

## Supporting Information

# PEGylated Paclitaxel Nanomedicine Meets 3D Confinement: Cytotoxicity and Cell Behaviors

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**Supplementary Table S1.** Percentage of cell disruption for cells without any treatment for 16 h in different microwells.

Microwell ( $\mu\text{m}^2$ )	without cover (%)	with cover (%)
50×50	11.63±5.79	7.26±2.62
100×100	11.43±4.57	6.61±2.65
150×150	10.47±3.65	7.65±2.19

**Supplementary Table S2.** Percentage of cell disruption after PTX and PEG-PTX NPs addition for 16 h in different microwells. Concentration of PTX and PEG-PTX NPs were both 5.85 nmol/mL.

Microwell ( $\mu\text{m}^2$ )		Control (%)	PTX (%)	PEG-PTX NPs (%)
without cover	50×50	5.69±5.43	13.40±6.62	10.50±2.84
	100×100	4.42±3.50	14.01±3.66	10.77±3.92
	150×150	4.50±2.67	14.82±4.86	10.96±2.68
with cover	50×50	3.21±2.58	16.08±5.94	12.41±4.80
	100×100	2.41±2.15	13.12±3.44	12.23±5.26
	150×150	3.37±2.63	15.56±3.96	12.68±3.87

**Supplementary Table S3.** Migration speed of NPC43 cells with PTX and PEG-PTX NPs treatments over 16 h in different microwells and on flat surface. Concentration of PTX and PEG-PTX NPs were both 5.85 nmol/mL.

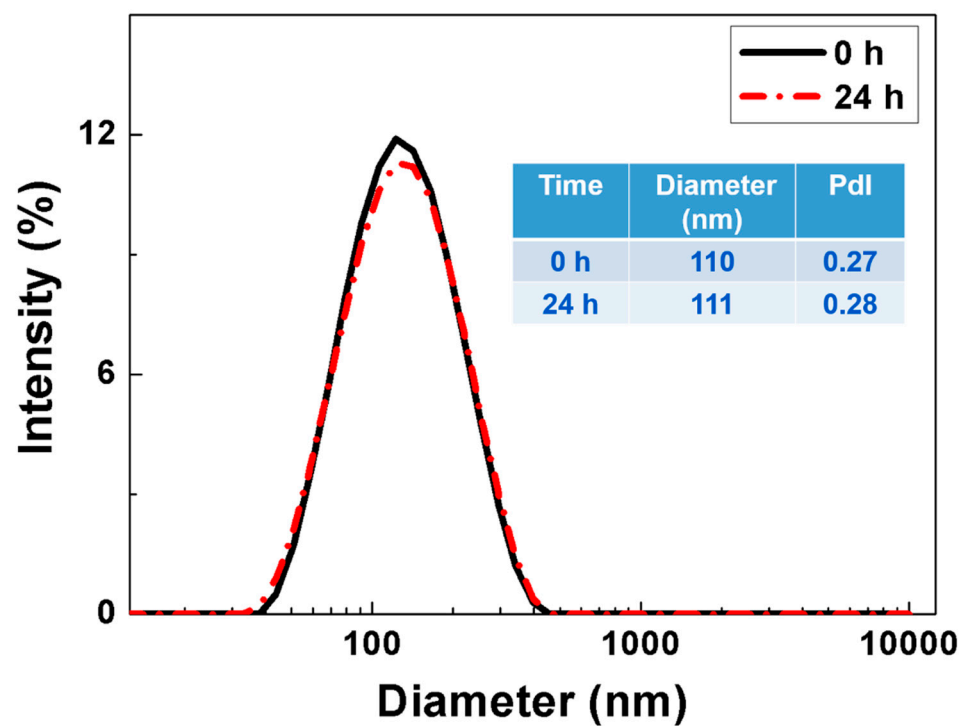
Microwell ( $\mu\text{m}^2$ )		Control ( $\mu\text{m}/\text{min}$ )	PTX ( $\mu\text{m}/\text{min}$ )	PEG-PTX NPs ( $\mu\text{m}/\text{min}$ )
without cover	Flat surface	0.32 $\pm$ 0.07	0.10 $\pm$ 0.03	0.17 $\pm$ 0.04
	50 $\times$ 50	0.34 $\pm$ 0.08	0.09 $\pm$ 0.03	0.13 $\pm$ 0.04
	100 $\times$ 100	0.33 $\pm$ 0.06	0.09 $\pm$ 0.02	0.13 $\pm$ 0.04
	150 $\times$ 150	0.36 $\pm$ 0.09	0.09 $\pm$ 0.03	0.16 $\pm$ 0.04
with cover	50 $\times$ 50	0.32 $\pm$ 0.07	0.08 $\pm$ 0.02	0.12 $\pm$ 0.03
	100 $\times$ 100	0.32 $\pm$ 0.07	0.10 $\pm$ 0.03	0.11 $\pm$ 0.03
	150 $\times$ 150	0.32 $\pm$ 0.07	0.10 $\pm$ 0.03	0.12 $\pm$ 0.04

**Supplementary Table S4.** Cell area after cells treated with PTX and PEG-PTX NPs for 16 h in different microwells. Concentration of PTX and PEG-PTX NPs were both 5.85 nmol/mL.

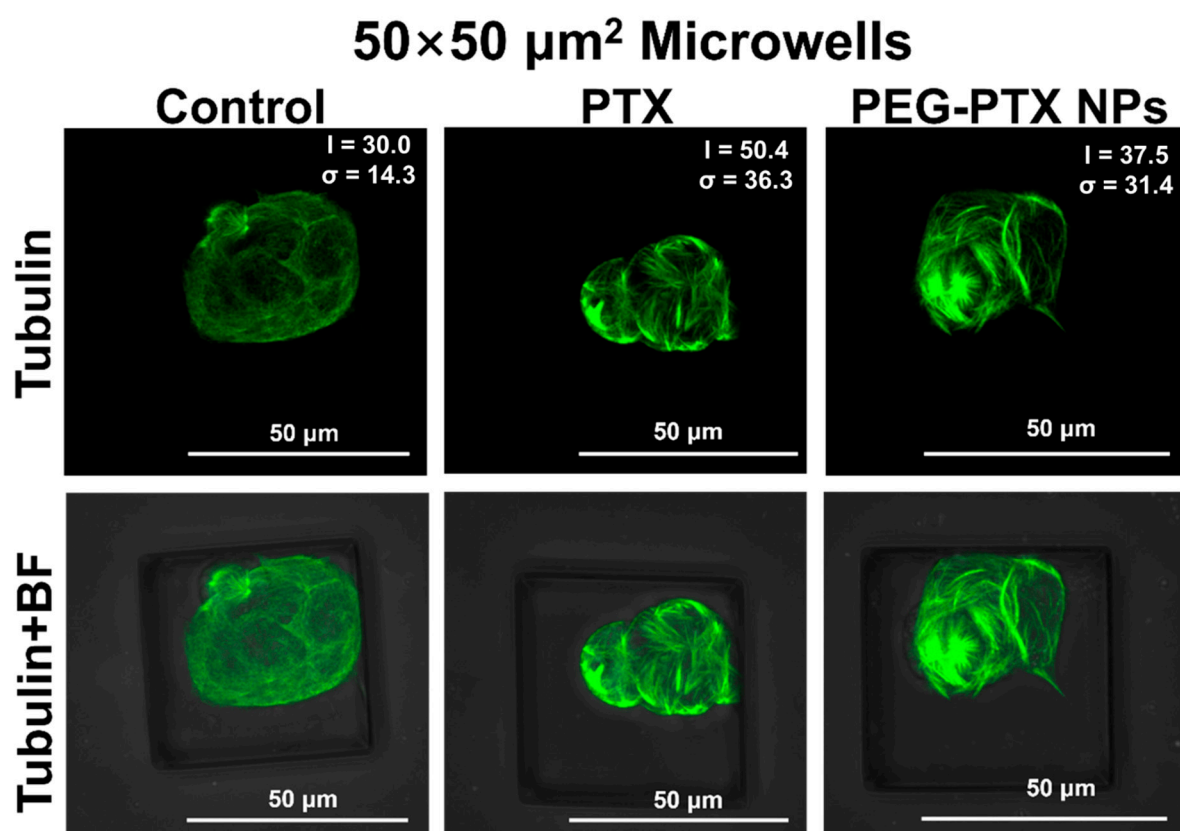
Microwell ( $\mu\text{m}^2$ )		Control ( $\mu\text{m}^2$ )	PTX ( $\mu\text{m}^2$ )	PEG-PTX NPs ( $\mu\text{m}^2$ )
without cover	50 $\times$ 50	329 $\pm$ 162	119 $\pm$ 41	145 $\pm$ 40
	100 $\times$ 100	345 $\pm$ 127	115 $\pm$ 39	165 $\pm$ 64
	150 $\times$ 150	334 $\pm$ 115	116 $\pm$ 43	166 $\pm$ 68
with cover	50 $\times$ 50	225 $\pm$ 88	114 $\pm$ 34	135 $\pm$ 39
	100 $\times$ 100	216 $\pm$ 63	139 $\pm$ 44	135 $\pm$ 44
	150 $\times$ 150	234 $\pm$ 64	152 $\pm$ 33	140 $\pm$ 52

**Supplementary Table S5.** Cell aspect ratio after NPC43 cells treated with PTX and PEG-PTX NPs for 16 h in different microwells. Concentration of PTX and PEG-PTX NPs were both 5.85 nmol/mL.

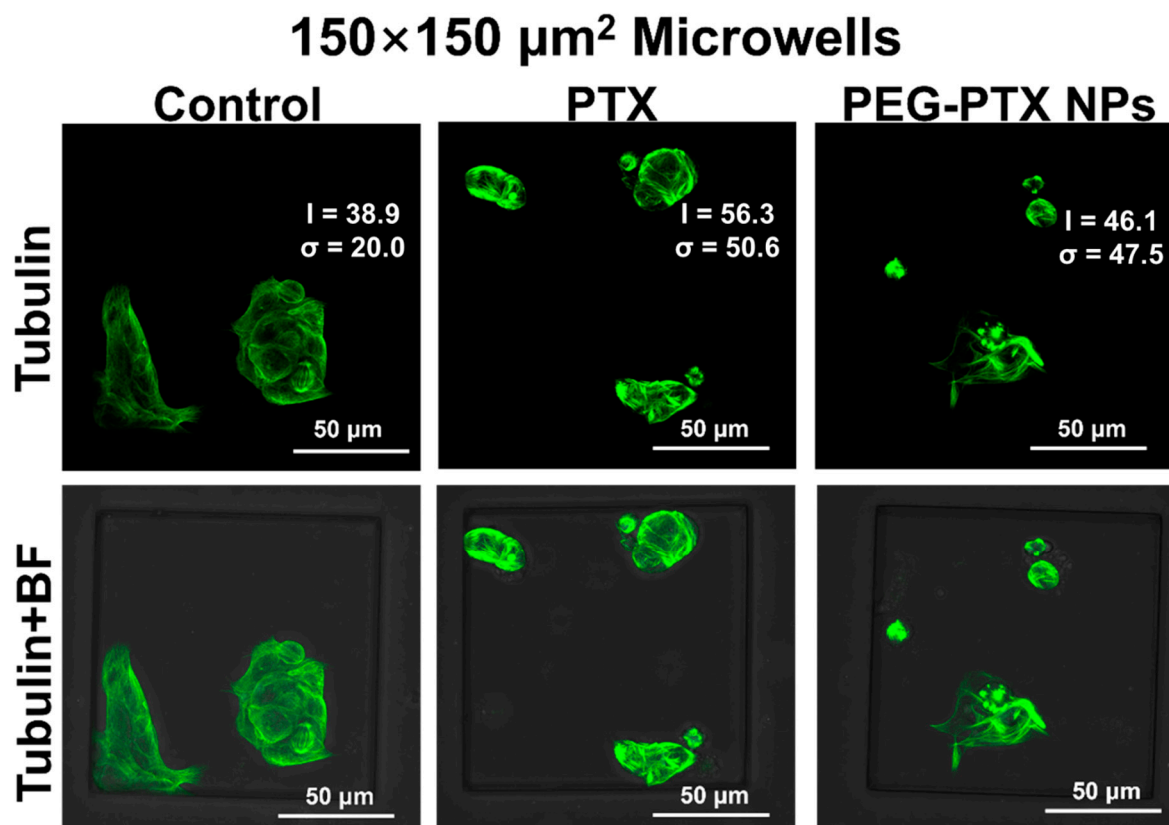
Microwell ( $\mu\text{m}^2$ )		Control	PTX	PEG-PTX NPs
without cover	50×50	1.77±0.65	1.31±0.20	1.55±0.35
	100×100	1.98±0.78	1.26±0.28	1.55±0.22
	150×150	1.79±0.58	1.22±0.43	1.60±0.30
with cover	50×50	1.93±0.78	1.22±0.18	1.31±0.22
	100×100	1.87±0.87	1.27±0.17	1.36±0.23
	150×150	1.71±0.47	1.21±0.16	1.29±0.20



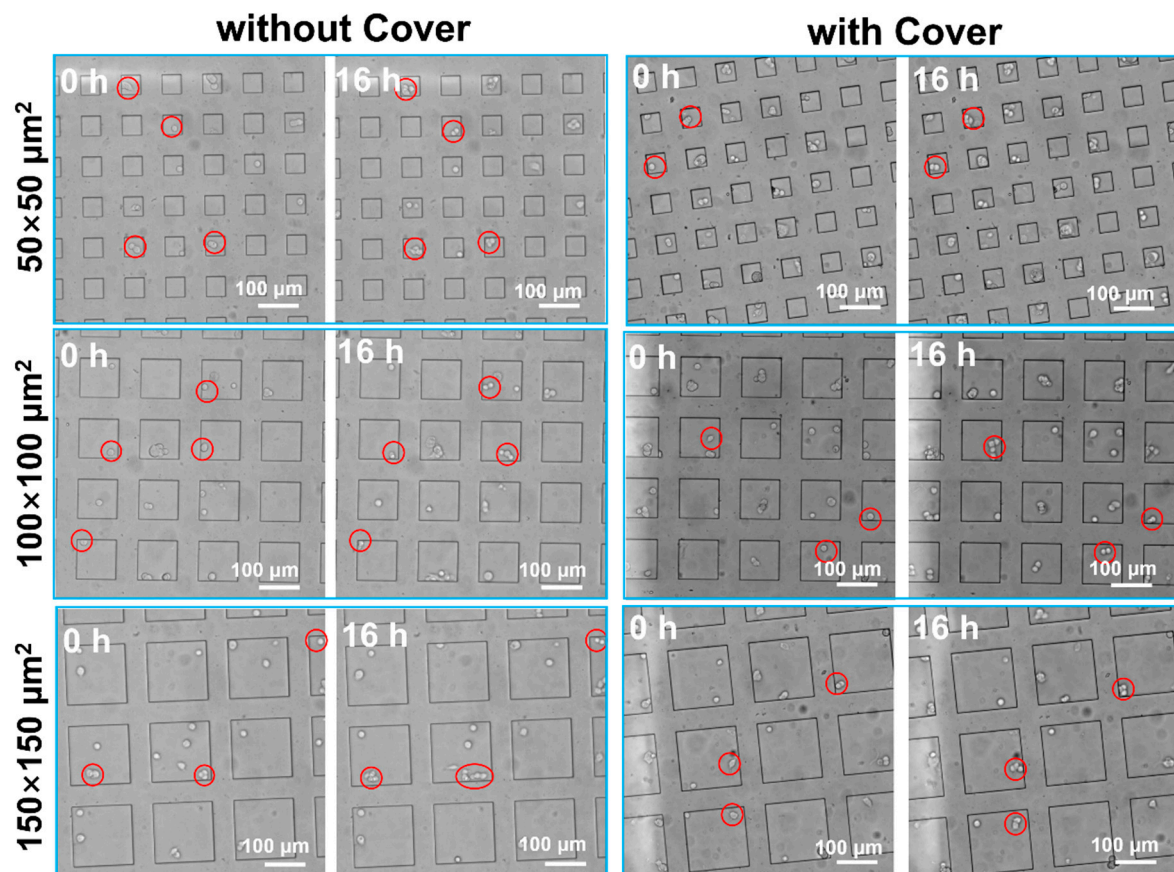
**Supplementary Figure S1.** Size distribution and stability of PEG-PTX NPs in cell culture medium measured by dynamic light scattering (DLS).



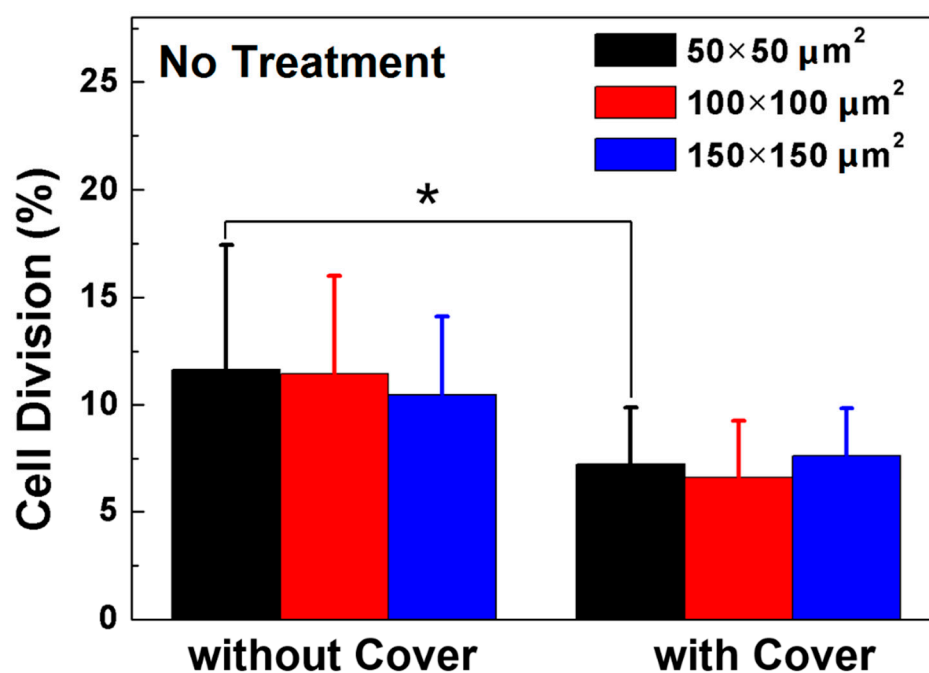
**Supplementary Figure S2.** Confocal laser scanning micrographs of NPC43 cells incubated with PTX and PEG-PTX NPs for 16 h in 50×50  $\mu\text{m}^2$  microwells. Tubulin in cells was stained by tubulin-tracker green (green fluorescence), and overlays of tubulin and bright field (BF) images. Average value ( $I$ ) and standard deviation ( $\sigma$ ) of fluorescent intensity were larger after drug treatment. Concentration of PTX and PEG-PTX NPs were both 5.85 nmol/mL.



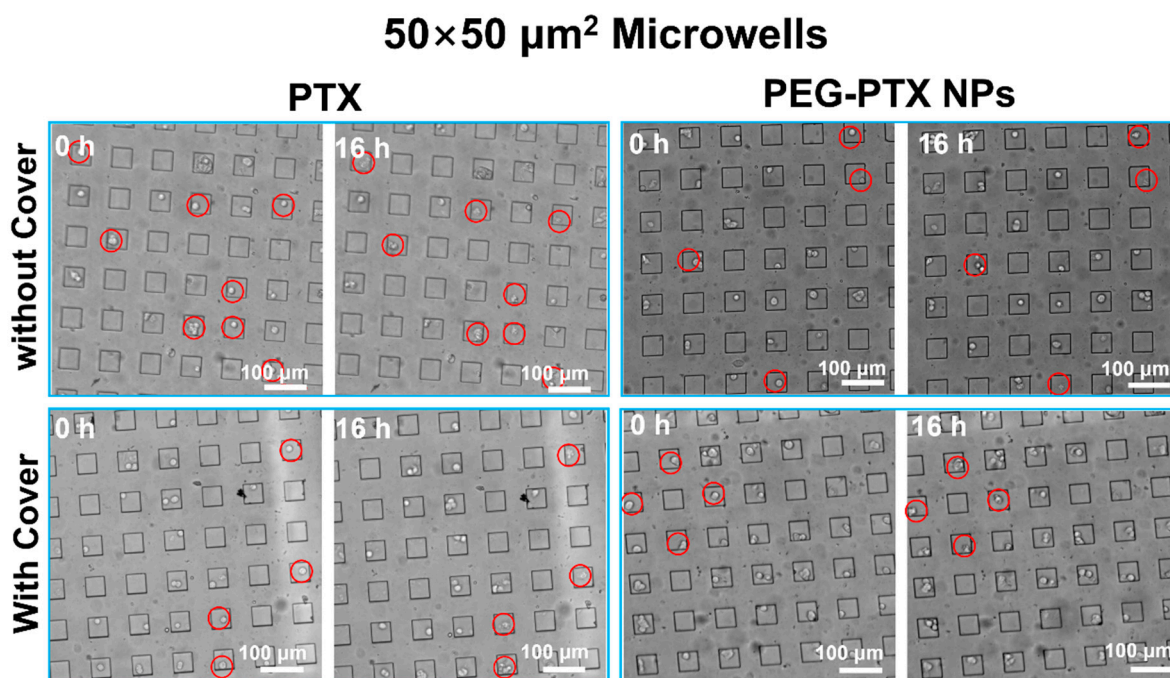
**Supplementary Figure S3.** Confocal laser scanning micrographs of NPC43 cells incubated with PTX and PEG-PTX NPs for 16 h in 150×150  $\mu\text{m}^2$  microwells. Tubulin in cells was stained by tubulin-tracker green (green fluorescence), and overlays of Tubulin and BF images. Average value ( $I$ ) and standard deviation ( $\sigma$ ) of fluorescent intensity were larger after drug treatment. Concentration of PTX and PEG-PTX NPs were both 5.85 nmol/mL.



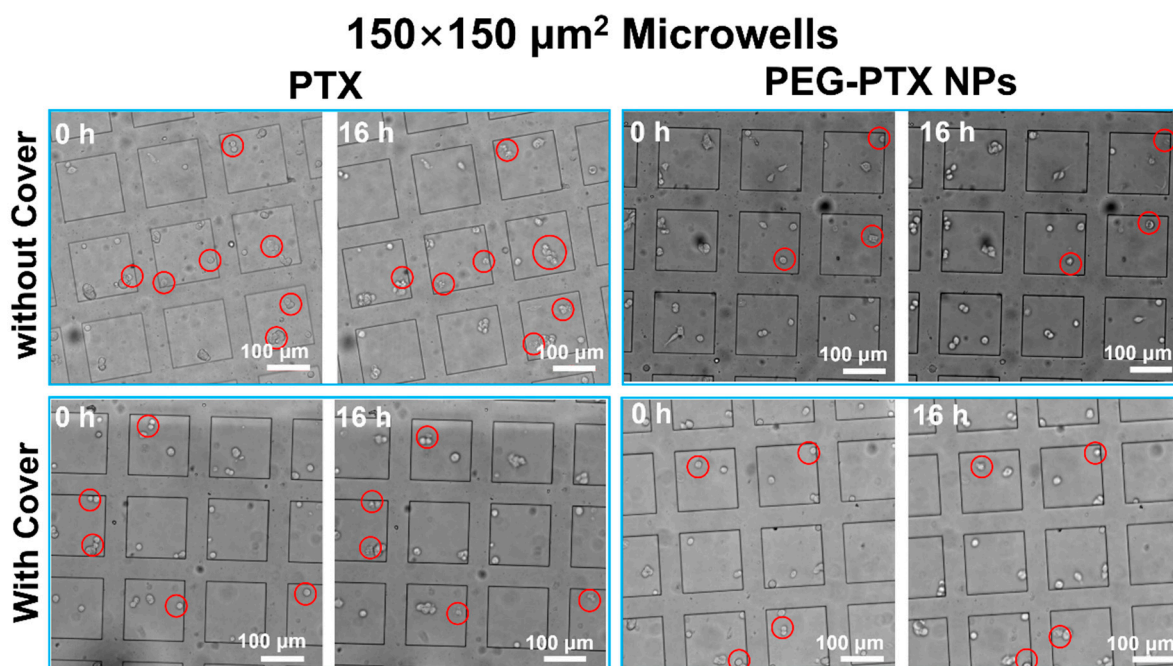
**Supplementary Figure S4.** Micrographs of NPC43 cells without any treatment for 16 h in microwells without and with cover. Cell division was highlighted in red circles.



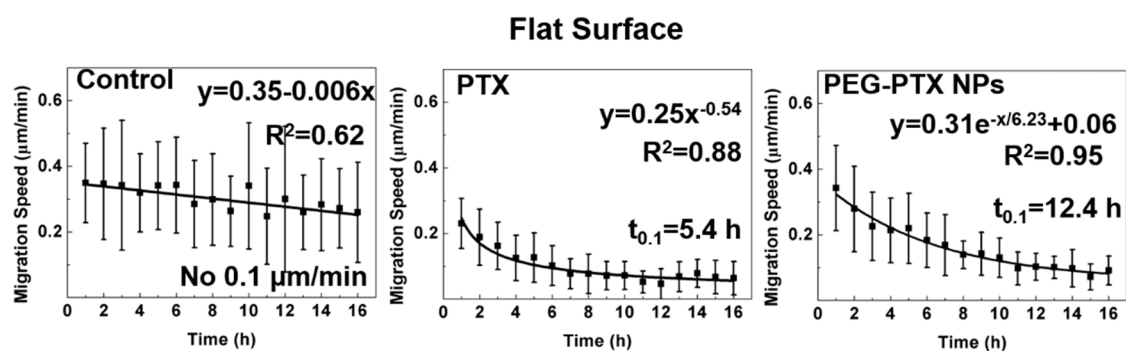
**Supplementary Figure S5.** Percentage of cell disruption for cells without any treatment for 16 h in different microwells. One-way ANOVA and Tukey's post hoc tests, \*p <0.05. Concentration of PTX and PEG-PTX NPs were both 5.85 nmol/mL.



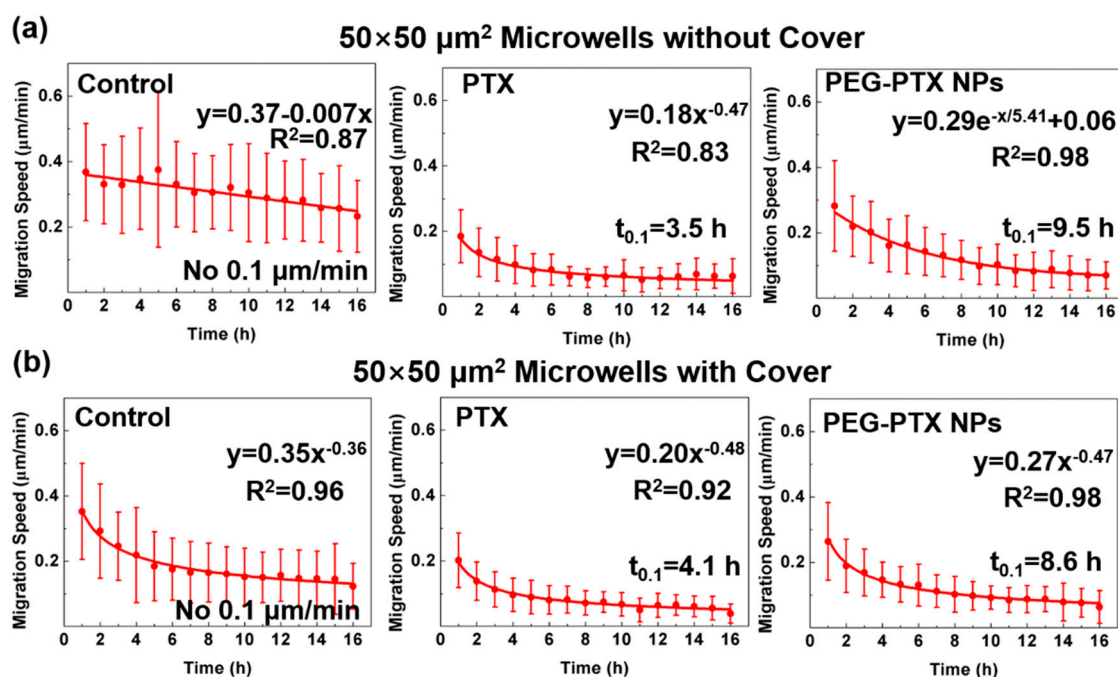
**Supplementary Figure S6.** Micrographs of NPC43 cells incubated with PTX or PEG-PTX NPs for 16 h in 50×50  $\mu\text{m}^2$  microwells. Cell disruption was highlighted in red circles. Concentration of PTX and PEG-PTX NPs were both 5.85 nmol/mL.



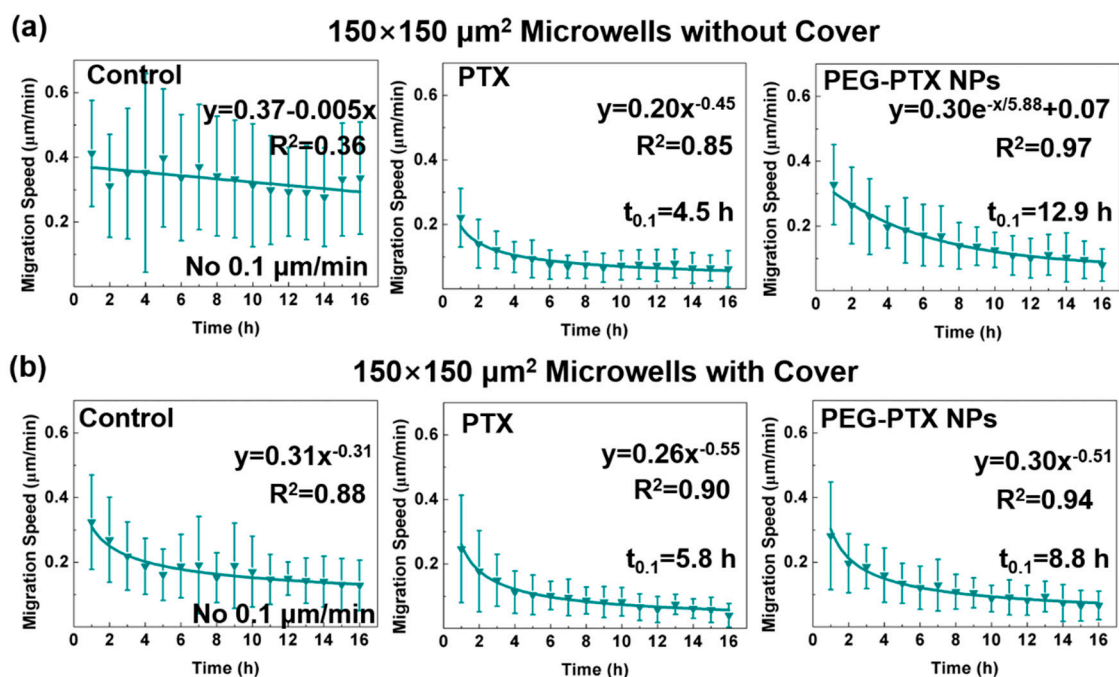
**Supplementary Figure S7.** Micrographs of NPC43 cells incubated with PTX or PEG-PTX NPs for 16 h in 150×150  $\mu\text{m}^2$  microwells. Cell disruption was highlighted in red circles. Concentration of PTX and PEG-PTX NPs were both 5.85 nmol/mL.



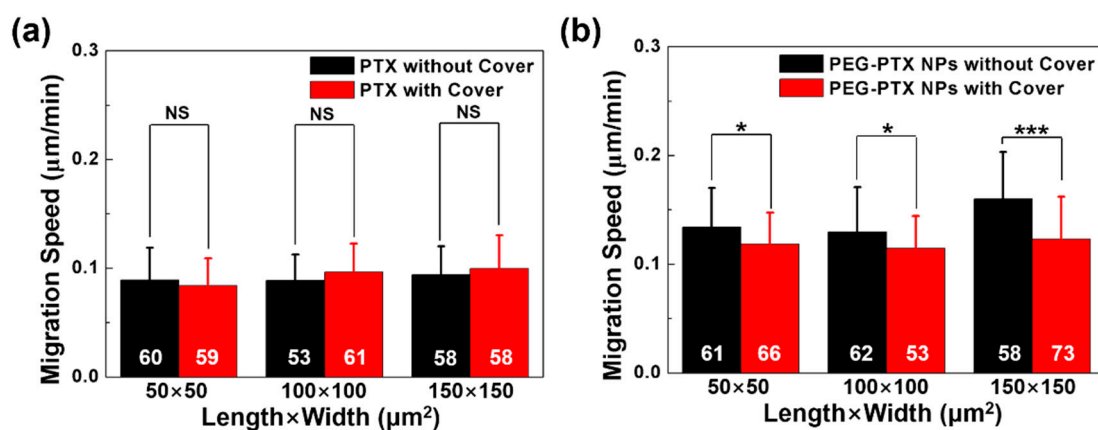
**Supplementary Figure S8.** Trends of NPC43 cell migration speed on flat surface after different treatments. These groups include control without any treatment, NPC43 cells treated with PTX, and NPC43 cells treated with PEG-PTX NPs.  $t_{0.1}$  represents time when migration speed was equal to 0.1  $\mu\text{m}/\text{min}$ . Concentration of PTX and PEG-PTX NPs were both 5.85 nmol/mL.



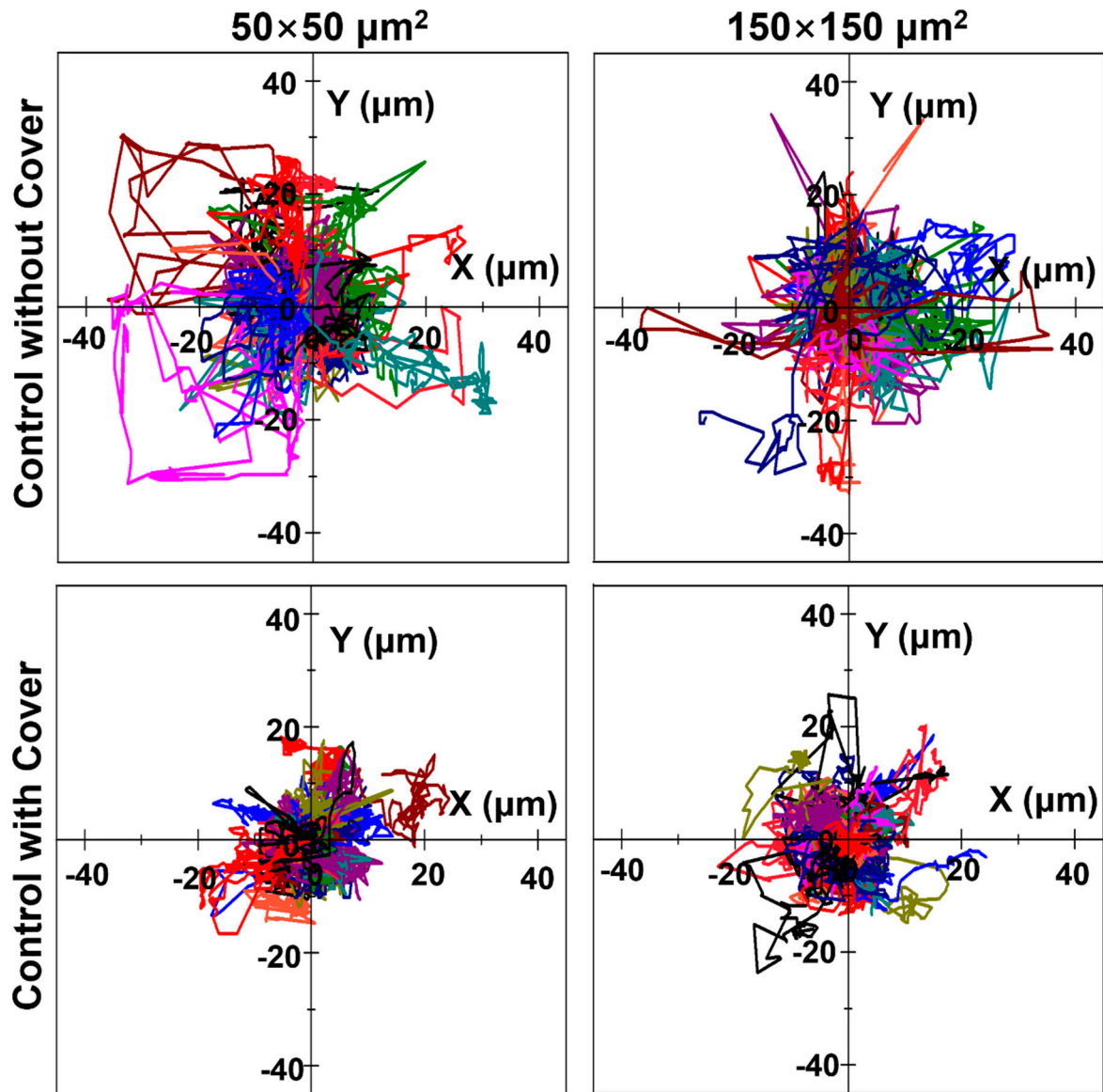
**Supplementary Figure S9.** Trends of NPC43 cell migration speed in 50×50  $\mu\text{m}^2$  microwells (a) without cover and (b) with cover after different treatments. These groups include control without any treatment, NPC43 cells treated with PTX, and NPC43 cells treated with PEG-PTX NPs.  $t_{0.1}$  represents time when migration speed was equal to 0.1  $\mu\text{m}/\text{min}$ . Concentration of PTX and PEG-PTX NPs were both 5.85 nmol/mL.



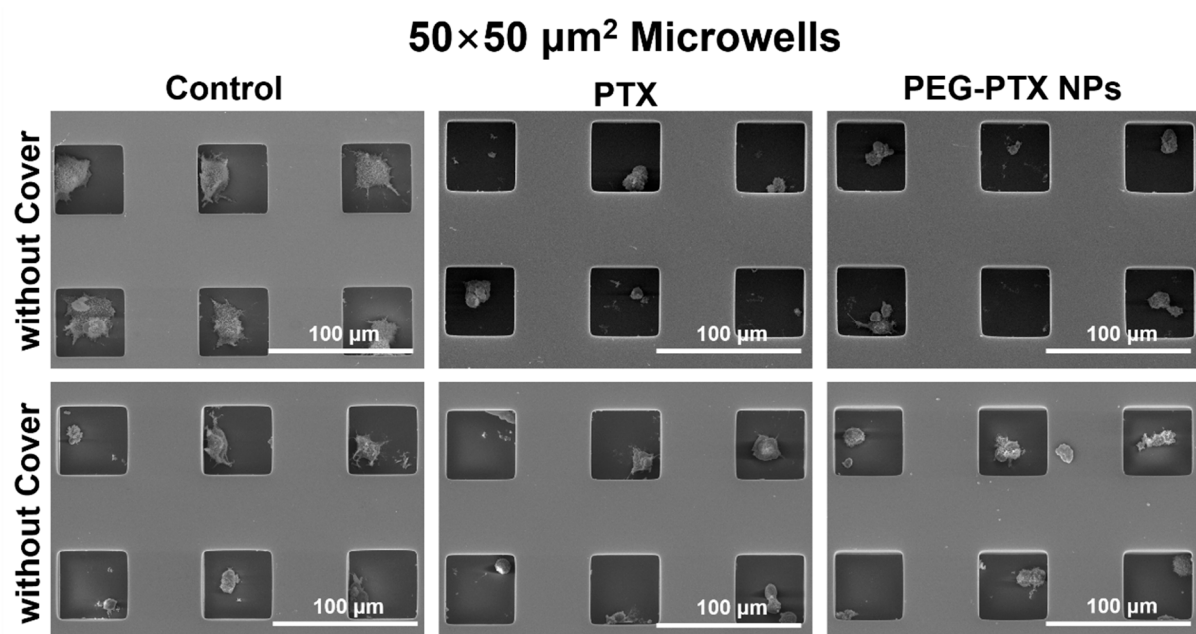
**Supplementary Figure S10.** Trends of NPC43 cell migration speed in 150×150  $\mu\text{m}^2$  microwells (a) without cover and (b) with cover after different treatments. These groups include control without any treatment, NPC43 cells treated with PTX, and NPC43 cells treated with PEG-PTX NPs.  $t_{0.1}$  represents time when migration speed was equal to 0.1  $\mu\text{m}/\text{min}$ . Concentration of PTX and PEG-PTX NPs were both 5.85 nmol/mL.



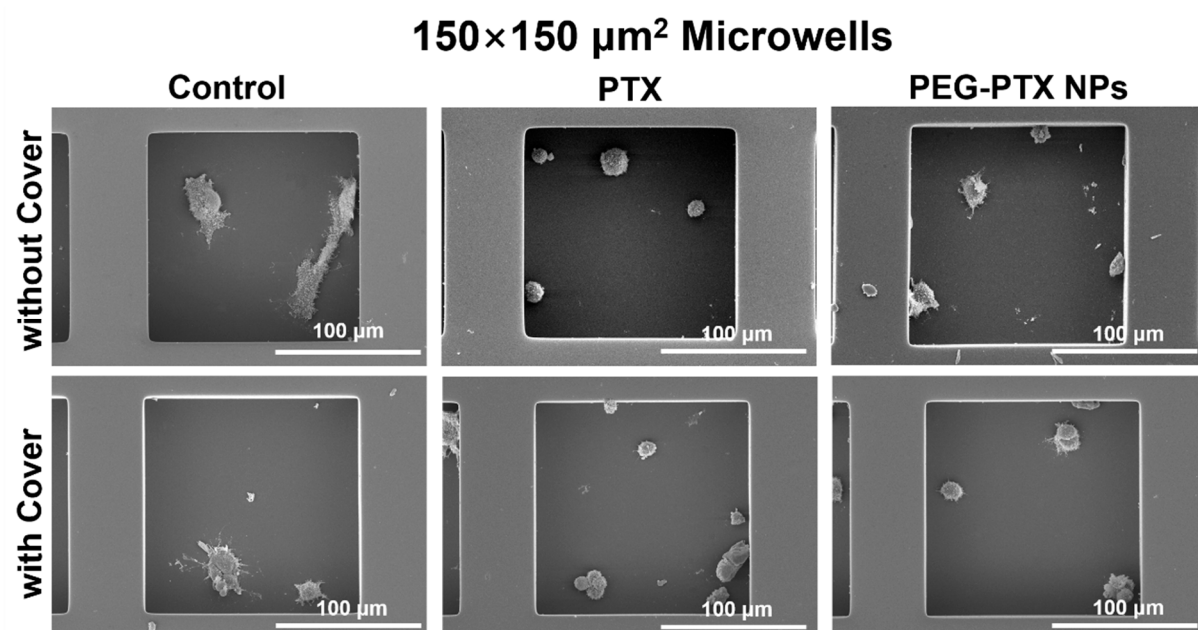
**Supplementary Figure S11.** NPC43 cell migration behavior in microwells with and without cover. (a) Migration speed of NPC43 cells with (a) PTX and (b) PEG-PTX NPs treatments over 16 h in microwells without cover and with cover. One-way ANOVA and Tukey's post hoc test, NS – not significant, \* $p < 0.05$ , and \*\*\* $p < 0.001$ . Number of NPC43 cells counted is marked in white. Concentration of PTX and PEG-PTX NPs were both 5.85 nmol/mL.



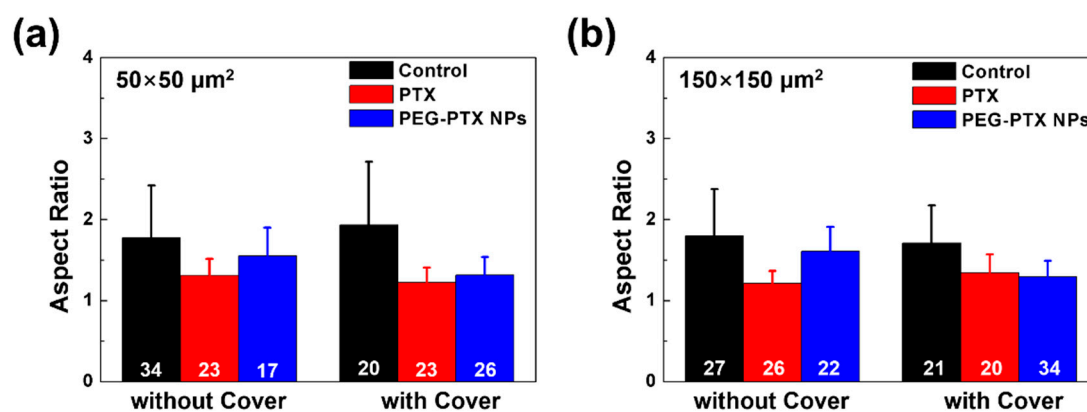
**Supplementary Figure S12.** Cell migration trajectories of NPC43 cells over 16 h without any treatment in  $50 \times 50$  and  $150 \times 150 \mu\text{m}^2$  microwells without and with cover. Starting points of cell migration trajectories are (0, 0).



**Supplementary Figure S13.** Scanning electron micrographs of NPC43 cells with PTX and PEG-PTX NPs added over 16 h in 50×50  $\mu\text{m}^2$  microwells without and with cover. Concentration of PTX and PEG-PTX NPs were both 5.85 nmol/mL.



**Supplementary Figure S14.** Scanning electron micrographs of NPC43 cells with PTX and PEG-PTX NPs added over 16 h in 150×150  $\mu\text{m}^2$  microwells without and with cover. Concentration of PTX and PEG-PTX NPs were both 5.85 nmol/mL.



**Supplementary Figure S15.** Cell aspect ratio after NPC43 cells treated with PTX and PEG-PTX NPs for 16 h in (a) 50×50  $\mu\text{m}^2$  and (b) 150×150  $\mu\text{m}^2$  microwells without and with cover. Number of NPC43 cells counted is marked in white. Concentration of PTX and PEG-PTX NPs were both 5.85 nmol/mL.