Supplementary Information

Size-Dependent Regulation of Intracellular Trafficking of Polystyrene Nanoparticle-Based Drug-Delivery Systems

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a

Carboxylate modified polystyrene NPs in PBS buffer and complete medium (cMEM, containing 10% foetal bovine serum)

Carboxylate modified polystyrene NPs	Mean hydrodynamic diameter (nm)		PDI		Zeta potential mV	
	PBS	cMEM	PBS	cMEM	PBS	cMEM
40 nm PS-NPs	52.3±5.6	74.2±12.1	0.022	0.168	-32.6±3	-11.5±2
150 nm PS-NPs	148.3±16.6	191.8±24.1	0.009	0.041	-26.63±1	-17.52±5

b

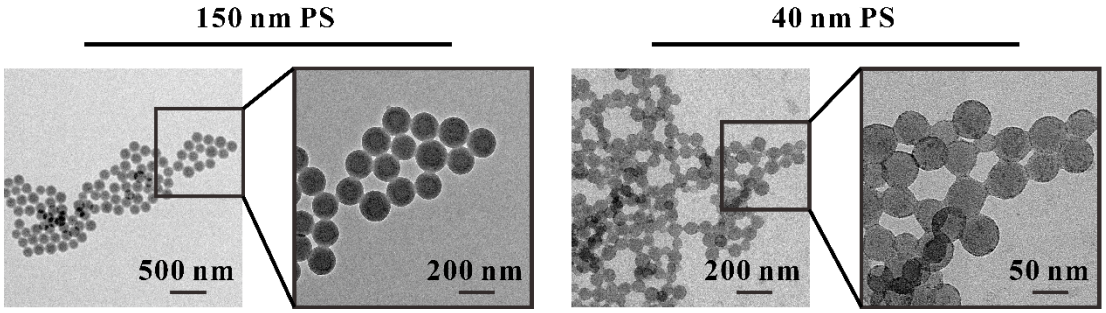

Figure S1. Characterization of PS nanoparticles. (a) Size and zeta potential of 40 nm PS and 150 nm PS in PBS or cMEM. (b) TEM images of 150 nm PS and 40 nm PS.

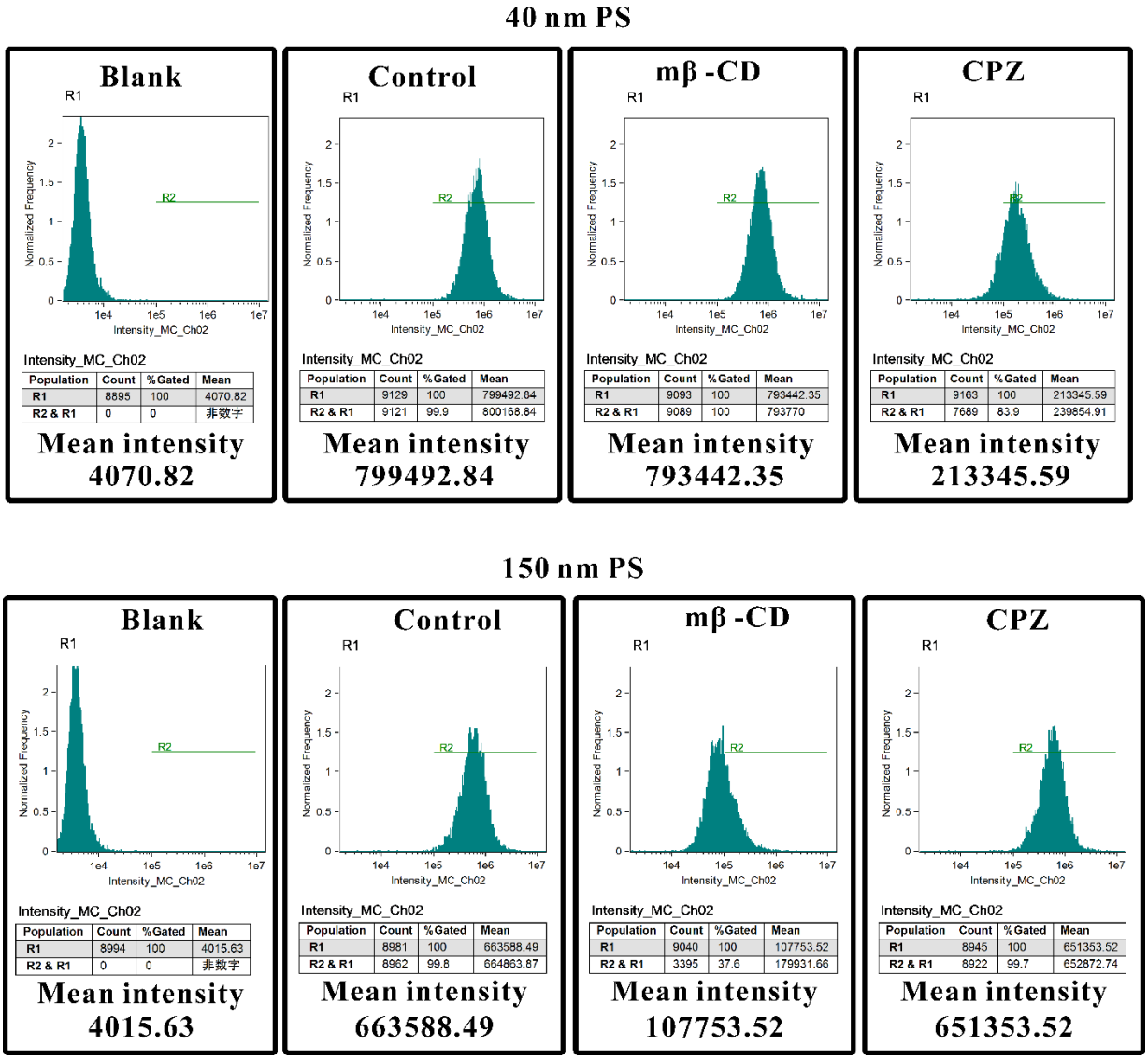

Figure S2. Representative original flow cytometry charts of intracellular fluorescence of HeLa cells incubated with 0.1 mM PS nanoparticles (40 nm or 150 nm) for 18 h in the presence or absence of selective inhibitor of either endocytosis pathways. Mean value represented mean fluorescence intensity per cell quantified by flow cytometry.

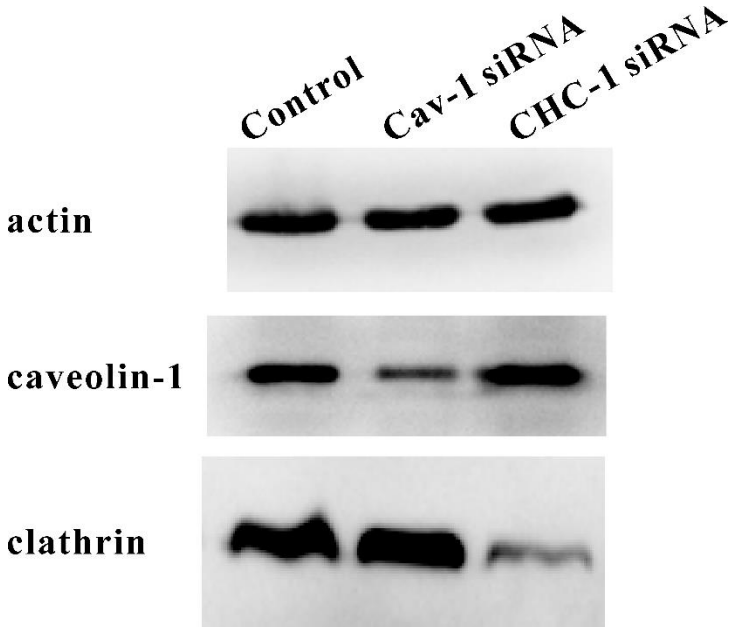

Figure S3. Knockdown of expression levels of caveolin-1 or clathrin heavy chain-1 by RNA interference. HeLa cells were transfected with siRNA against target gene (Cav-1 and CHC-1) for 48 hours. Whole-cell extracts were analyzed by western blotting. Actin was included as loading control.

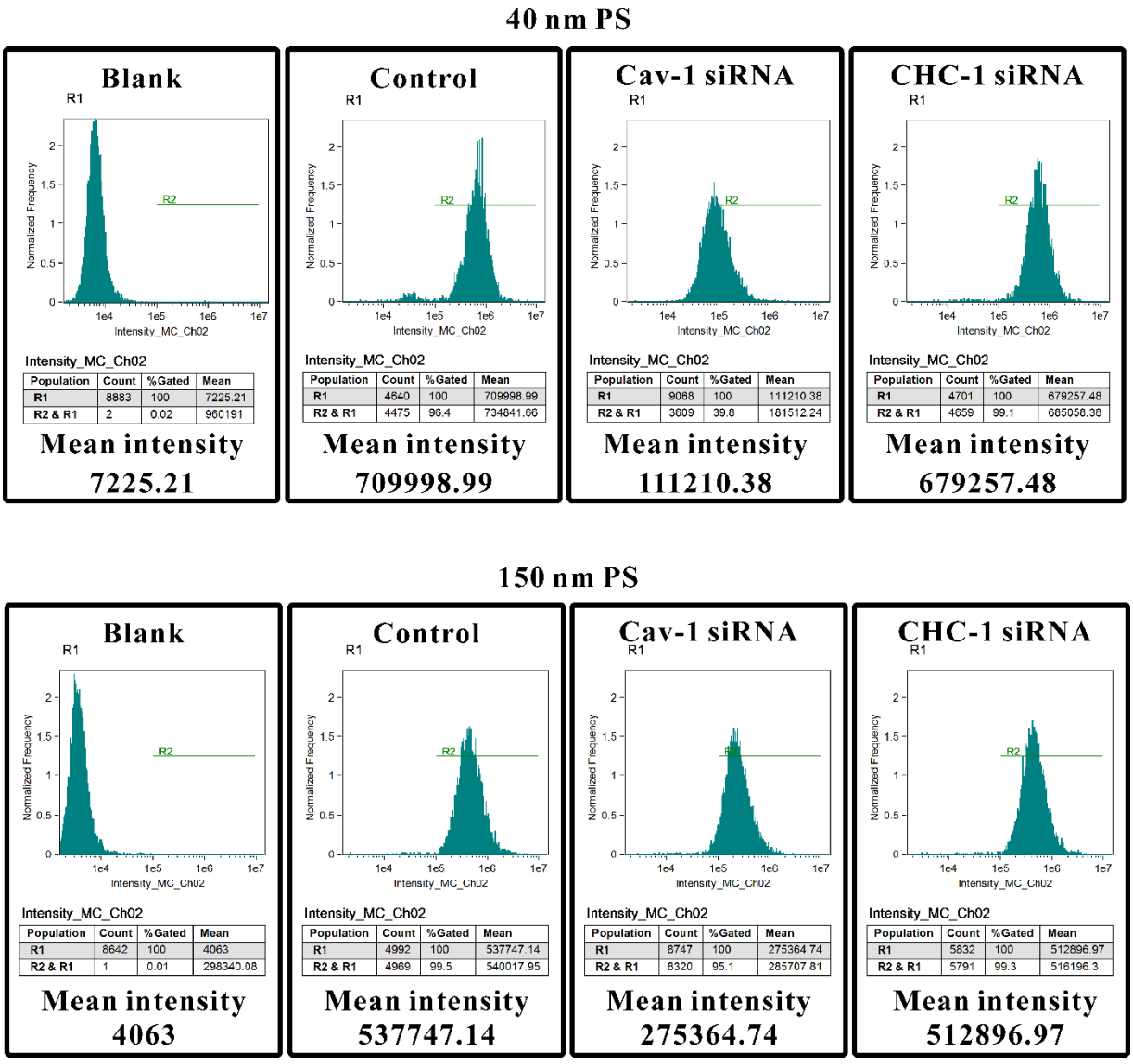

Figure S4. Representative original flow cytometry charts of intracellular fluorescence of HeLa cells transfected with siRNA targeting caveolin-1 or clathrin and incubated with 0.1 mM PS nanoparticles (40 nm or 150 nm) for 18 h. Mean value represented mean fluorescence intensity per cell quantified by flow cytometry.

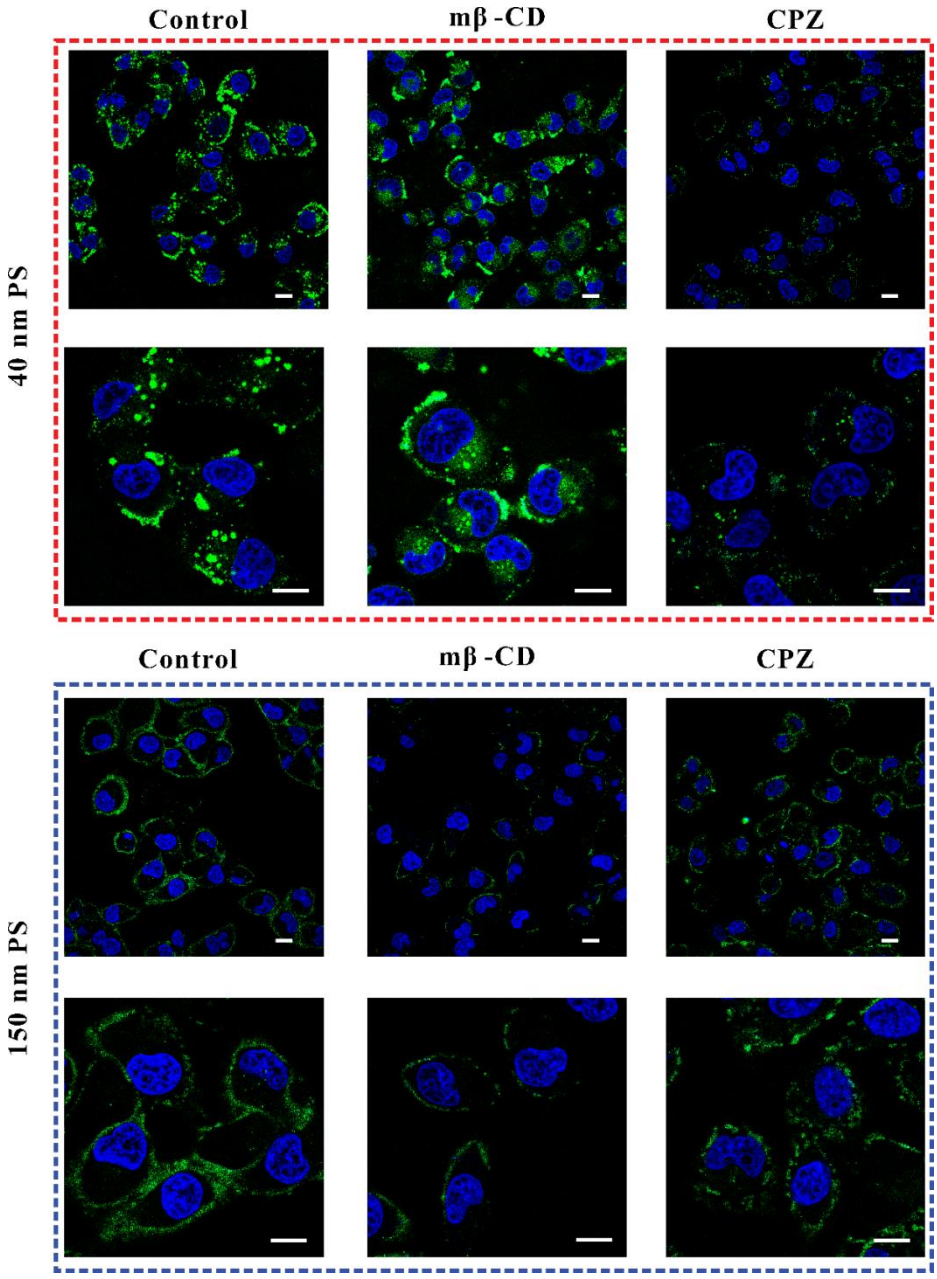

Figure S5. MCF-7 cells were incubated with 0.1 mM PS nanoparticles (40 nm or 150 nm) for 4h in the presence or absence of selective inhibitors and imaged with a confocal microscope. Scale bar: 30 μm.

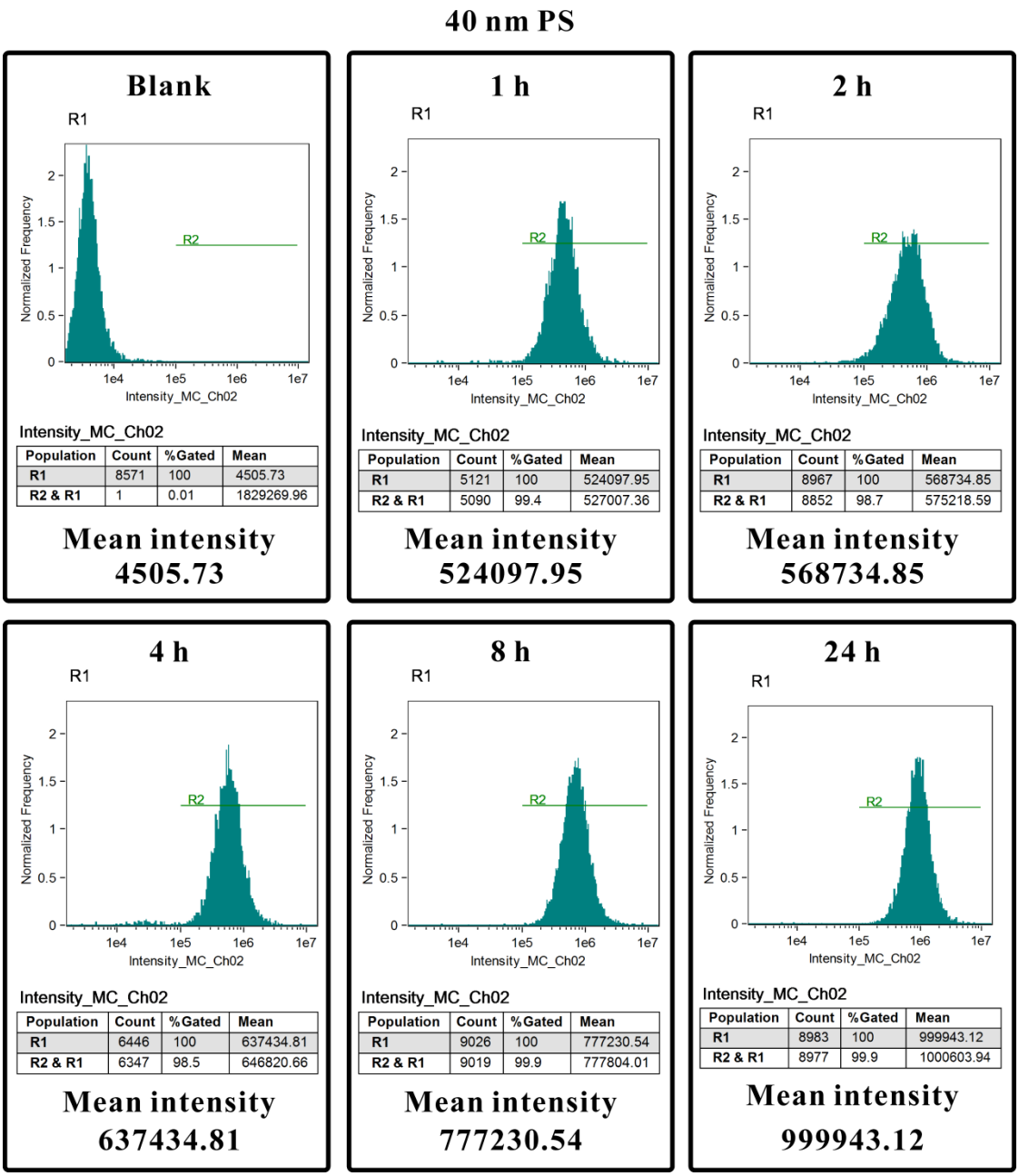

Figure S6. Representative original flow cytometry charts of intracellular fluorescence of HeLa cells incubated with 40 nm PS NPs for indicated time. Mean value represented mean fluorescence intensity per cell quantified by flow cytometry.

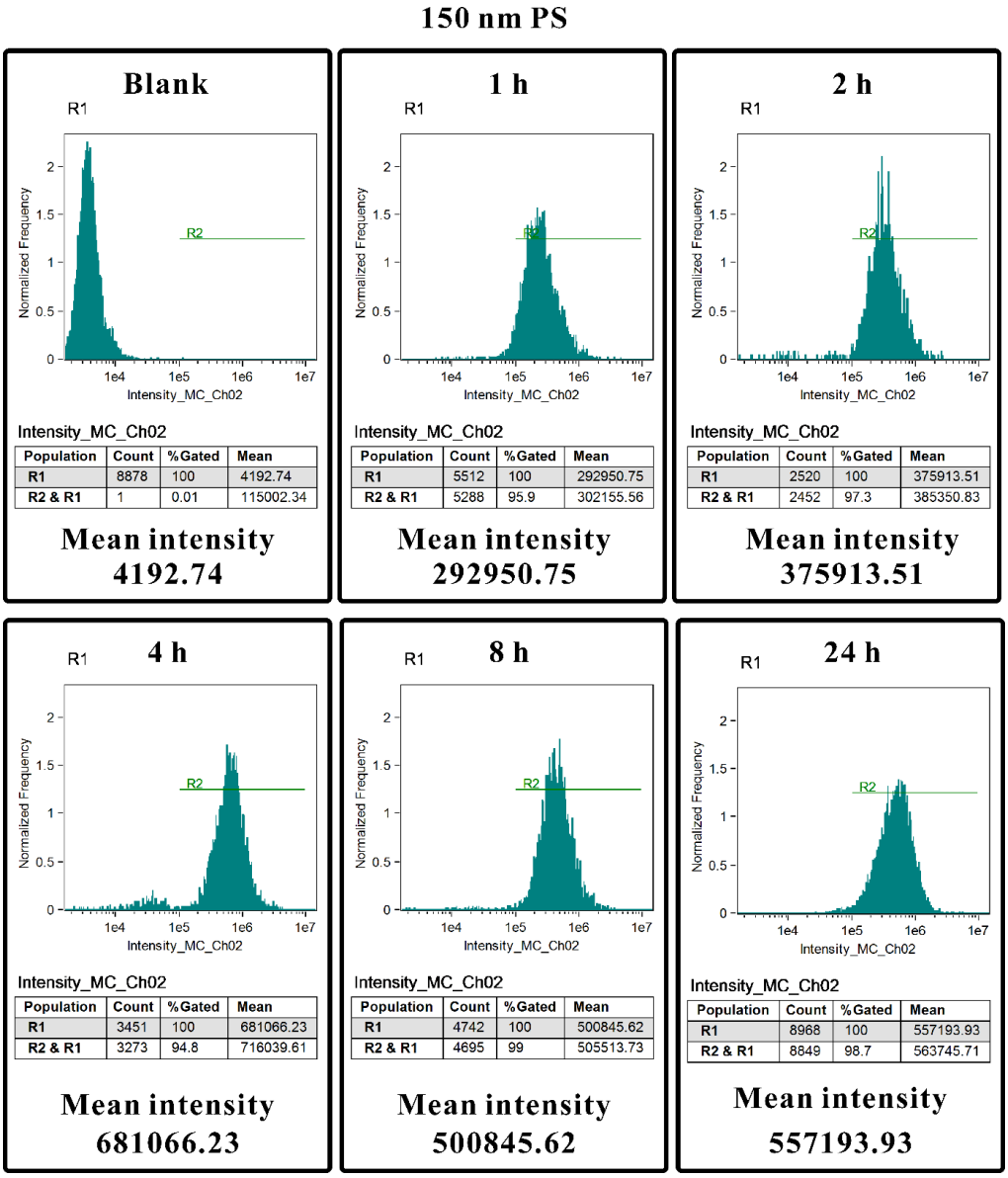

Figure S7. Representative original flow cytometry charts of intracellular fluorescence of HeLa cells incubated with 150 nm PS NPs for indicated time. Mean value represented mean fluorescence intensity per cell quantified by flow cytometry.

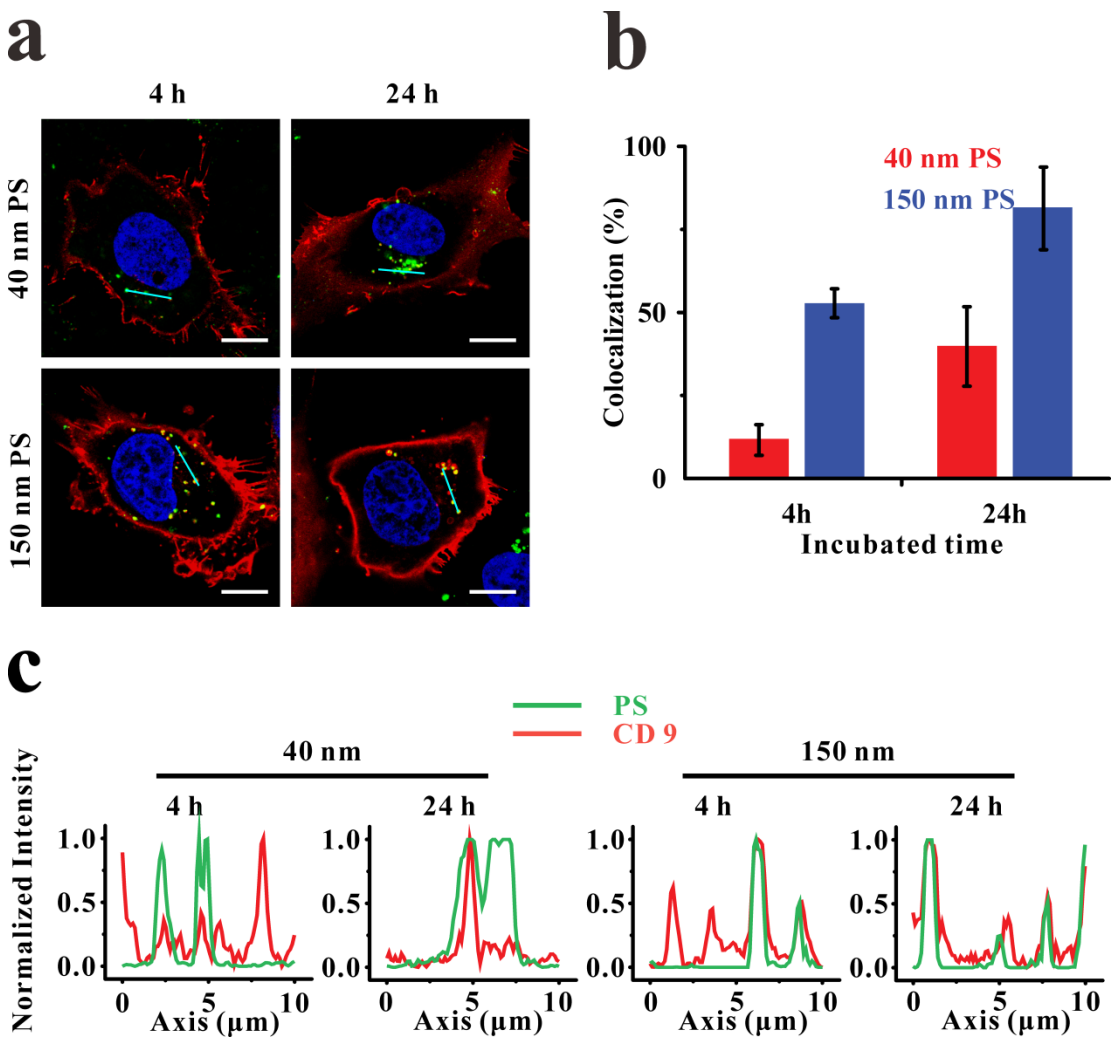

Figure S8. 150 nm PS nanoparticles colocalized with CD9 in MCF-7 cells. (a) MCF-7 cells expressing RFP-CD9 were incubated with NPs (40 nm and 150 nm) and imaged by confocal microscope at 4 h or 24 h. (b) Percentages of PS nanoparticles (green) colocalized with CD9 (red) were quantified using the ImageJ software. (c) The colocalization between CD9 and PS nanoparticles were analyzed by line profiling fluorescence intensity of RFP-CD9 (red) and PS (green) along the line selected in (a). Scale bar: 10 μm.