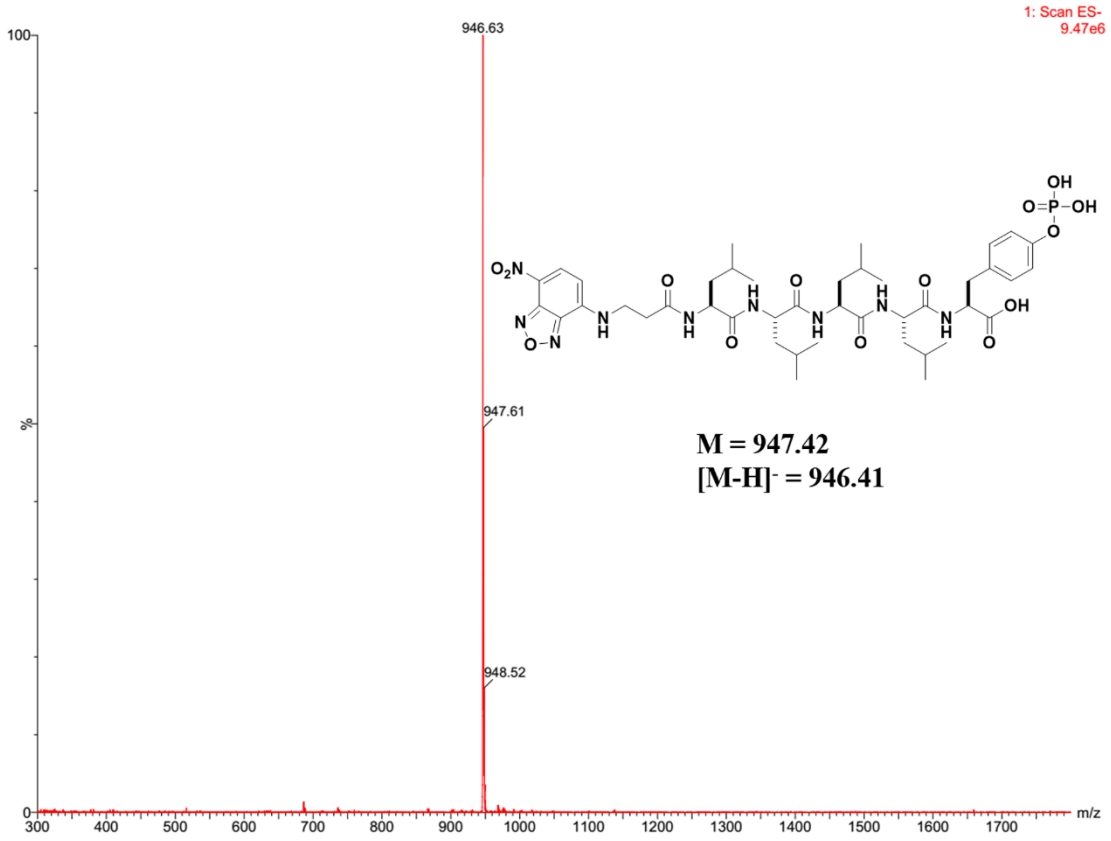


Figure S5. Mass spectrum of **5** (M/Z = 946.63).

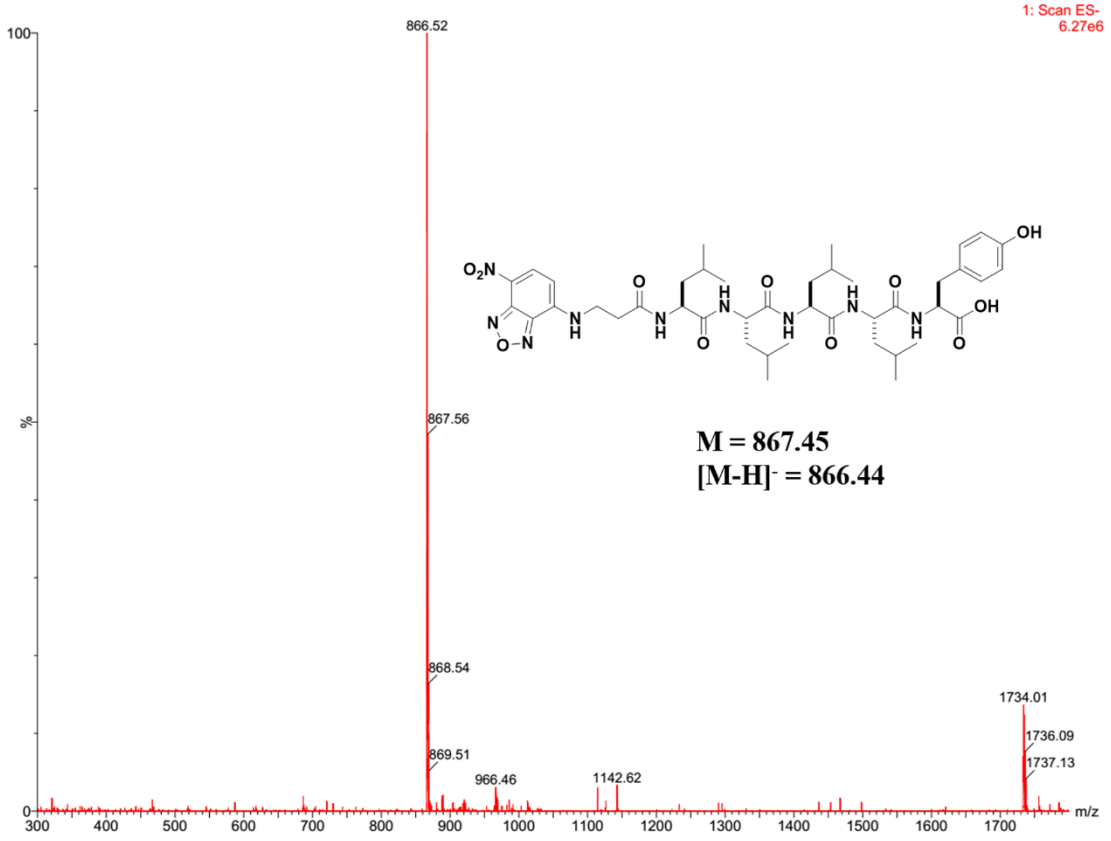


Figure S6. Mass spectrum of **6** (M/Z = 866.52).

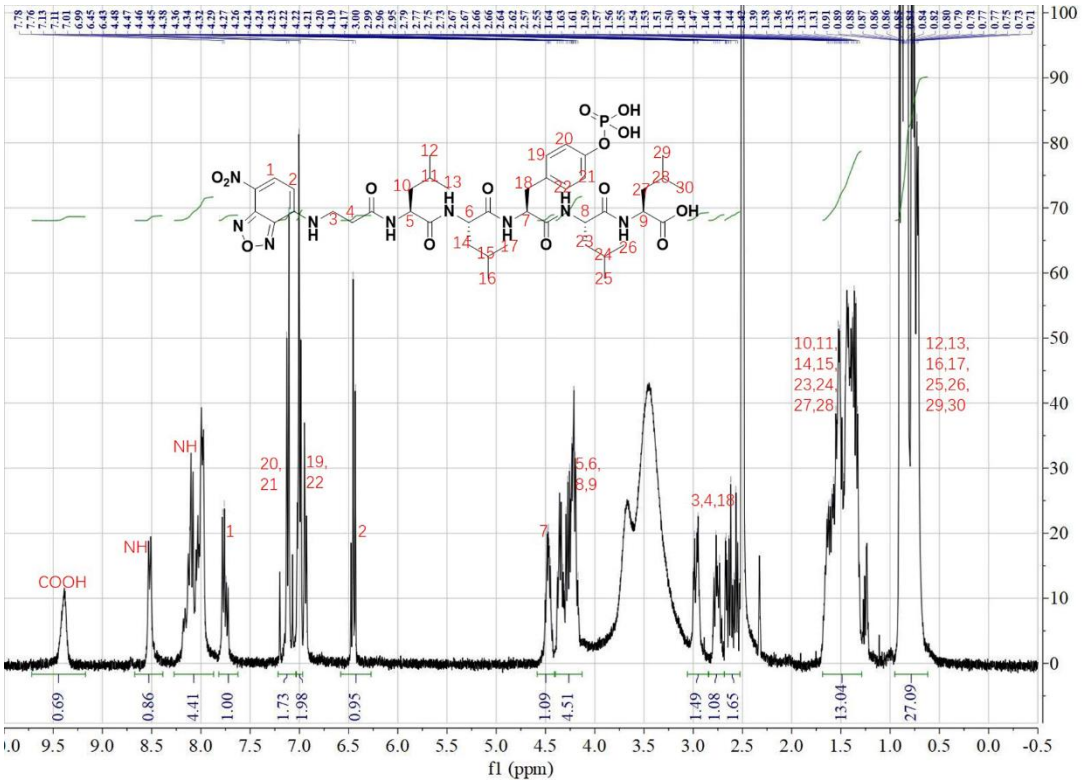


Figure S7. ^{1}H NMR spectrum of **7**.

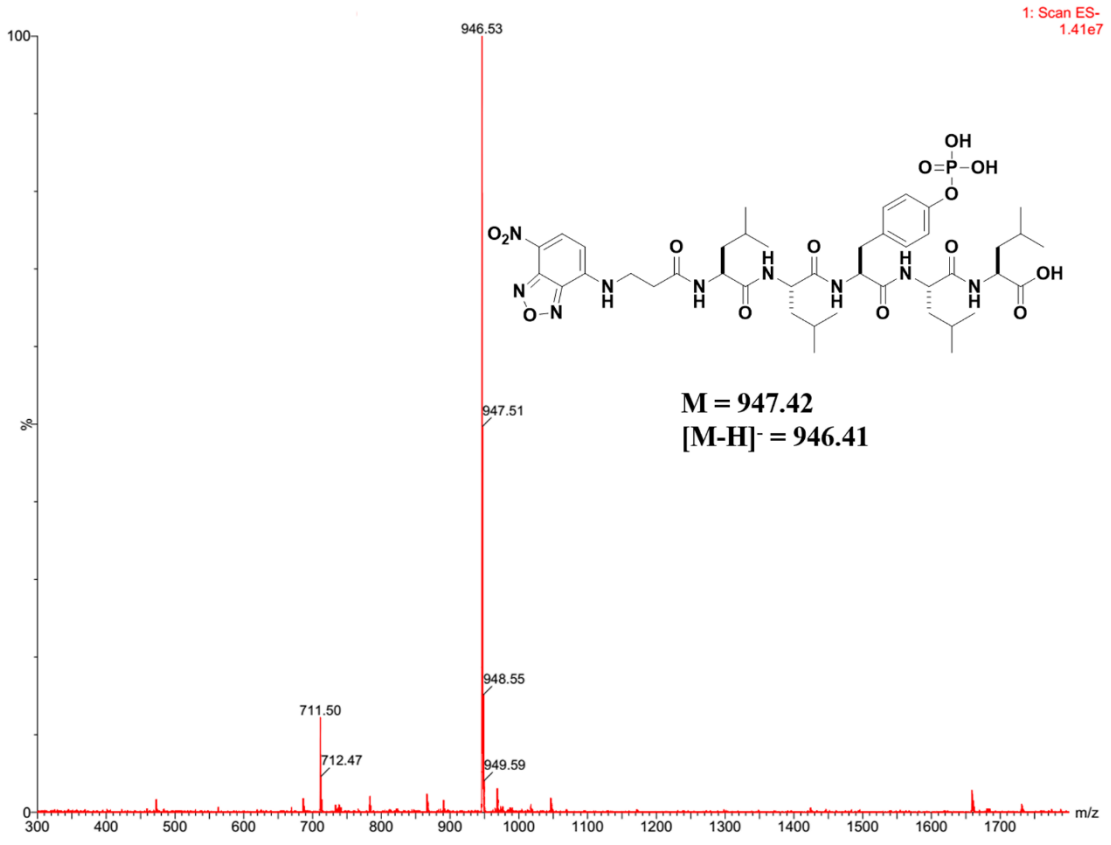


Figure S8. Mass spectrum of **7** (M/Z = 946.53).

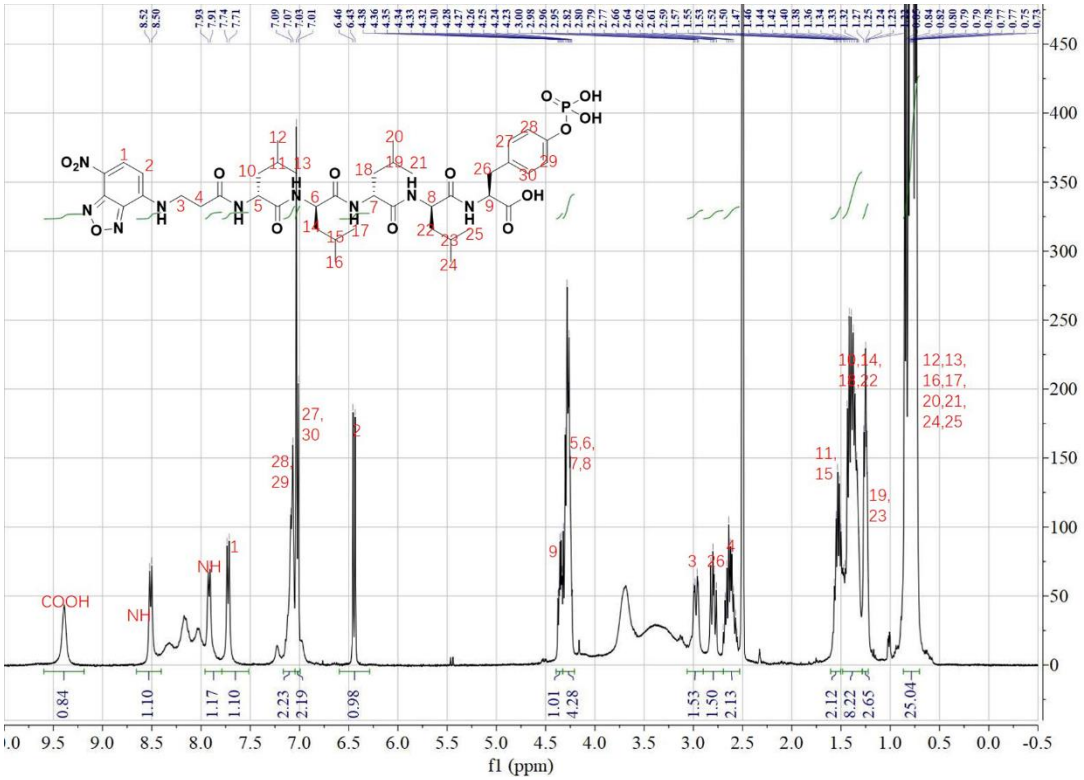


Figure S9. ^{1}H NMR spectrum of **9**.

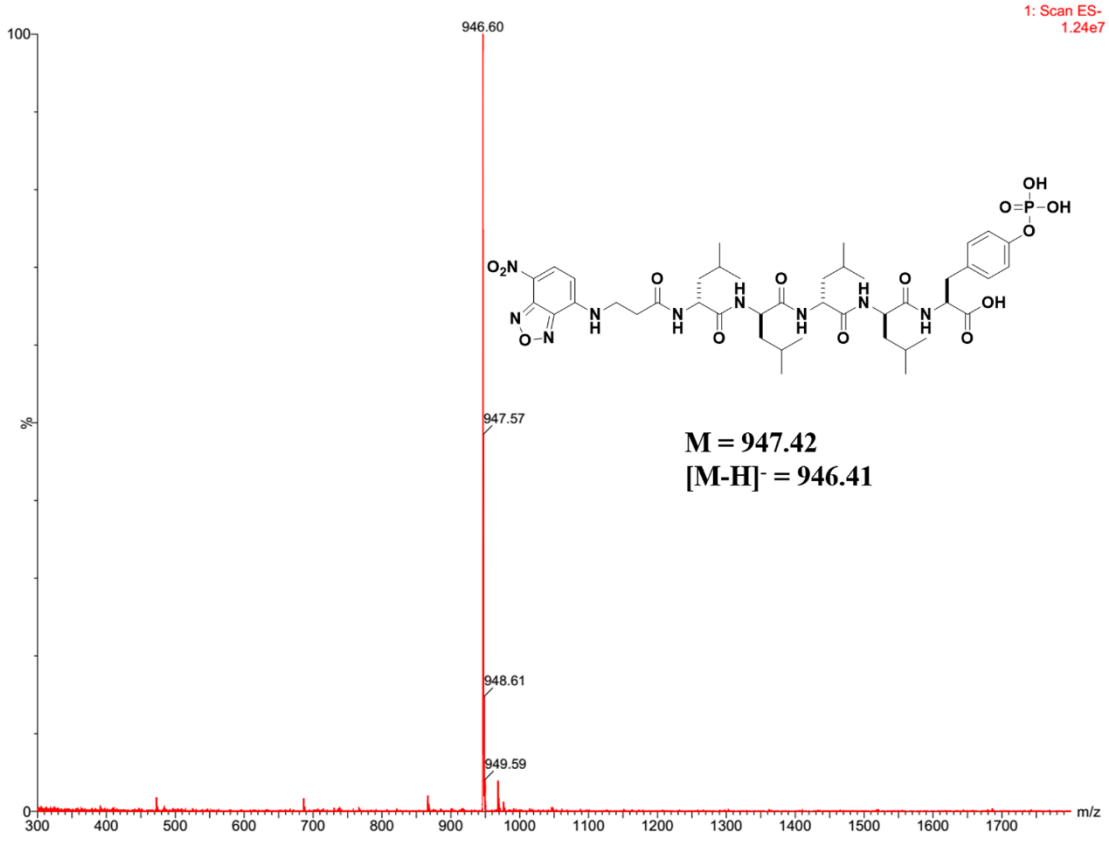


Figure S10. Mass spectrum of **9** (M/Z = 946.60).

S3. TEM sample preparation

After placing 5 μL samples on 400 mesh copper grids coated with continuous thick carbon film (~35 nm) which is 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 Morgagni 268 transmission electron microscope.

S4. 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 Synergy H1 hybrid multi-mode microplate reader. Plot I_{378}/I_{393} against the concentrations of compounds. The concentration at the turning point is the CMC.

S5. Dephosphorylation rate measurement

To 100 μL solution of **5**, **7** or **9** in PBS, ALP was added, and the mixtures were shaken at 37°C. At different time point, 900 μL methanol was added to quench the enzyme reaction. The reaction mixtures were analyzed with LC-MS.

S6. Cell culture

Human induced pluripotent stem cell line A21 was generated from human normal dermal fibroblasts by using the StemRNATM-NM Reprogramming kit (Stemgent, Cat # 00-0076). hiPSCs were routinely cultured and passaged on 6-well plates coated with 0.25μg/cm^2 iMatrix-511 (Recombinant Laminin-511) (ReproCell) with NutriStem XF/FFTM medium (Biological Industries). HS-5 cell line and HEK293 cell line were purchased from American Type Culture Collection (ATCC, USA). HS-5 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. HEK293 cells were cultured in Minimal Essential Medium (MEM) 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.

S7. Differentiation of human iPSC to iPS-derived hematopoietic progenitor cells (HPCs)

iPSCs were differentiated into hemopoietic progenitor cells (HPCs) by using a 3D-bioreactor platform[1]. HPCs released form iPSC-spheroids after 9-10 days' differentiation were collected and characterized. Hematopoietic lineage specific marker expression of harvested HPCs were analyzed by flow cytometry. About 97.6% of these HPCs were CD31+CD43+ double positive, but only about 13% are OCT4+, indicative of commitment to hematopoietic lineage (Figure S20). These iPS-derived HPCs were used for **5** (400 uM, 2hr) cytotoxicity assay.

S8. Cell viability

iPSCs were plated in 6-well plates and incubated for 24 to 48 hours, then media were replaced with fresh one (2 ml) supplemented with PBS (control) **5** (200 μM, 300 μM, and 400 μM), **7** (400 μM) or **9** (400 μM), and incubated for 2 hours. Media were removed and cells were rinsed with PBS once, fresh normal cultural media were added and incubated for 30 min. All cells were collected and stained with trypan blue, live cells were counted using Cellometer Auto 2000 (Nexcelom Bioscience). Data were obtained by from three independent wells (n = 3).

iPS-derived HPCs were plated in 12-well plates and incubated overnight, then media were replaced with fresh one (2 ml) supplemented with PBS (control) **5** (400 μM) incubated for 2 hours. All cells were collected and stained with trypan blue, live cells were counted using Cellometer Auto 2000 (Nexcelom Bioscience). Data were obtained by from three independent wells (n = 3).

We determined the cytotoxicity against HS-5 cells and HEK293 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 different concentration of **5** (immediately diluted from fresh prepared 10 mM stock solution). After 1/2/3 hours, the culture medium with **5** 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 DTX880 Multimode Detector. The results were calculated as cell viability percentage relative to untreated cells. Data were obtained by from three independent wells (n = 3).

S9. 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 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^{-1}) for 10 minutes. After being washed with culture medium (2 mL × 2), the cells were incubated with fresh medium containing precursor **5** in a Tokai Hit stage top incubator (STXF-WSKMX-SET) to be used for CLSM imaging.

All the CLSM images were obtained using Zeiss LSM 880 confocal microscopy at the lens of 63× with oil. The lasers used are 405 nm and 488 nm.

S10. Degradation

5 million HS-5 cells were made into 1 mL lysate by freeze-thaw lysis method. To 100 μL lysate, **5** (200 μM) and rhodamine 6G (inner standard) were added. The mixtures were shaken at 37°C. At different time point, 900 μL methanol was added to quench the reaction. The reaction mixtures were analyzed with LC-MS.

S11. Supporting data

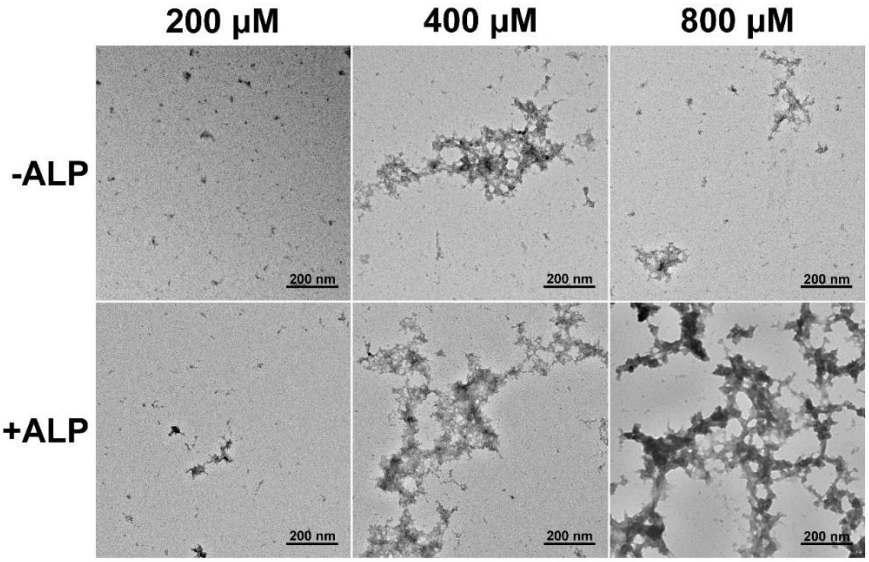


Figure S11. Transmission electron microscopes (TEM) images of **3** (200, 400, and 800 μM, PBS) before and after dephosphorylation by ALP (0.5 U/mL) for 24 h.

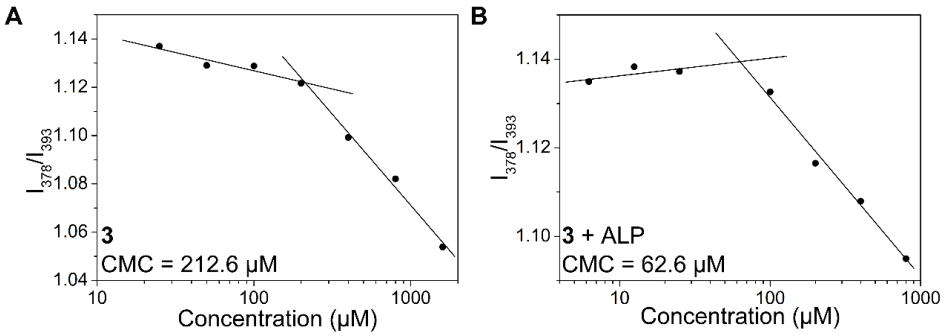


Figure S12. Critical micelle concentration (CMC) of (A) **3** and (B) **3** + ALP (0.5 U/mL, 37°C, 24 h).

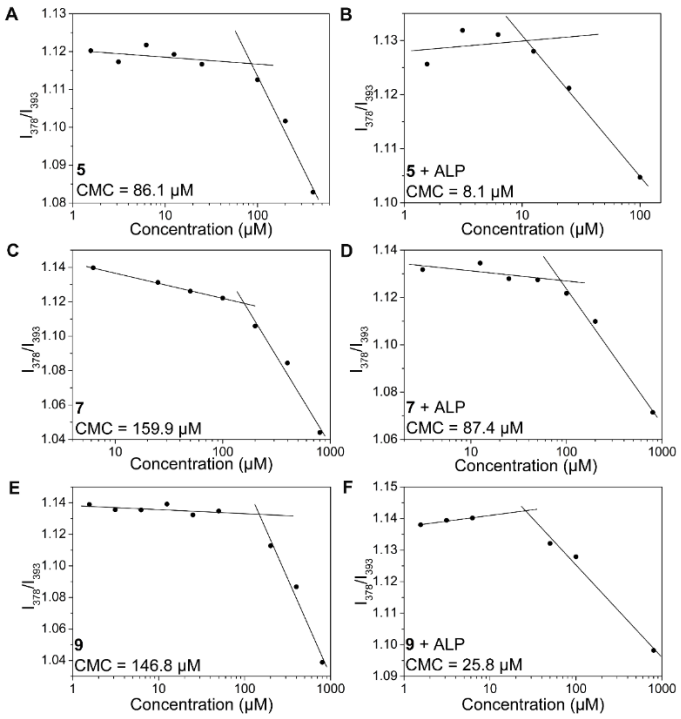


Figure S13. Critical micelle concentration (CMC) of (A) **5** (PBS), (B) **5** + ALP, (C) **7** (PBS), (D) **7** + ALP, (E) **9** (PBS) and (F) **9** + ALP. ALP concentration is 0.5 U/mL (PBS, 37°C, 24 h).

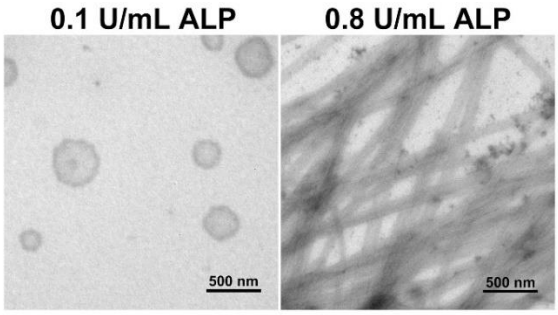


Figure S14. Transmission electron microscopes (TEM) images of **5** (200 μM, PBS) after dephosphorylation by ALP (0.1 U/mL or 0.8 U/mL) for 1 h.

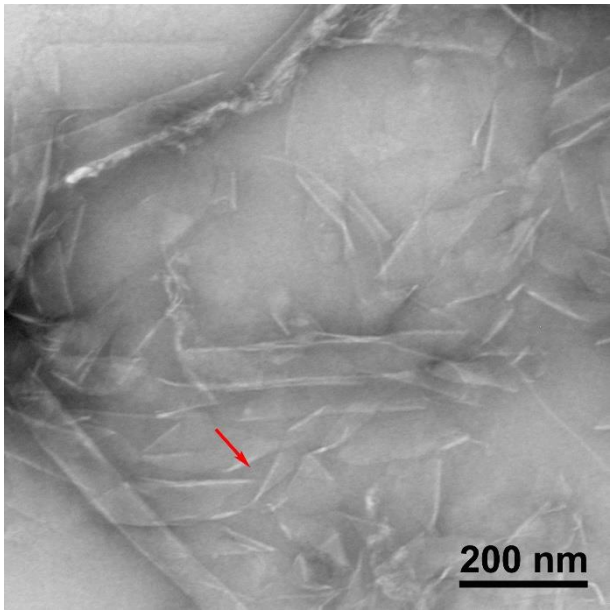


Figure S15. Transmission electron microscopes (TEM) image of **5** (400 μM, PBS) after dephosphorylation by ALP 0.8 U/mL for 2 h. The red arrow shows the folding of nanoribbons, indicating that the self-assembly nanostructures are more like nanoribbons than nanotubes.

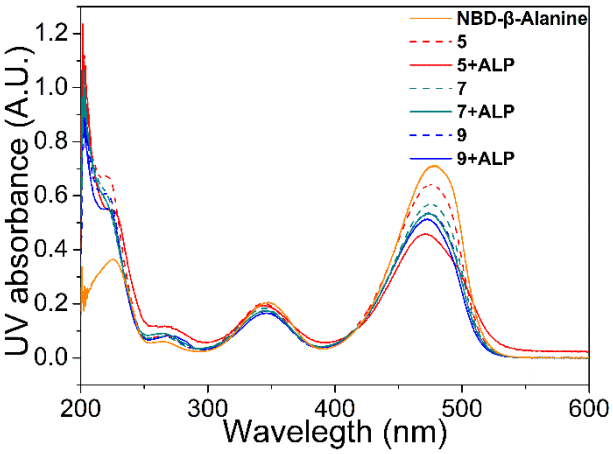


Figure S16. UV spectra of NBD-β-alanine, **5**, **7** and **9** (25 μM, PBS) before and after dephosphorylation by ALP (0.5 U/mL, 37°C, 24 h).

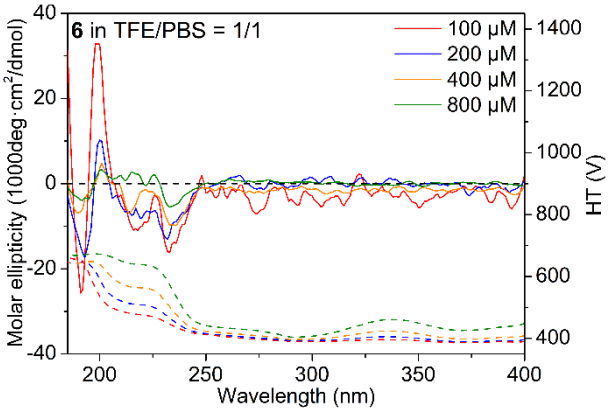


Figure S17. Circular dichroism (CD) spectra (solid line) and HT voltages (dash line) of **6** in TFE/PBS = 1/1.

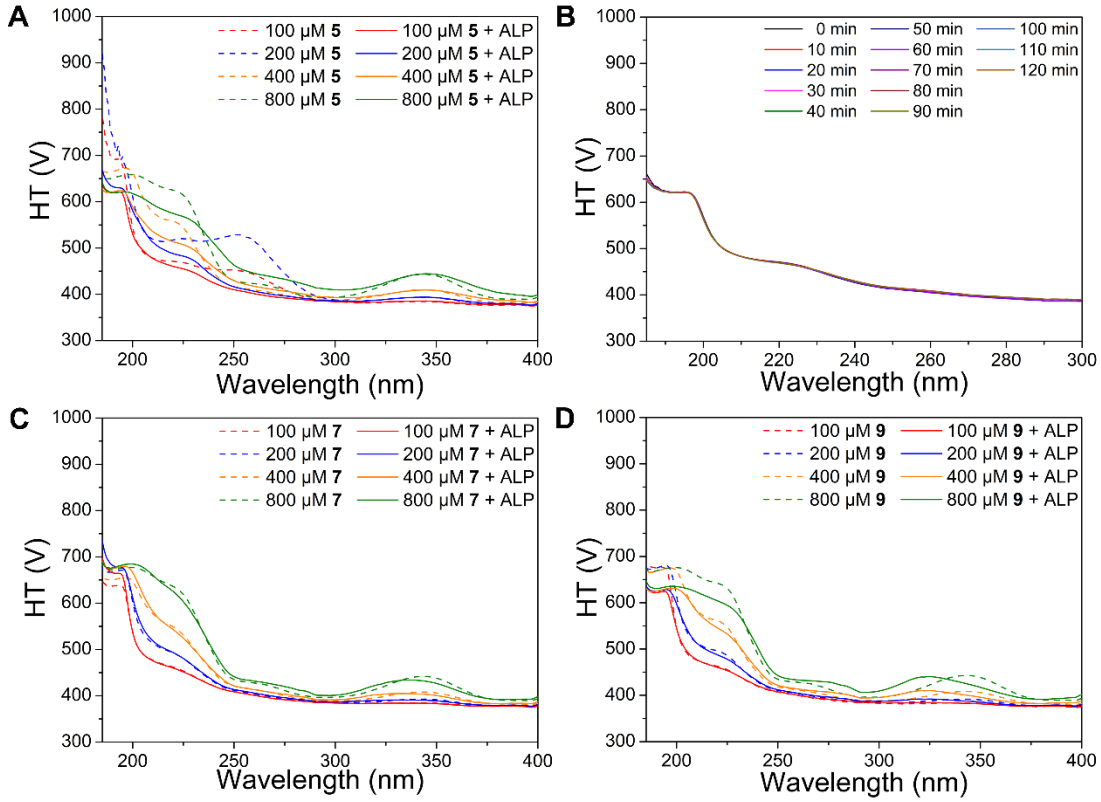


Figure S18. HT voltages of CD spectra (A) Figure 6A, (B) Figure 6B, (C) Figure 7A, and (D) Figure 7B.

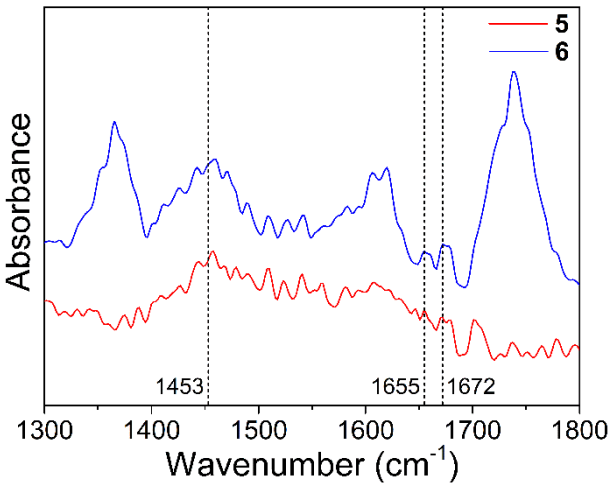


Figure S19. Fourier-transform infrared (FTIR) spectra for 20 mM **5** and **6** in D_2O.

Table S1. iPSC number after treatment of **5** (200 μM, 300 μM, 400 μM) for 2 h

Concentration of **5**	Ctrl			200 μM			300 μM			400 μM		
Cell No. ($\times10^4$)	76.4	66.6	70.5	24.7	20.5	21.1	13.4	12.0	14.1	4.24	4.26	6.36

Table S2. iPSC number after treatment of **7**, **9** (400 μM) for 2 h

Compounds	Ctrl			**7**			**9**		
Cell No. ($\times10^4$)	72	114	108	97	119	98	101	100	88

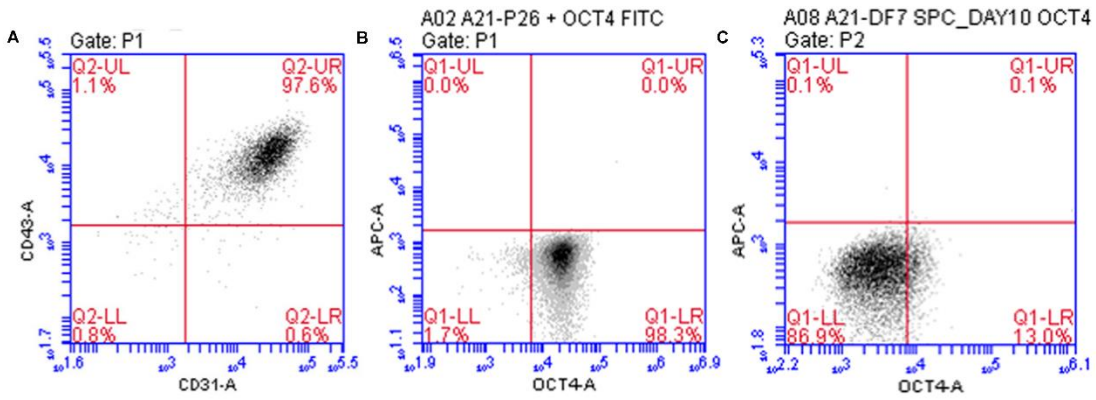


Figure S20. (A) CD31 and CD43 expression of human iPS-derived HPCs. OCT4 expression in (B) human iPSCs and (C) human iPS-derived HPCs.

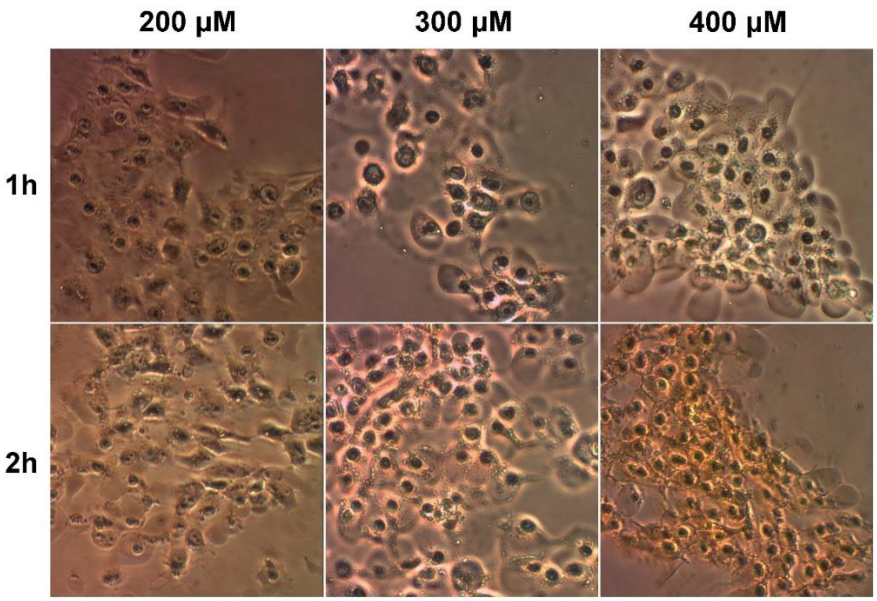


Figure S21. Optical photos of iPSCs after incubating with **5**.

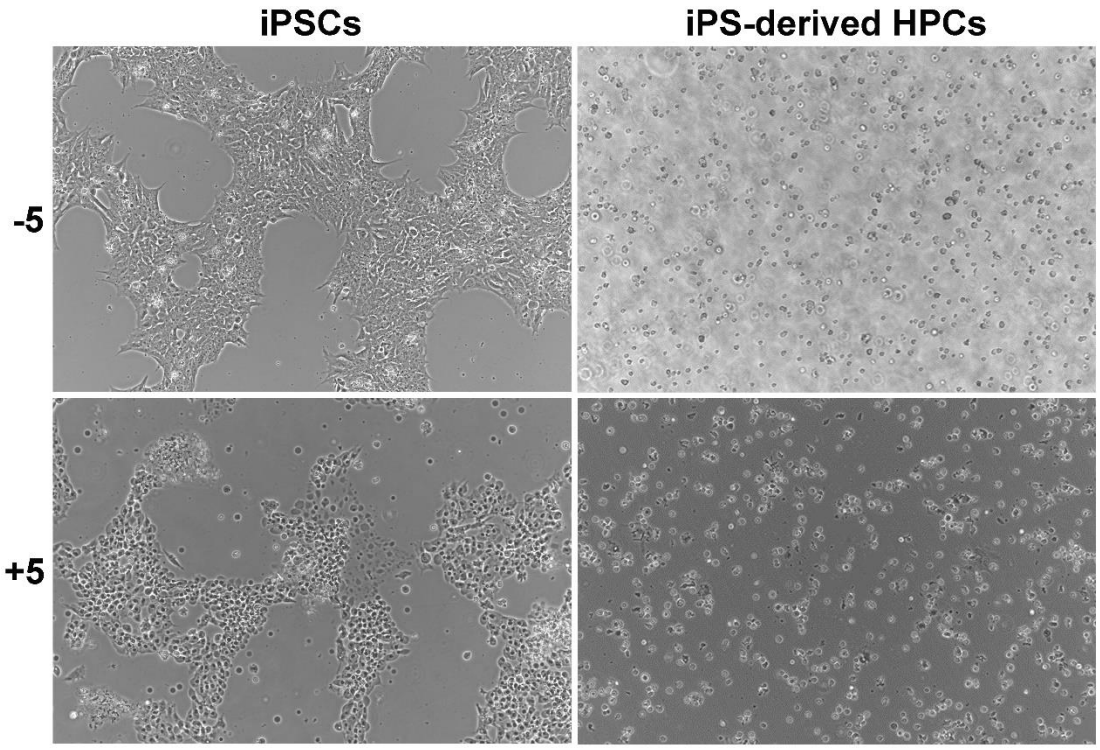


Figure S22. Optical images of iPSCs and iPS-derived HPCs before and after incubating with **5** (400 μM) for 2 h.

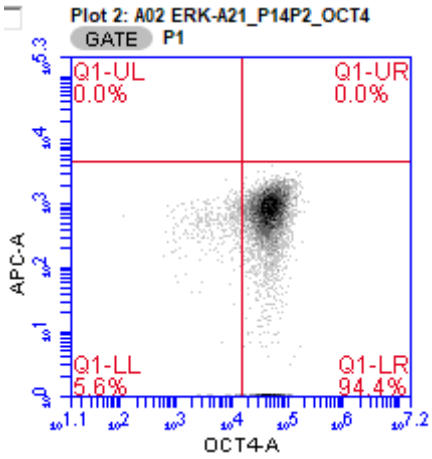


Figure S23. OCT4 expression in human iPSCs derived from A21.

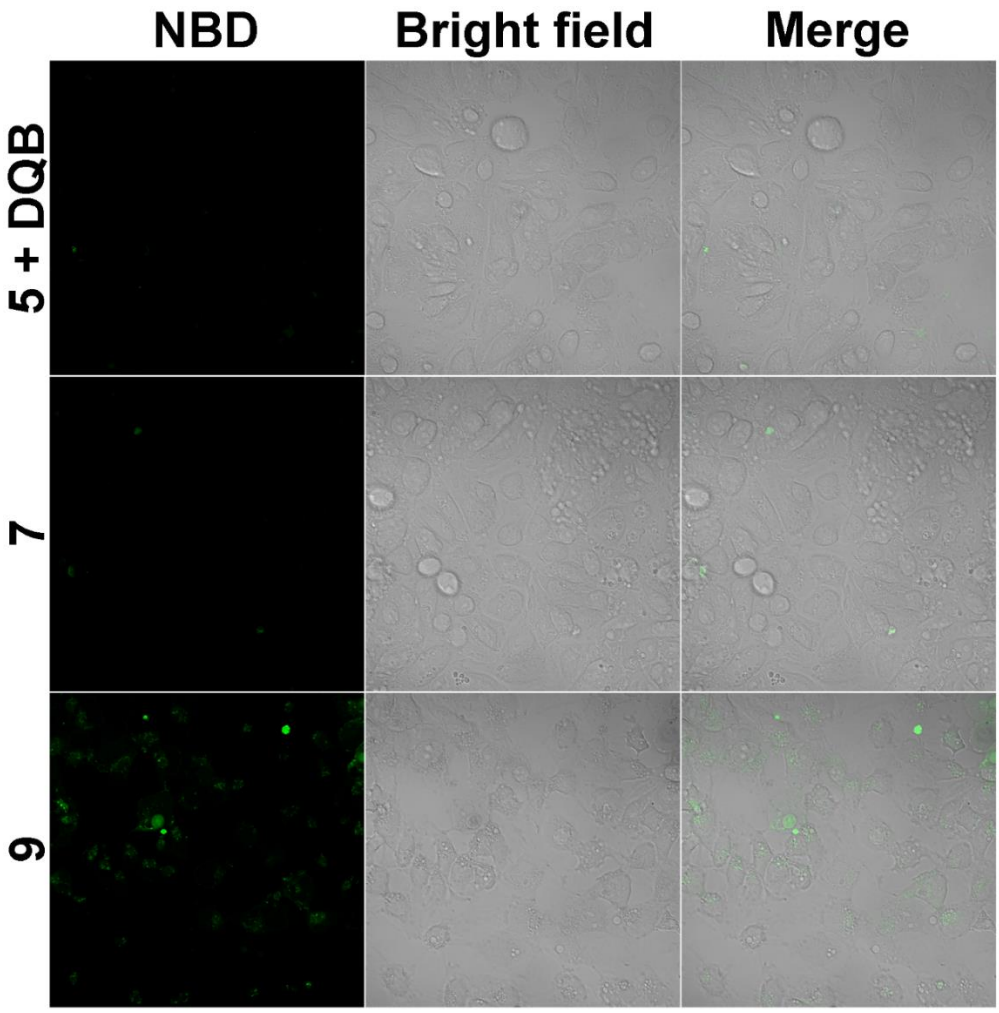


Figure S24. Confocal laser scanning microscopy (CLSM) images of iPS cells after being treated by **5** (400 μM) with DQB (5 μM), **7** (400 μM) or **9** (400 μM) for 2 h.

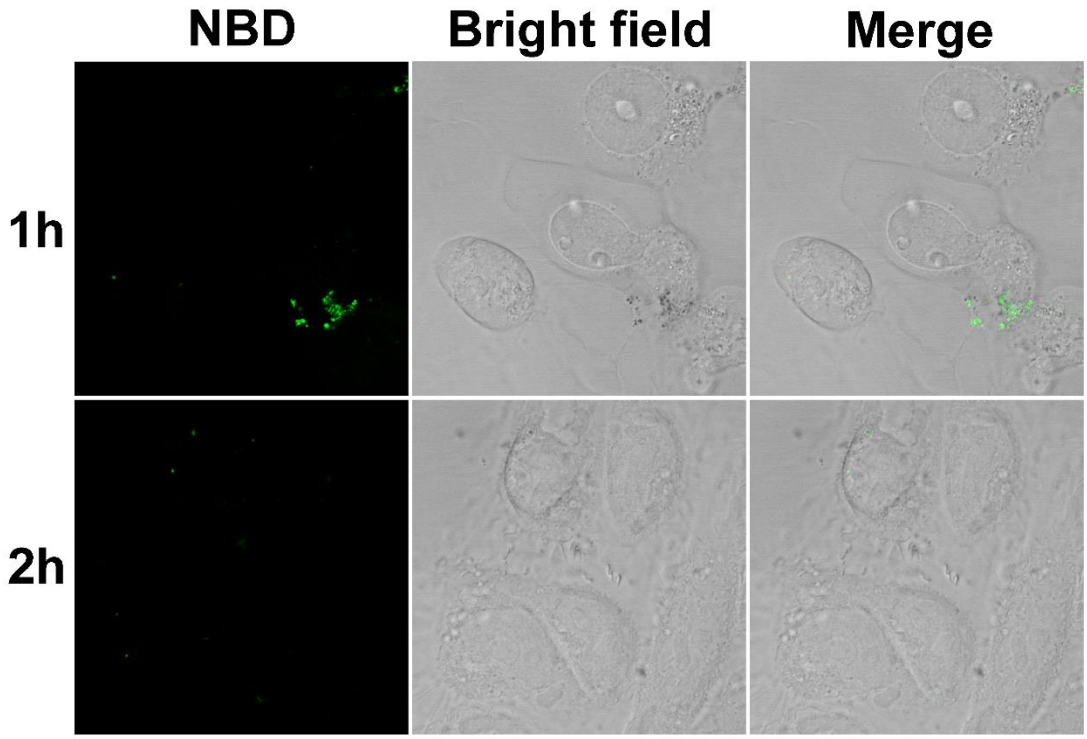


Figure S25. CLSM images of iPSCs after incubating with **5** (200 μM).

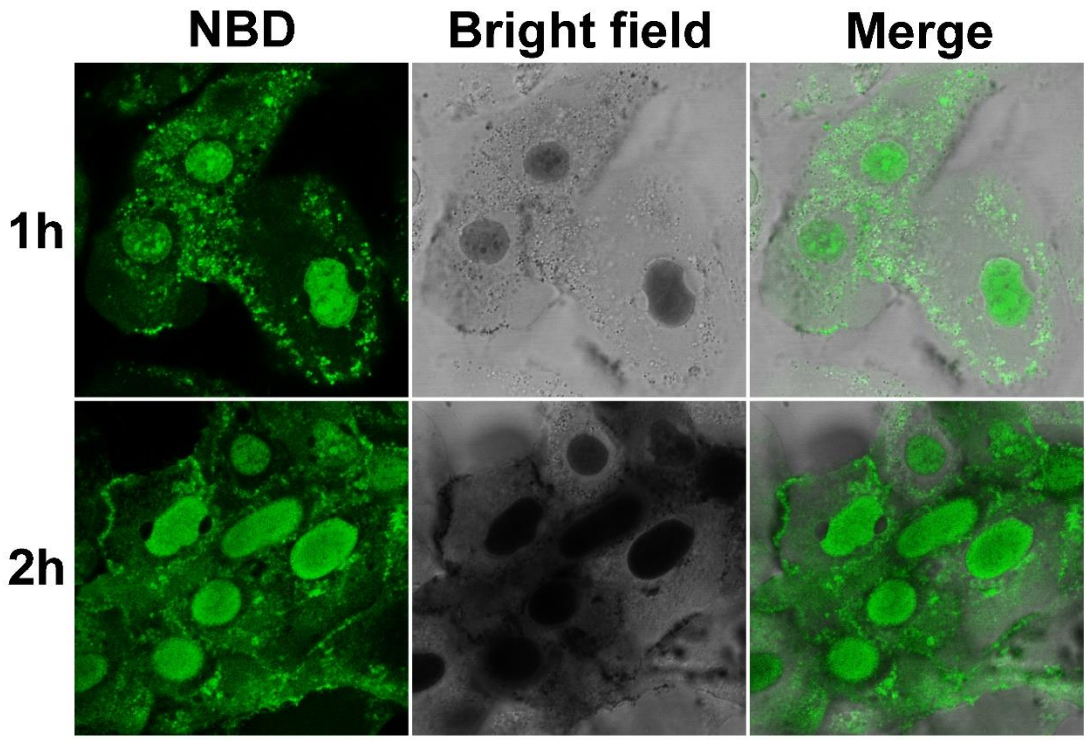


Figure S26. CLSM images of iPSCs after incubating with **5** (400 μM).

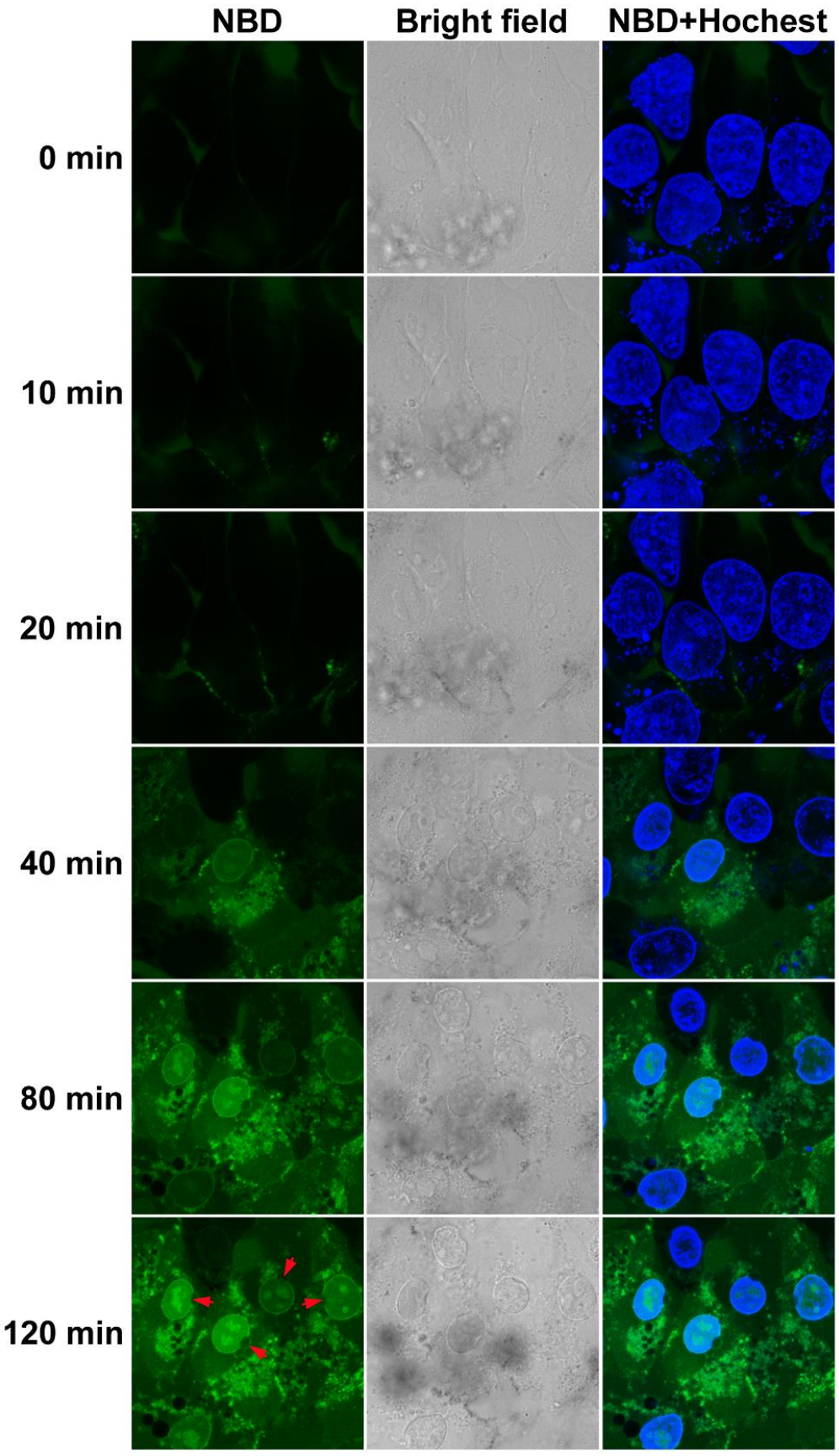


Figure S27. CLSM images of iPSCs after incubating with **5** (400 μM) for different time, the red arrows show the disruption of nucleus membrane.

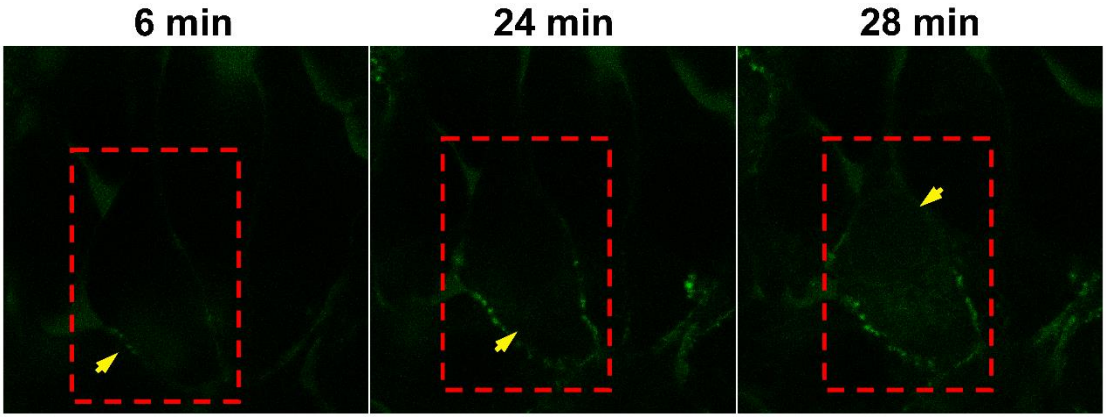


Figure S28. CLSM images of iPCs after incubating with **5** (400 μM) for 6 min, 24 min and 28 min. The red dash squares show the exampled cell. The yellow arrows show the fluorescent point on cell membrane, fluorescence in cytoplasm or nucleus.

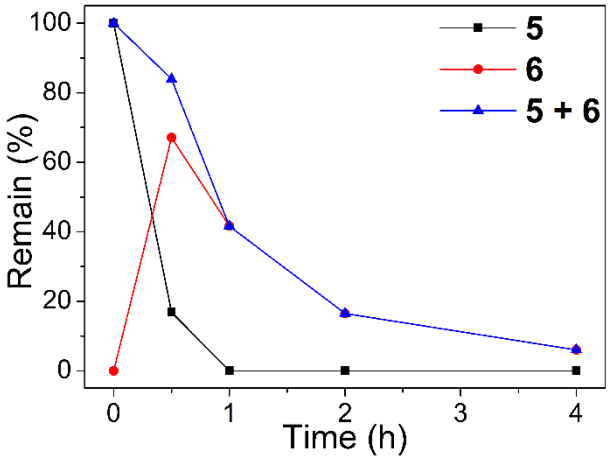


Figure S29. The remain percentages of **5** and **6** after **5** (200 μM, PBS) being treated by HS-5 lysate (5 million cells in 1 mL) for different time.

Table S3. LLLLY motif in the transmembrane domain of membrane proteins

Gene	Uniprot No.	Starting position of LLLLY	Location
ABCA1	O95477	1741	endosome, cell membrane
ABCA3	Q99758	1158	late endosome membrane, lysosome membrane
ABCA7	Q8IZY2	1622	cell membrane, Golgi membrane, ruffle membrane, early endosome membrane

ADCK2	Q7Z695	113	membrane
ADCY9	O60503	989	cell membrane
ATP13A3	Q9H7F0	44	late endosome membrane, recycle endosome membrane, early endosome membrane
CSF1R	P07333	534	cell membrane
GOSR1	O95249	243	Golgi membrane
GPR156	Q8NFN8	234	plasm membrane
GPR18	Q14330	156	cell membrane
ICMT	O60725	104	endoplasmic reticulum membrane
KCNS2	Q9ULS6	329	plasm membrane
MFSD9	Q8NBP5	364	membrane
PHTF1	Q9UMS5	103	endoplasmic reticulum membrane, cell membrane
PIGZ	Q86VD9	370	endoplasmic reticulum membrane
SPATA31E1	Q6ZUB1	81	membrane
SPPL2A	Q8TCT8	344	late endosome membrane, lysosome membrane
TSPO2	Q5TGU0	110	endoplasmic reticulum membrane, cell membrane

Reference

1. Feng, Q.; Zhang, M.; and Lu, S., Methods and systems for manufacturing hematopoietic linage cells. PCT/US2019/057929, WIPO PCT WO 2020/086889 A1.