Enzymatic Transformation of Phosphate Decorated Magnetic Nanoparticles for Selectively Sorting and Inhibiting Cancer Cells

*Xuewen Du, Jie Zhou, Liheng Wu, Shouheng, Sun, and Bing Xu**

Supplementary Information

Materials and Methods

Instruments: Transmission electron microscope (TEM) images were taken on Morgagni 268 transmission electron microscope. Confocal images were obtained on a Leica TCS SP2 Spectral Confocal Microscope. Magnetic studies were carried out using a Lakeshore 7404 high sensitivity vibrating sample magnetometer (VSM). Samples were dried with Labconco Freezone 4.5 Plus vacuum lyophilizer. The cells were counted by Bio-Rad TC 20^{TM} Automated cell counter.

Phosphate assay: We utilize the phosphate assay kit (colorimetric) (ab65622, abcam) to quantify the amount of phosphate on MNP_pY. First, we get the phosphate standard curve. We dilute 10 µl of the 10 mM phosphate standard to 990 µl dH_2O and mix well to generate 100 µM working phosphate standard. After adding 0, 10, 20, 30, 40, 50 µl of the 100 µM working phosphate standard to one 96 well plate, we adjust the volume to 200 µl with dH_2O to generate 0, 1, 2, 3, 4, 5 nmol of phosphate standard. Prior to read the absorbance at 620 nm using a plate reader, 30 µl phosphate reagent is added into all standard wells and the mixture is incubated at room temperature for 30 min. When quantifying the amount of phosphate on MNP_pY, we follow the similar protocol. First, we incubate 40 µg MNP_pY with 100 µl dH_2O or 100 µl dH_2O containing 30 U alkaline phosphatase (ALP) for 24 hrs. After centrifugation, we take 20 µl treated solution out and adjust the volume to 200 µl with dH_2O. With the treatment with 30 µl phosphate reagent for 30 min at room temperature, we read the absorbance by using a plate reader.
The result indicates that the absorbance of 20 µl solution treated with ALP is 1.36 and that of 20 µl solution just with dH_2O is 0.2843. According to the phosphate standard curve in Figure S2, we can conclude that there are 6.829 nmol tyrosine phosphates in 20 µl treated solution. Since we incubate MNP_pY with 100 µl dH_2O containing ALP, there are around 34.145 nmol tyrosine phosphates on 40 µg MNP_pY. According to the data from iron oxide nanoparticles (NanoTech Ocean), there are 6.9 nmol particles of 1 mg iron oxide, which means 40 µg MNP_pY contains 0.276 nmol nanoparticles. Followed by this estimation, we can get the conclusion that there are at least 34.145 nmol tyrosine phosphates on 0.276 nmol MNP_pY nanoparticles, which indicates that, in average, there are at least 124 D-tyrosine phosphate molecules on each MNP_pY nanoparticle.

Cell culture: All cell lines were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). The HeLa-GFP and HS-5 cells were propagated in Dulbecco's Modified Eagle Medium (DMEM, high glucose, Invitrogen Life Technologies 10829-018) supplemented with 10% fetal bovine serum (FBS, Invitrogen Life Technologies 10082-147), 100 U ml^{-1} penicillin and 100 µg ml^{-1} streptomycin (Invitrogen Life Technologies 15070-063) in a fully humidified incubator containing 5% CO_2 at 37°C.

Confocal microscopy: 1.0×10^6 cells in exponential growth phase were seeded in 6 cm cell culture dish. The cells were allowed for attachment for 12 h at 37 ºC, 5% CO_2. The culture

medium was removed, and new culture medium containing MNP_pY or MNP at 40 μg/mL was added. After 4 h of incubation, cells were washed with growth medium for 3 times and detached with 0.25% (w/v) Trypsin- 0.53 mM EDTA solution. As shown in Scheme 2, after harvesting the cells, we used one magnet to collect extraction from supernatant and rinsed each group of cells for three times with growth medium before seeding them back onto the confocal dishes. With four-hour attachment, the cells were rinsed for three times in PBS, and then kept in the PBS buffer for imaging.

Magnetic characterization: Similar to the method for confocal microscopy, 1.0×10^6 cells were treated with growth medium containing 200 μg MNP_pY or MNP for 4 h, and then washed with growth medium for 3 times and detached with 0.25% (w/v) trypsin- 0.53 mM EDTA solution. The collected cell pellets after centrifugation were dried with the vacuum lyophilizer. Magnetic studies were carried out using a Lakeshore 7404 high sensitivity vibrating sample magnetometer (VSM) with fields up to 1.5 tesla at room temperature.

Cell viability assay: Cells in exponential growth phase were seeded in a 96 well plate at a concentration of 1.0×10^4 cell/well. The cells were allowed to attach to the wells for 12 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 24, 48, 72 h, the growth medium in each well was removed and 50 μL 0.25% (w/v) trypsin- 0.53 mM EDTA solution was added. 4 min later, the cells were counted by using automated cell counter. Data represent the mean ± standard deviation of three independent experiments.

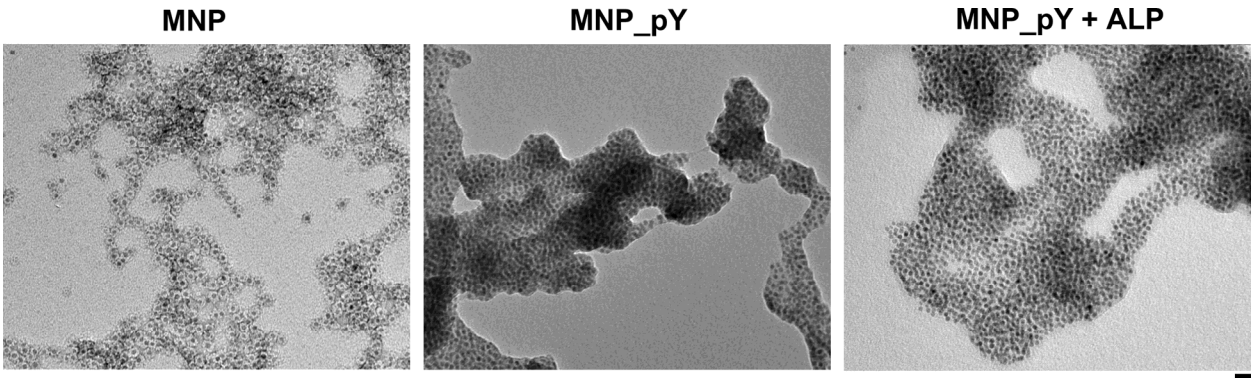

Figure S1. Transmission electron microscope (TEM) images of iron oxide nanoparticles MNP (Left), MNP_pY (Middle), and MNP_pY + ALP. The nanoparticles are dissolved in water at the concentration of 2,000 μg/mL (pH = 7.4). The scale bar is 20 nm.

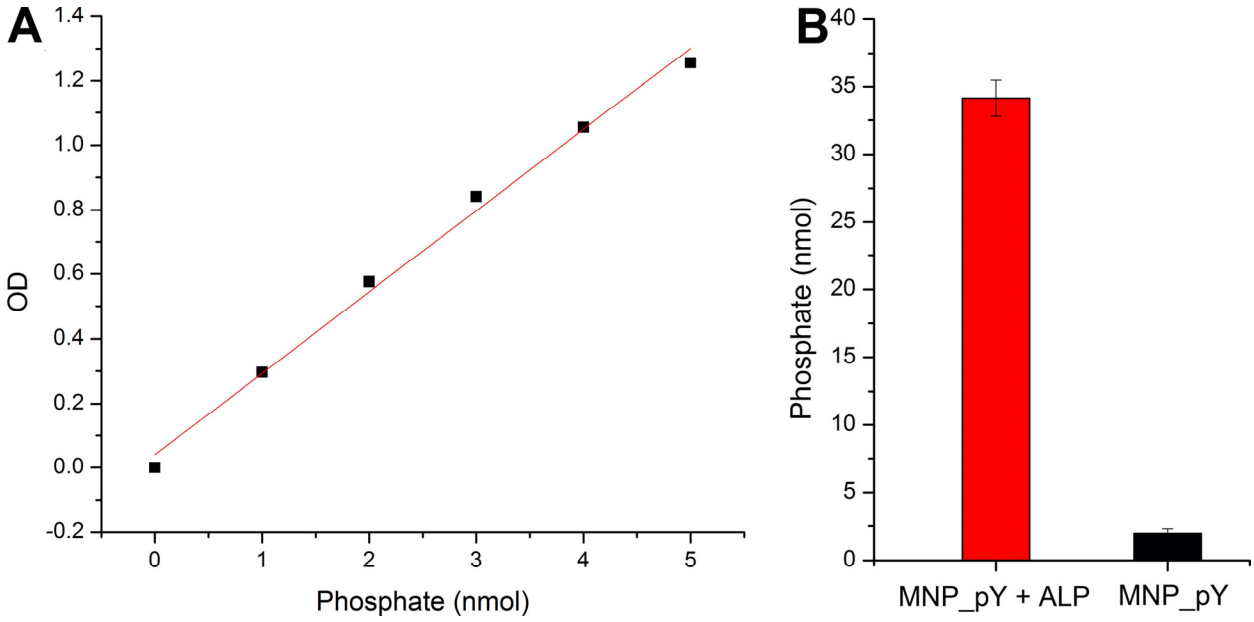

Figure S2. (A) Phosphate standard curve performed according to the phosphate assay. (B) Average of phosphate containing in 40 μg MNP_pY. Red bar indicates amount of phosphate in MNP_pY treated with ALP for 24 hrs; and black bar indicates amount of phosphate treated with di H_2O for 24 hrs.

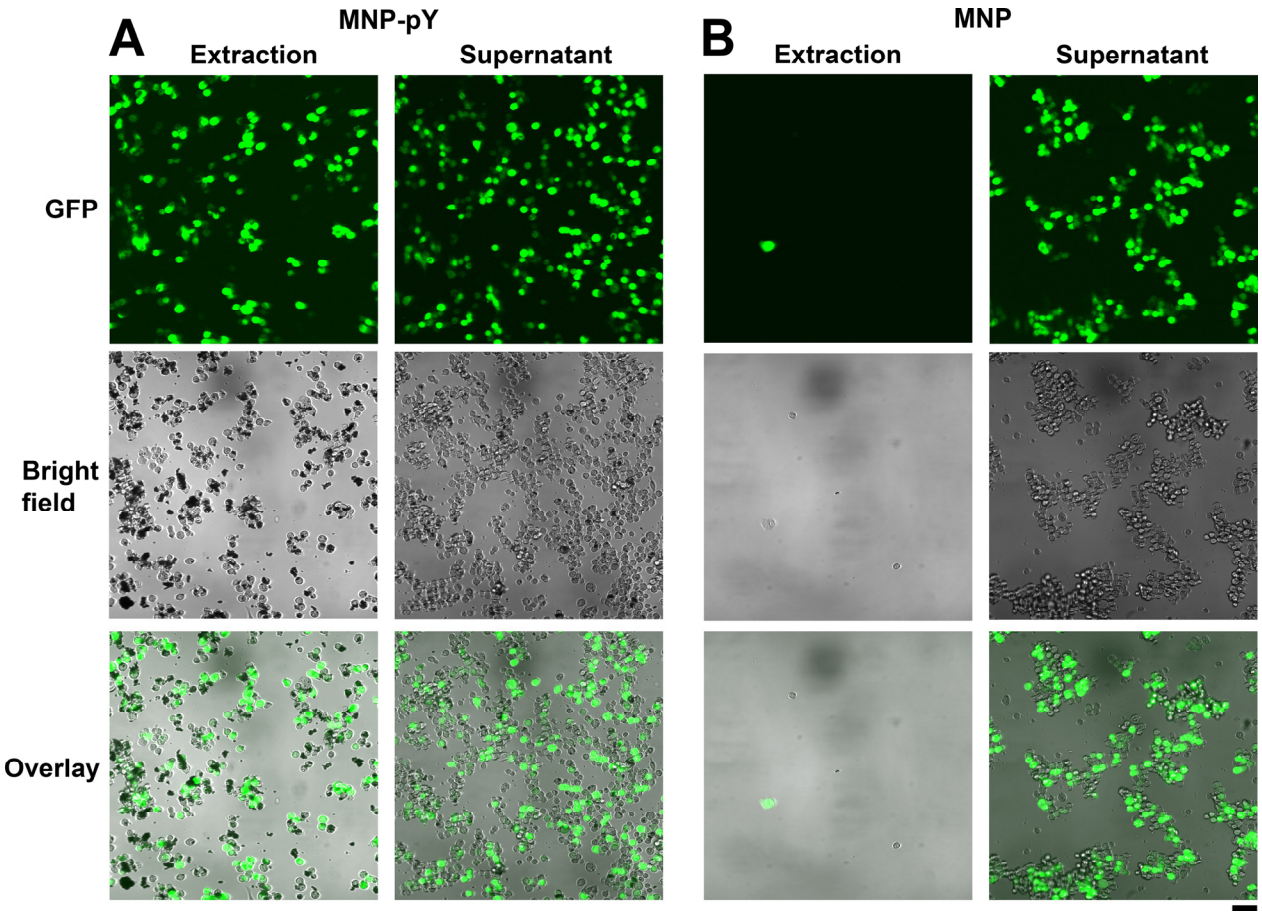

Figure S3. The overlaid confocal fluorescent microscope images (×20 dry objective lens) of co-culture of HeLa-GFP and HS-5 cells treated with (A) MNP_pY and (B) MNP show the fluorescence emission of the cells (Left indicates the extraction by magnet; and right indicates supernatant). Cells were incubated with the growth medium, Dulbecco's Modified Eagle Medium, containing 40 μg/mL nanoparticles for 4 hrs. After separation by magnet, extraction or supernatant of cells are seeded back onto the confocal dishes. The initial number of cells is about 1.0×10^6 per 6 cm culture dish. The scale bar is 100 μm.

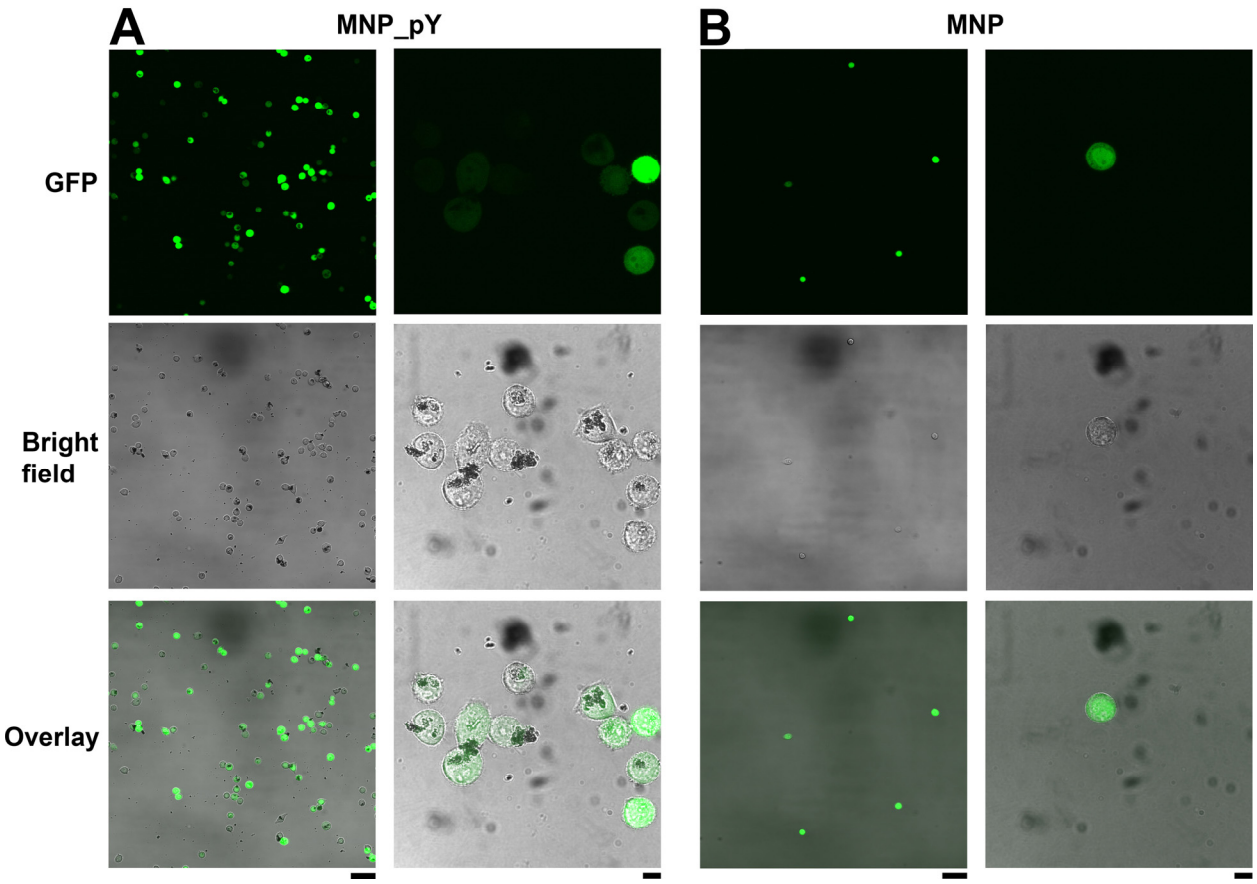

Figure S4. The overlaid confocal fluorescent microscope images of HeLa-GFP cells treated with (A) MNP_pY and (B) MNP at low magnification (Left, the scale bar is 100 μm) or high magnification (Right, the scale bar is 10 μm) of the cells show the fluorescence emission. The images here indicate the extraction of cells by magnet. Cells were incubated with the growth medium, Dulbecco's Modified Eagle Medium, containing 40 μg/mL nanoparticles for 4 hrs. After separation by magnet, extracted cells are seeded back onto the confocal dishes. The initial number of cells is about 1.0×10^6 per 6 cm culture dish.

We also compared the rate of sorting HeLa-GFP by FACS with the use of MNP_pY. After 4h, FACS collects 1.8 x 10^5 HeLa-GFP cells, and the use 40 μg/mL MNP_pY captures about 2 x 10^6 HeLa-GFP cell. This result confirm the effectiveness of MNP_pY based sorting.

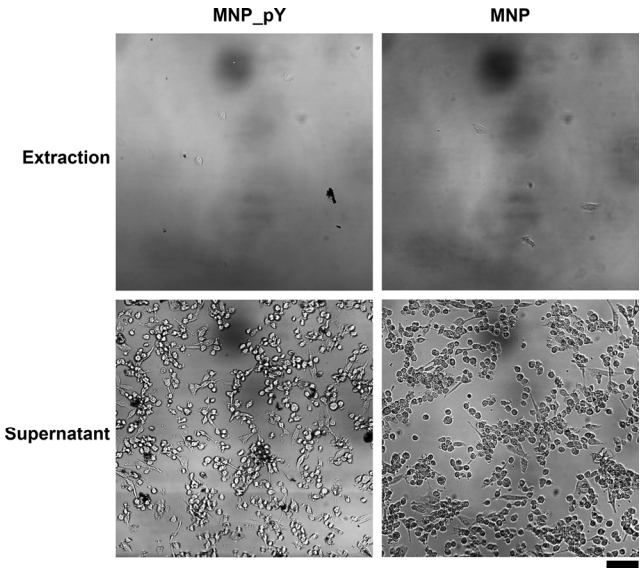

Figure S5. The bright field microscope images (×20 dry objective lens) of HS-5 cells after magnetically capture by incubating the cells with MNP_pY (Left) and MNP (Right) show the cells distribution (Up indicates the extraction by magnet; and down indicates supernatant). Cells were incubated with the growth medium, Dulbecco's Modified Eagle Medium, containing 40 μg/mL nanoparticles for 4 hrs. After separation by magnet, extraction or supernatant of cells are seeded back onto the confocal dishes. The initial number of cells is about 1.0×10^6 per 6 cm culture dish. The scale bar is 100 μm.

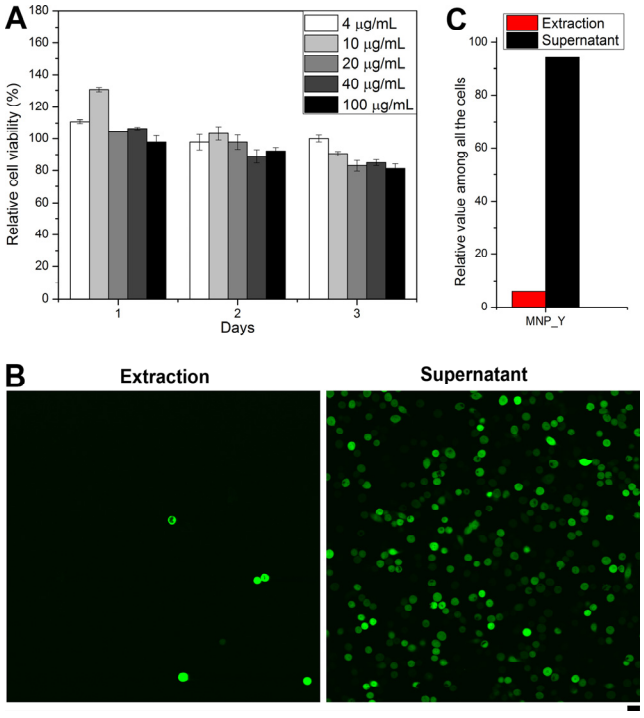

Figure S6. MNP_Y results from the treatment of MNP_pY with ALP for 24h. (A) Relative cell viability (determined by counting the cell numbers; 100% represents the control, i.e., 0 μg/mL of the compound) of the HeLa-GFP cells incubated with MNP_Y at the concentrations of 4, 10, 20, 40, 100 μg/mL. The initial number of cells is 1.0×10^4/well. (B) The confocal fluorescent images (×20 dry objective lens) of HeLa-GFP cells treated with MNP_Y show the fluorescence emission of the cells (Left indicates the extraction by magnet; and right indicates supernatant). Cells were incubated with the growth medium, Dulbecco's Modified Eagle Medium, containing 40 μg/mL nanoparticles for 4 hrs. After separation by magnet, extraction or supernatant of cells are seeded back onto the confocal dishes. The initial number of cells is about 1.0×10^6 per 6 cm culture dish. The scale bar is 50 μm. (C) Relative amount of cells (%) in the extraction or supernatant of all the cells collected after the treatment by 40 μg/mL MNP_Y and the magnetic capture.

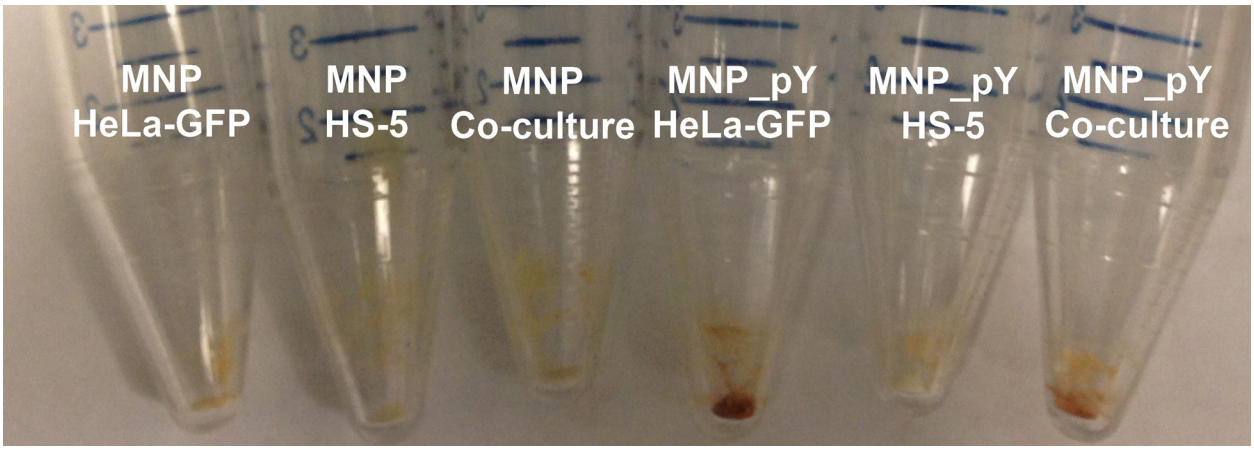

Figure S7. The optical images of pellets collected from co-culture of HeLa-GFP and HS-5 cells, HeLa-GFP cells or HS-5 cells treated with 200 μg MNP_pY or MNP. The initial number of cells is about 1.0×10^6 per 6 cm culture dish.

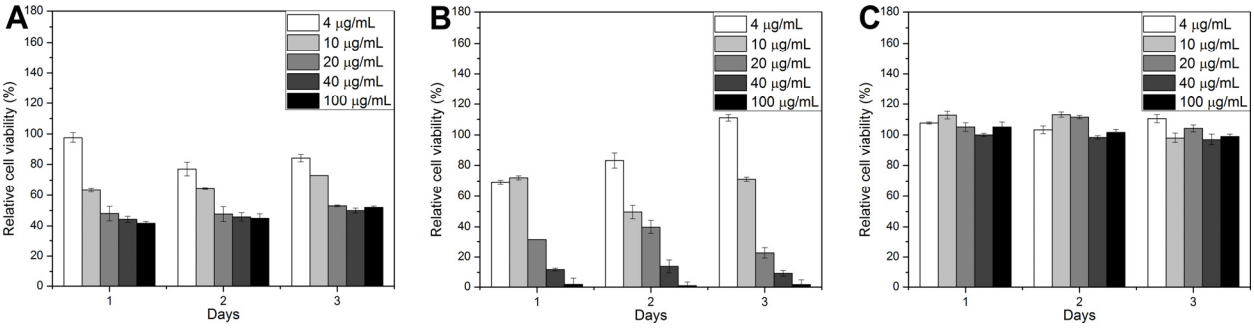

Figure S8. Relative cell viability (determined by counting the cell numbers; 100% represents the control, i.e., 0 μg/mL of the compound) of the (A) co-culture of HeLa-GFP and HS-5 cells; (B)

HeLa-GFP cells; and (C) HS-5 cells incubated with MNP_pY at the concentrations of 4, 10, 20, 40, 100 μg/mL. The initial number of cells is 1.0×10^4/well.

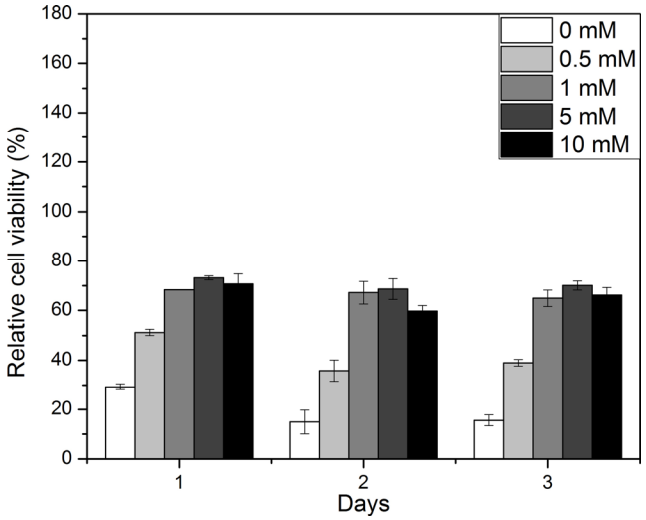

Figure S9. Relative cell viability (determined by counting the cell numbers; 100% represents the control, i.e., 0 μg/mL of the compound) of the HeLa-GFP cells incubated with 40 μg/mL MNP_pY and different concentration of L-phenylalanine (e.g., 0, 0.5, 1, 5, 10 mM). The initial number of HeLa-GFP cells is 1.0×10^4/well.

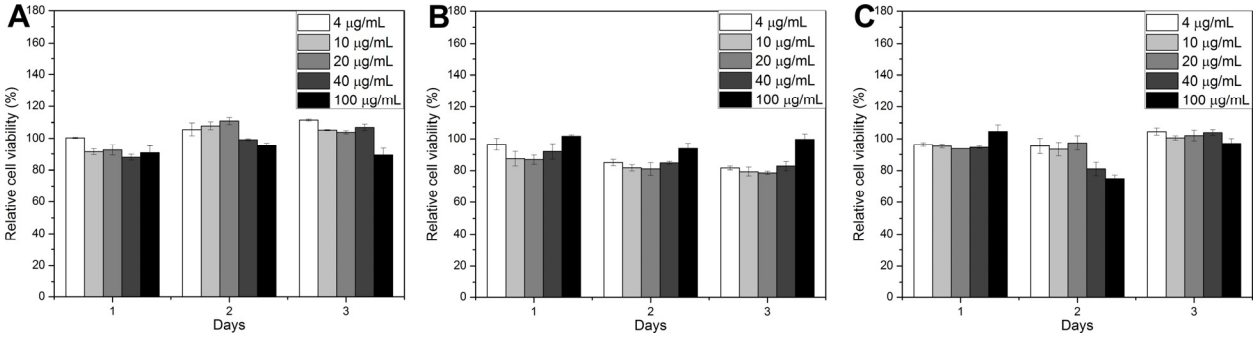

Figure S10. Relative cell viability (determined by counting the cell numbers; 100% represents the control, i.e., 0 μg/mL of the compound) of the (A) co-culture of HeLa and HS-5 cells; (B) HeLa-GFP cells; and (C) HS-5 cells incubated with the control (MNP) at the concentrations of 4, 10, 20, 40, 100 μg/mL. The initial number of cells is 1.0×10^4/well.