Supporting Information

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S1. Experimental materials and instruments

All the solvents and chemical reagents were used directly as received from the commercial sources without further purification. All of the products (**TPD-2p**, **TPD-1p**, **fTPD-2p** and **fTPD-1p**) were purified with Water Delta600 HPLC system, equipped with an XTerra C18 RP column and an in-line diode array UV detector. ^{1}H-NMR spectra were obtained on Varian Unity Inova 400, LC-MS spectra on a Waters Acouity ultra performance LC with Waters MICRO-MASS detector, TEM images on Morgagni 268 transmission electron microscope, confocal microscopy images on Leica TCS SP2 spectral confocal microscope or Marianas Spinning Disk confocal microscope.

S2. Synthesis and characterizations

We prepared all the compounds by solid phase peptide synthesis (SPPS) in fair yields (60-80%) and reasonable scales (0.1-0.5 g). The standard solid-phase peptide synthesis (SPPS)[1] uses 2-chlorotriyl chloride resin (100-200 mesh and 0.3-0.8 mmol/g) and N-Fmoc-protected amino acids with side chains properly protected. Before that, we prepared NBD-COOH, which was directly used in SPPS, from NBD-Cl based on literature[2]. The following scheme (Figure S1) illustrates the synthetic procedure of **fTPD-1p** and **TPD-1p**. The synthetic route of others is the same with that of **TPD-1p** and **fTPD-1p**.

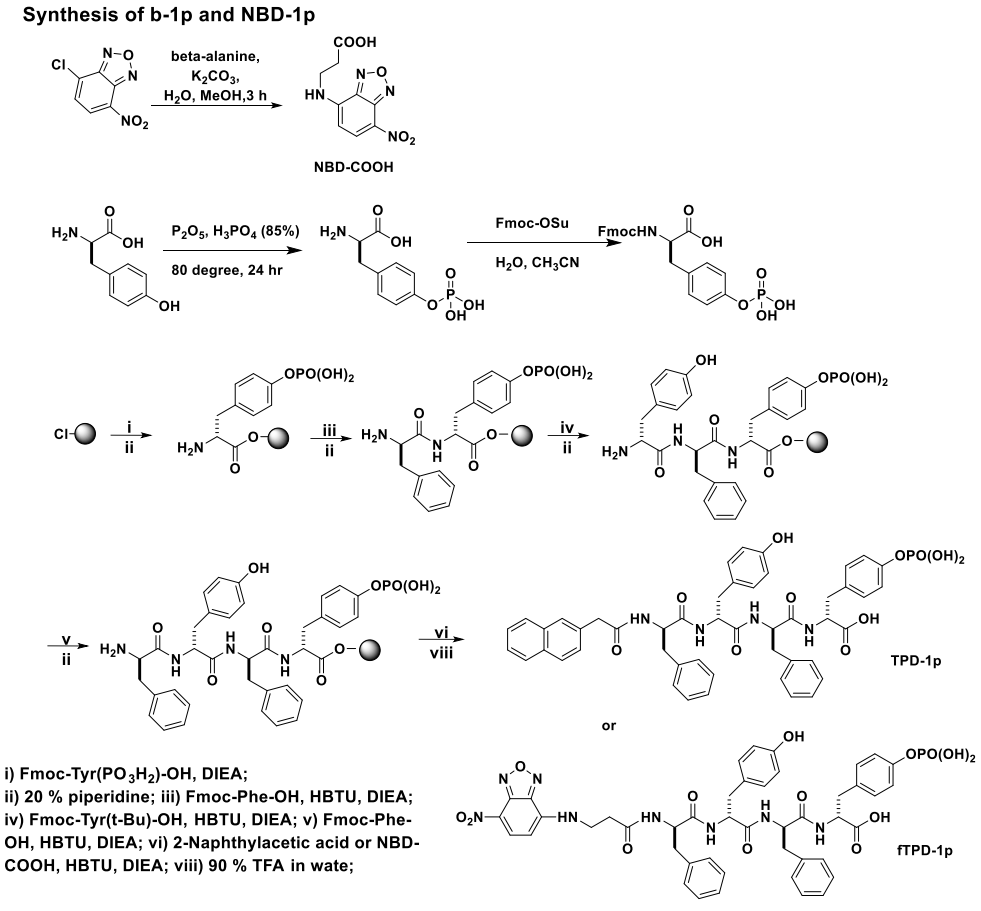

Figure S1. Synthesis route of **TPD-1p** and **fTPD-1p**

The NMR spectra of precursors and hydrogelators.

TPD-1p: ^{1}H-NMR (400 MHz, DMSO-d_6) δ 8.29 (d, J = 8.0 Hz, 1H), 8.20 (d, J = 8.0 Hz, 1H), 8.03 (d, J = 8.0 Hz, 1H), 7.94 (d, J = 8.0 Hz, 1H), 7.80 (d, J = 8.0 Hz, 1H), 7.70 (dd, J = 8.0, 20.0 Hz, 2H), 7.52 (s, 1H), 7.42 (p, J = 8.0 Hz, 2H), 7.20-7.03 (m, 20 H), 6.92 (d, J = 8.0 Hz, 2H), 6.56 (d, J = 8.0 Hz, 2H), 4.57 (dd, J = 8.0, 12.0 Hz, 1H), 4.47-4.35 (m, 4H), 4.47 (dd, J = 16.0, 44.0 Hz, 2H), 2.99 (d, J = 8.0 Hz, 2H), 2.90-2.73 (m, 5H), 2.66-2.56 (m, 3H).

TPD-2p: ^{1}H-NMR (400 MHz, DMSO-d_6) δ 8.37 (d, J = 8.0 Hz, 1H), 8.26 (d, J = 8.0 Hz, 1H), 8.15 (d, J = 8.0 Hz, 1H), 8.06 (d, J = 8.0 Hz, 1H), 7.80 (d, J = 8.0 Hz, 1H), 7.70 (dd, J = 8.0, 20.0 Hz, 2H), 7.52 (s, 1H), 7.42 (m, 2H), 7.24-7.03 (m, 22 H), 6.96 (d, J = 12.0 Hz, 2H), 4.58 (dd, J = 8.0, 16 Hz, 1H), 4.44 (m, 3H), 3.47 (dd, J = 16, 44 Hz, 2H), 3.11-3.29 (m, 4H), 2.91-2.88 (m, 4H), 2.77 (dd, J = 8, 12 Hz, 1H), 2.66-2.60 (m, 2H).

fTPD-1p: ^{1}H-NMR (400 MHz, DMSO-d_6) δ 9.31 (s, 1H), 8.47 (d, J = 8.0 Hz, 1H), 8.32 (d, J = 8.0 Hz, 1H), 8.19 (d, J = 8.0 Hz, 1H), 8.03 (dd, J = 4.0, 16.0 Hz, 2H), 7.23-7.03 (m, 21H), 6.96 (d, J = 4.0 Hz, 3H), 6.58 (d, J = 8.0 Hz, 3H), 6.32 (d, J = 8.0 Hz, 1H), 4.6 (m, 1 H), 4.49-4.38 (m, 4H), 3.53 (d, J = 12.0 Hz, 4H), 3.02 (d, J = 12.0 Hz, 3H), 2.93-2.77 (m, 6H), 2.61 (m, 3H).

fTPD-2p: ^{1}H-NMR (400 MHz, DMSO-d_6) δ 9.34 (s, 1H), 8.47 (d, J = 8.0 Hz, 1H), 8.44 (d, J = 8.0 Hz, 1H), 8.28 (d, J = 8.0 Hz, 1H), 7.79-8.72 (m, 3H), 7.26-6.93 (m, 21H), 6.34 (d, J = 8.0 Hz, 1H), 4.69-4.63 (m, 1H), 4.53-4.46 (m, 5H), 3.57 (t, J = 4.0 Hz), 3.12-3.04 (m, 3H), 2.94-2.75 (m, 4H), 2.67-2.60 (m, 2H).

LC-MS (ESI):

TPD-2p (m/z): $C_{48}H_{48}N_4O_{14}P_2$, calc. 966.26; observed (M+1)+ 967.08, (M-1)- 965.20.

TPD-1p (m/z): $C_{48}H_{47}N_4O_{11}P_1$, calc. 886.30; observed (M+1)+ 887.27, (M-1)- 885.32.

fTPD-2p (m/z): $C_{45}H_{46}N_8O_{17}P_2$, calc. 1032.25; observed (M+1)+ 1033.20, (M-1)- 1031.67.

fTPD-1p (m/z): $C_{45}H_{45}N_8O_{14}P$, calc. 952.28; observed (M+1)+ 953.43, (M-1)- 951.68.

S3. General procedures for hydrogel preparation.

Enzymatic gelation: We dissolved precursors (4 mg) into distilled water (700 μL), and adjusted pH of the solution, monitored by pH paper, carefully by adding 1M NaOH. After the pH of the solution reaches 7.4, we then added extra distilled water to make the final concentration of 0.5 wt%, followed by the addition of alkaline phosphatase (ALP).

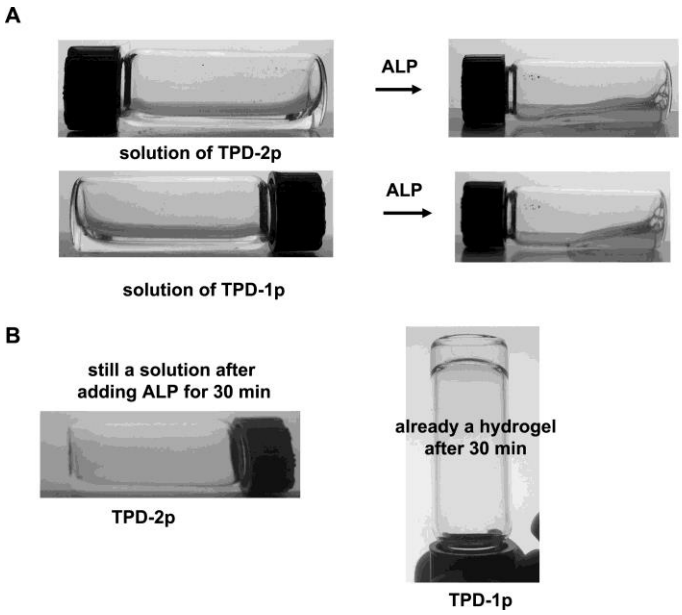

Figure S2. (A) Optical images of the solutions of **TPD-2p** and **TPD-1p** at the concentration of 500 μM in PBS buffer, and the viscous solutions formed after ALP treatment. (B) The solution of **TPD-2p** still remains as a solution and the solution of **TPD-1p** turns into hydrogel after ALP treatment for 30 min. pH = 7.4, 0.5 wt%.

S4. TEM sample preparation.

In this paper, we used negative staining technique to obtain the TEM images. We first glowed discharge the 400 mesh copper grids coated with continuous thick carbon film (~ 35 nm) prior to use to increase the hydrophilicity. After loading samples (4 μL) on the grid, we then rinsed grid by dd-water for twice or three times. Immediately after rinsing, we stained the grid containing sample with 2.0 % w/v uranyl acetate for three times. Afterwards, we allowed the grid to dry in air.

S5. Cell culture

All cell lines were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). The HepG2 cells were propagated in Minimum Essential Media (MEM) supplemented with 10% fetal bovine serum (FBS) and antibiotics in a fully humidified incubator containing 5% CO_2 at 37 ℃. The Saos-2 cells were propagated in McCoy's 5A supplemented with 15% fetal bovine serum (FBS), and antibiotics, in a fully humidified incubator containing 5% CO_2 at 37 ℃.

S6. Cell viability assay

Cells in exponential growth phase were seeded in a 96 well plate at a concentration of 1×10^4 cell/well. The cells were allowed to attach to the wells for 24 h at 37 ℃, 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 ℃, 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 ℃ 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.

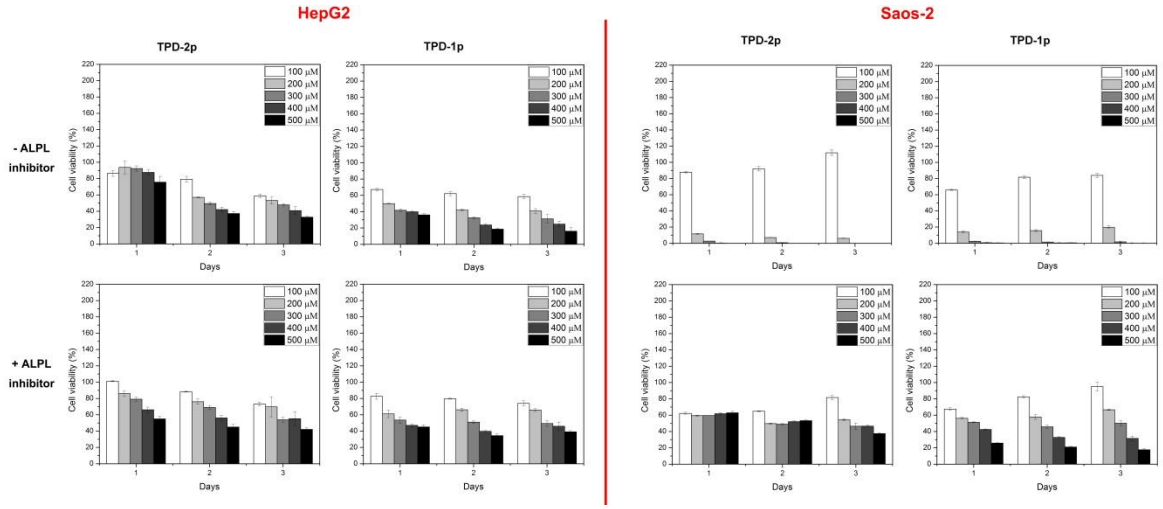

Figure S3. Cell viability of HepG2 and Saos-2 cells treated with **TPD-2p** and **TPD-1p** at different concentration (500-100 μM). The cell viability is determined by MTT cell viability assay, with initial cell number 10,000 cells/well. The concentration of ALPL inhibitor, **DQB,** is at 2 μM.

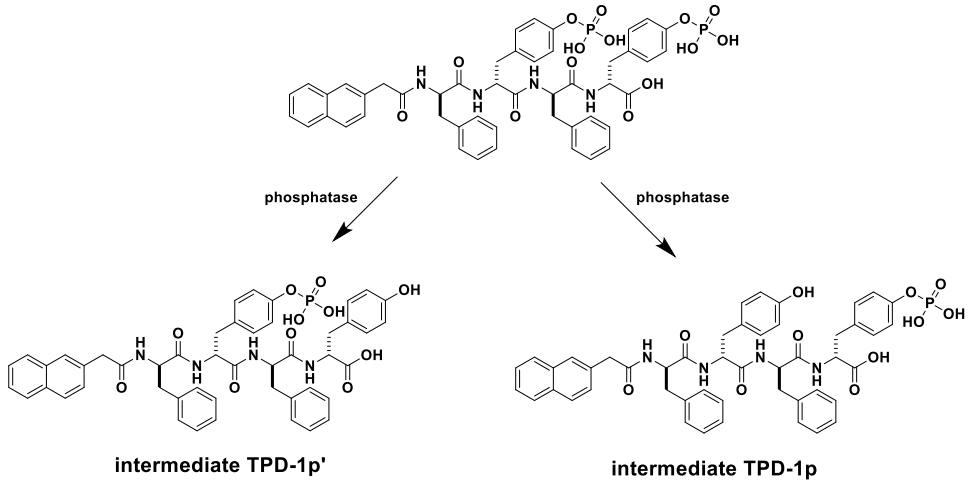

Figure S4. Chemical structures of intermediates **TPD-1p** and **TPD-1p'**, resulted from enzymatic dephosphorylation of **TPD-2p**.

S7. Sample preparation for confocal microscopy

Live cell imaging: HepG2 or Saos-2 cells in exponential growth phase were seeded in glass bottomed culture chamber at 1 × 10^5 cells/well. The cells were allowed for attachment for overnight at 37 ℃, 5% CO_2. The culture medium was removed, and new culture medium containing **fTPD-2p/1p** at 500 μM was added. After incubation for certain time, cells were stained with 1.0 μg/mL Hochst 33342 for 10 min at 37 ℃ in dark. After that, cells were rinsed three times by PBS buffer, and then kept in the live cell imaging solution (Invitrogen Life Technologies A14291DJ) for imaging.

Antibody staining:

- Seed cell (200,000 cell/3 cm confocal dish) and allow attachment (overnight)
- Wash by PBS buffer 3X and 4% formaldehyde fixed (15 min)
- Wash by PBS buffer 3X and incubated in 1.0 %BSA / 10% normal goat serum / 0.3M glycine in PBS for 1h block non-specific protein-protein interactions.
- Wash by PBS buffer 3X and incubated with the antibody (1/100) (e.g., ALPP and ALPL) overnight at +4 ℃.
- Wash by PBS buffer 3X and the secondary antibody (green) was ab150077 Alexa Fluor® 488 goat anti-rabbit IgG (H+L) used at 2 μg/mL (1/1000) for 1h.
- Hoechst 33342 was used to stain the cell nuclei (blue).
- Wash by PBS buffer 3X and mount for imaging.

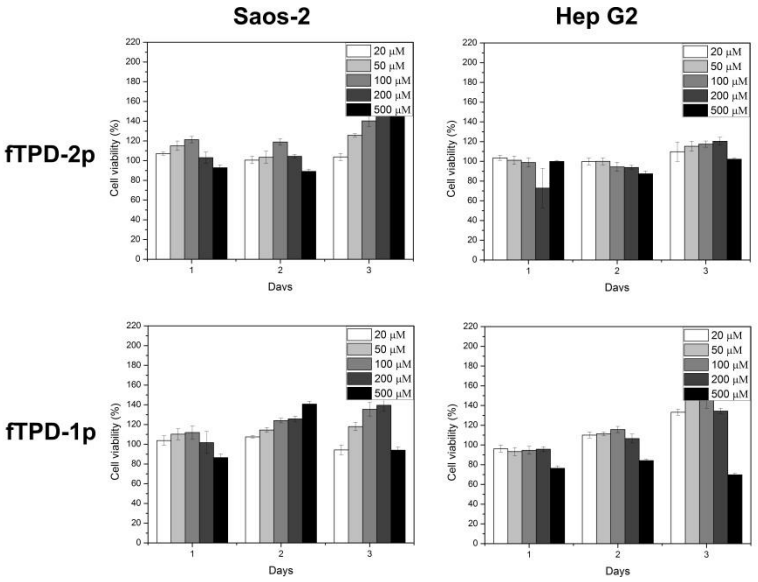

Figure S5. Cell viability determined by MTT assay of HepG2 and Saos-2 cells treated with **fTPD-2p** and **fTPD-1p** at different concentrations (100, 200, 300, 400, 500 μM).

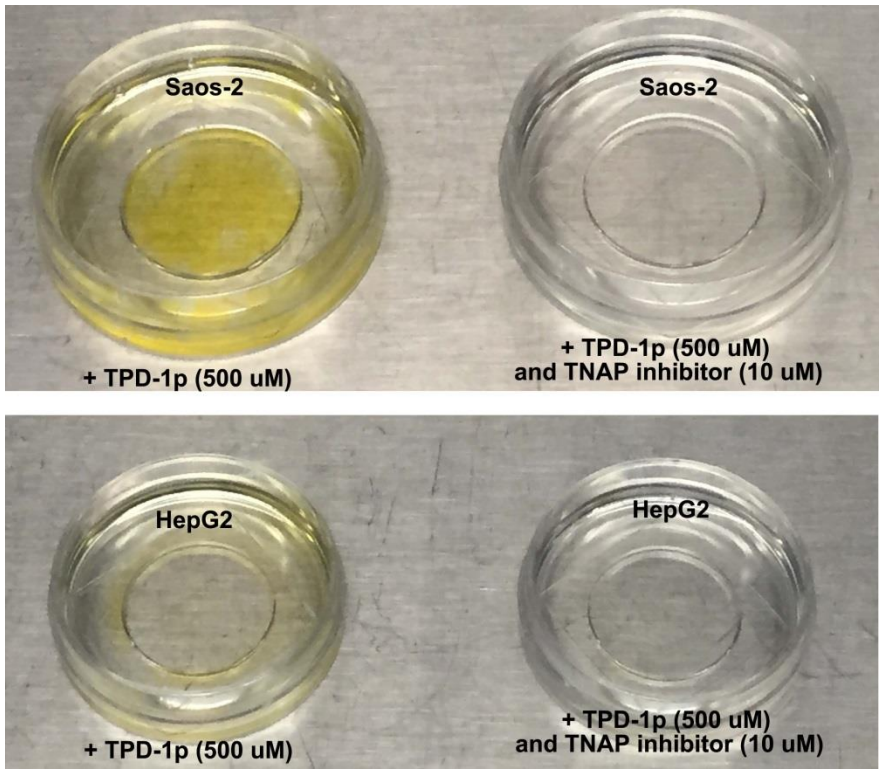

Figure S6. Optical images of confocal dishes cultured with Saos-2 and HepG2 cells treated with **fTPD-1p** (500 μM) for 4 hours (Saos-2) and 8 (HepG2). The optical images are taken after the dishes washed with PBS buffer for 3 times. The addition of ALPL inhibitor (**DQB,** 10 μM) significantly inhibits the EISA of **fTPD-1p.**

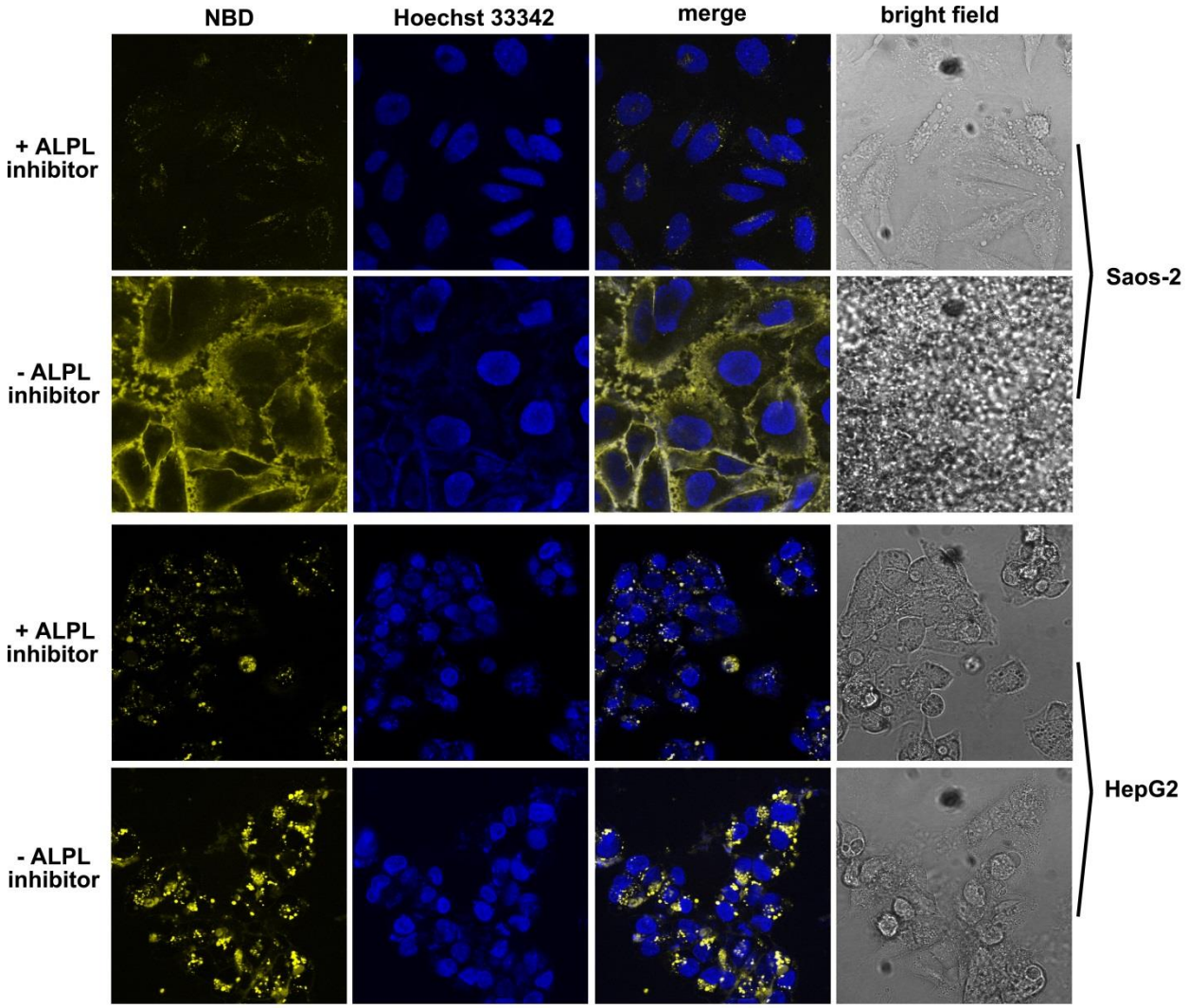

Figure S7. Confocal microscopy images of Saos-2 cells and HepG2 cells treated with **fTPD-1p** at the concentration of 500 μM for 4 hours. The addition of ALPL inhibitor (**DQB,** 10 μM) significantly inhibits the EISA of **fTPD-1p**. Nuclei are stained by Hoechst 33342.

S8. The preparation of cell lysate

Cell Lysis Buffer (10X) (non-denature) was purchased at Cell Signaling Technology.

- Seed cell in 10 cm petri-dish and wait until confluent culture.
- Wash plate with PBS to remove residual media.
- Add 400 μL of 1x lysis buffer/ 10 cm dish.
- Incubate plate on ice for 5 minutes.
- Scrape cells.
- Sonicate/vortex briefly.
- Spin extract 10 minutes at 14,000 x g in a cold microfuge.
- Collect supernatant for use and add protease inhibitor.

- Protein quantification by Coomassie protein quantification assay, using BSA Standard.

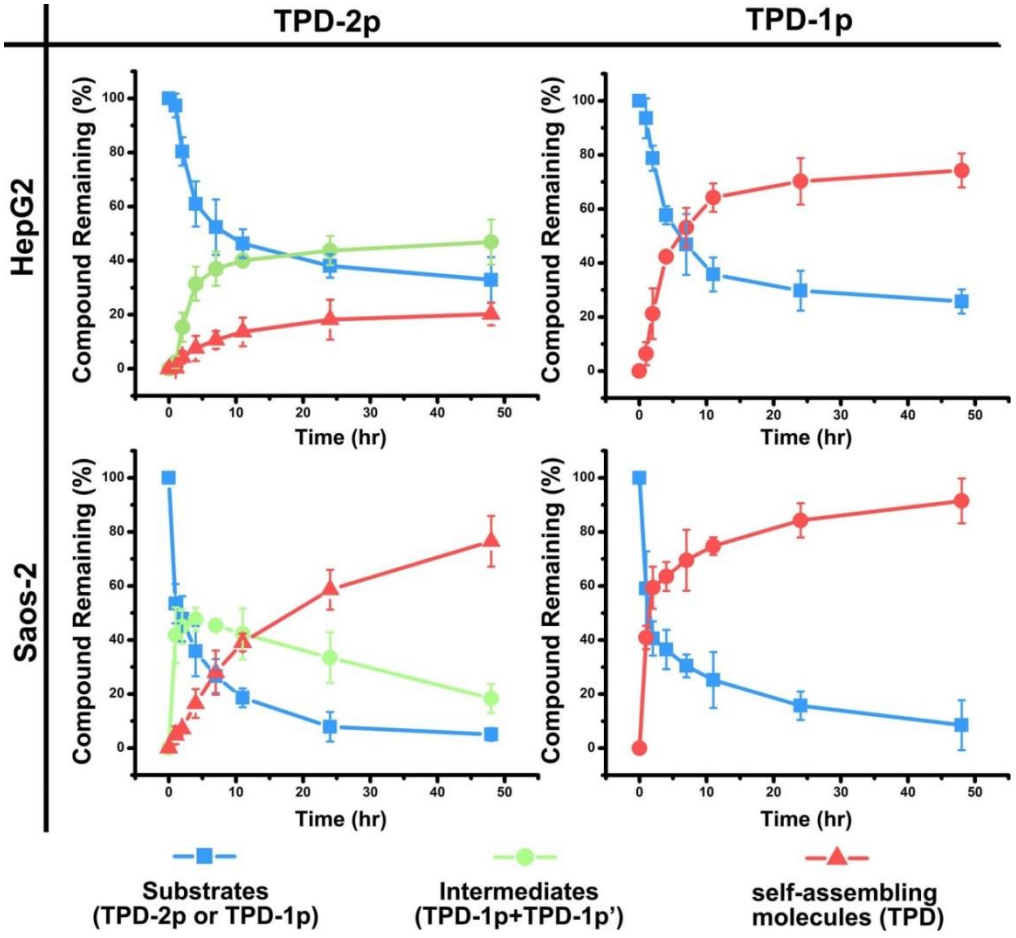

Figure S8. The time-dependent curves for dephosphorylation of **TPD-2p** and **TPD-1p** (500 μM) after incubation with the cell lysate of HepG2 and Saos-2 at 37 ℃ in PBS buffer.

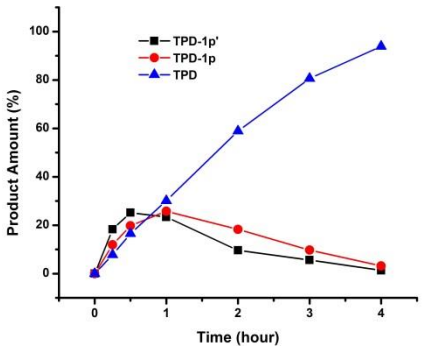

Figure S9. The time-dependent curves show the accumulation of hydrogelator **TPD** and intermediates **TPD-1p** and **TPD-1p'** after treating the solution of **TPD-2p** (500 μM) in PBS buffer with ALP (0.1 U/mL) treatment at 37 ℃.