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Electronic Supporting Information

In vitro cellular behaviors and toxicity assays of small-sized fluorescent silicon nanoparticles

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1. Reagents and Methods

Reagents. Sodium azide, chlorpromazine hydrochloride, methyl-β-cyclodextrin, 5-(N-Methyl-N-isobutyl)amiloride (MIBA), nocodazole, dihydrocytochalasin B (DHCB), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (All from Sigma-Aldrich); sucrose (Sinopharm Chemical Reagent Co., Ltd); calcein-AM and cytotoxicity LDH assay kit-WST (Dojindo, China); propidium iodode (PI) (purchased from J&K Scientific Ltd); Cell Titer-Glo Luminescent Cell Viability Assay kit (Promega); LysoTracker-Red DND-99, DiD, CellLight Early Endosomes-RFP, BacMam 2.0 and CellLight Late Endosomes-RFP, BacMam 2.0 were purchased from Life Invitrogen. pDsRed-Golgi and pDsRed2-ER were obtained from Clontech.

Instrumentation. The characterizations of SiNPs were through transmission electronic microscopy (TEM) and high-resolution TEM (HRTEM) (Philips CM 200 electron microscope, 200kV). UV-vis absorption spectra were measured from 750 UV-vis near-infrared spectrophotometer (Perkin-Elmer lambda). Photoluminescence (PL) was obtained by spectro-fluorimeter (HORIBA JOBIN YVON FLUORMAX-4). A Fourier-transform infrared (FTIR) spectrometer (Bruker HYPERION) was used for FTIR spectra. Delsa™ nano submicron particle size was employed for the analysis of dynamic light scattering (DLS). Fluorescence imaging experiments were performed on a confocal laser scanning microscope (Leica, TCS-SP5 II). Flow cytometry evaluation was realized via a flow cytometer (FACS Caliber, Becton, Dickinson and Company).

Cytotoxicity assessment. In vitro cytotoxicity was systematically determined through established MTT, ATP, LDH (lactate dehydrogenase) and calcein-AM/PI staining assay, respectively. In brief, HeLa cells were seeded in 96-well plates at 4×10^3 or 8×10^3 cells per well overnight. Then, various concentrations of SiNPs were introduced to cells for 24 h or 48 h, with untreated cells as control. For MTT assay, 10 μL stock MTT (5 mg/mL) was added and incubated for another 4 h at 37 °C and later, acidified SDS was used to lyse the cells. The absorbance at 570 nm was tested by using the microplate reader (Bio-Rad 680, U.S.A.). For ATP assay, 20 μL Cell Titer-Glo Luminescent Cell Viability Assay kit (Promega) regent was added, shaking for 5 min and incubated for another 10 min, and then luminescent was measured by SpectraMax M5. For LDH assay, HeLa cells were first washed with PBS, and then treated with lysis buffer (10 μL) at 37 ^{o}C for 30min. Afterward, the LDH working solution (100 μL) was added into each well for another 30 min at room temperature. The absorbance measurement was performed at 490 nm. On the other

hand, live/dead cells staining were examined using the calcein-AM and propidium iodode (PI), respectively. Cells were trypsinized, collected and re-suspended in 200 μL PBS, and incubated with 2 μM calcein-AM and 2 μM PI for 30 min at room temperature. Lastly, 20 μL samples were dropped on glass slides and analyzed with CLSM. The excitation wavelength was set as 488 nm (20% power of argon laser) for calcein-AM and 543 nm (30% power of He/Ne laser) for PI; the corresponding emission window was set as 500-550 nm or 550-620 nm. All independent experiments were carried out three times at least.

2. Characterization of SiNPs

2.1 Photo-/ Storage-/pH-stability of SiNPs

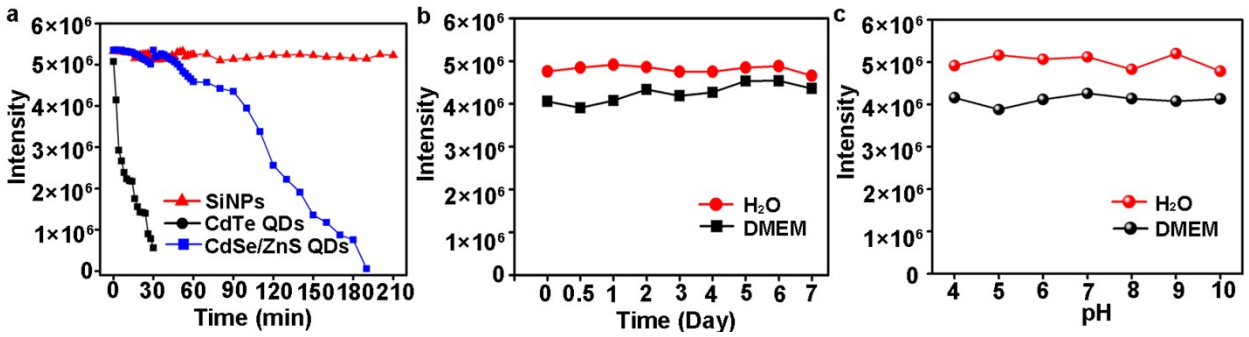

Figure S1. (a) Photostability comparison of SiNPs and QDs under continuous UV irradiation (365 nm, 450 W xenon lamp). (b) The fluorescent intensity of SiNPs in water and DMEM during seven days at 37°C. (c) The fluorescent intensity of SiNPs dispersed in water and DMEM under various pH values.

The SiNPs possess extremely high photo-/storage-/pH-stability. The fluorescence of SiNPs remains stable during up to 210 min of continuous irradiation with high-power UV light (365 nm, 450 W) (Figure S1a) or in different incubation conditions (*i.e.*, water and culture medium) during seven-day test period at 37 °C (Figure S1b). Also, the intensities of their fluorescence keep nearly unchanged under various pH values (*i.e.*, pH 4-10) in water or DMEM (Figure S1c).

2.2. Fourier transform infrared (FTIR) spectrum

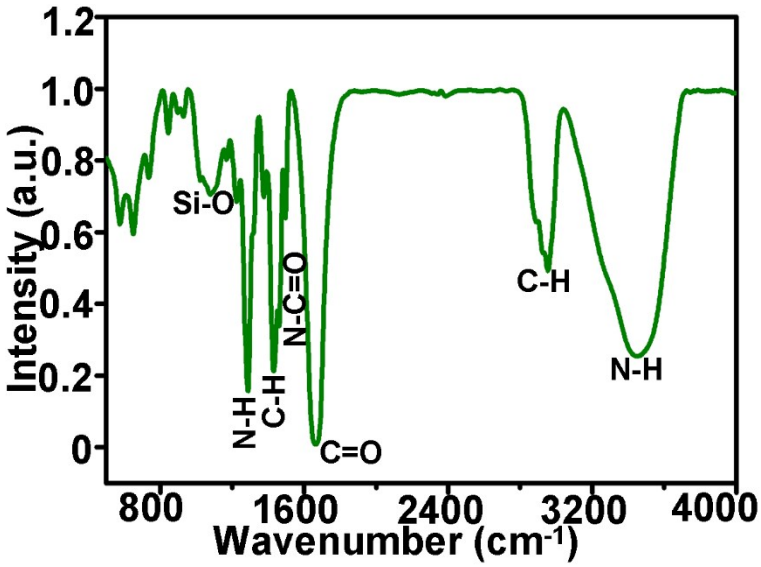

Figure S2. FTIR spectrum of SiNPs

The FTIR spectrum shows surface chemical bonding of SiNPs. Strong absorbance peaks at 1080, 1290, 1421-1440, 1525, 1664, 2958 and 3470 cm^{-1} were credited to the vibrational stretch of Si-O bonding, N-H deformation vibration, the C-H aromatic skeletal vibration, N-C=O vibration, C=O stretching vibration, C-H vibration, and the N-H stretching vibration, respectively.

2.3 Hydrodynamic diameter of SiNPs in water and DMEM

Table S1. Hydrodynamic diameter of SiNPs in water and DMEM during seven-day incubation at 37^{o}C.

Day Size (nm)	0	1	2	3	4	5	6	7
Water	4.363	4.421	4.315	4.322	4.104	4.565	4.340	4.509
DMEM	5.893	5.804	5.948	5.789	5.999	5.805	6.607	5.920

2.4 DLS-determined size of SiNPs in water and DMEM

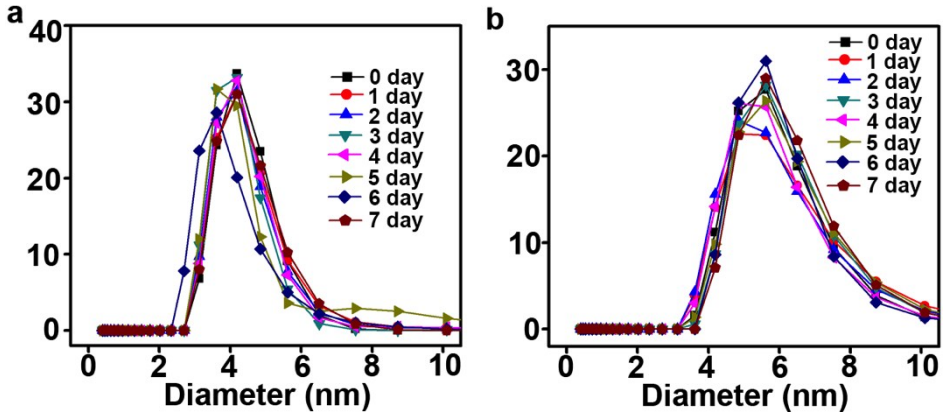

Figure S3. DLS size of SiNPs in water (a) and DMEM (b) during seven-day incubation at 37°C.

The DLS-determined size of SiNPs maintains nearly the same during seven-day incubation, indicating that the SiNPs dispersed in water and DMEM culture medium suffer no obvious aggregation. The size of SiNPs in DMEM is slightly larger than that of SiNPs in water, which is possibly due to biomolecules (*e.g.*, amino acid, vitamins, and sugar contained in DMEM medium) absorption on the surface of SiNPs.[1,2]

2.5 Coomassie Blue stained gel lanes analysis

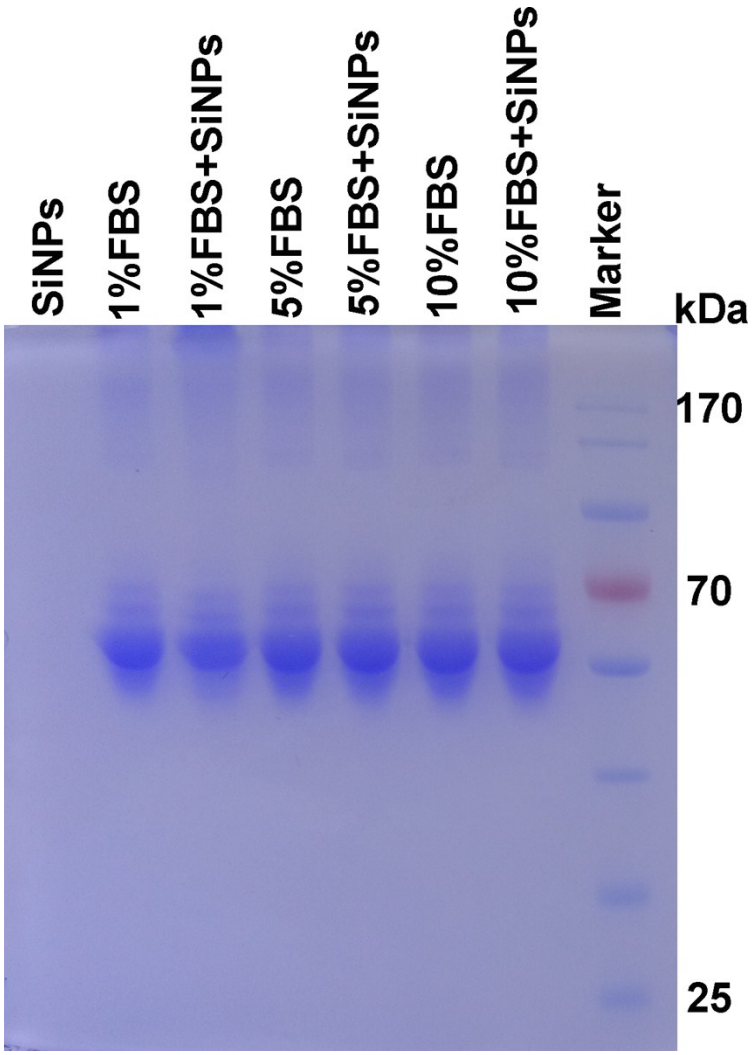

Figure S4. Coomassie Blue stained gel lanes analysis. 0.8 mg/mL SiNPs were incubated with different concentrations of FBS, then 10% Native-PAGE was used for protein separation.

For the SiNPs with a concentration of 0.8 mg/mL (*i.e.*, the concentration used in our experiment), no significant interaction between SiNPs and serum protein is happened at various concentrations of FBS ranging from 1% to 10%, indicating negligible protein adsorption on SiNPs' surface in our experiment conditions.[3,4]

3. Time-dependent uptake of SiNPs by flow cytometry

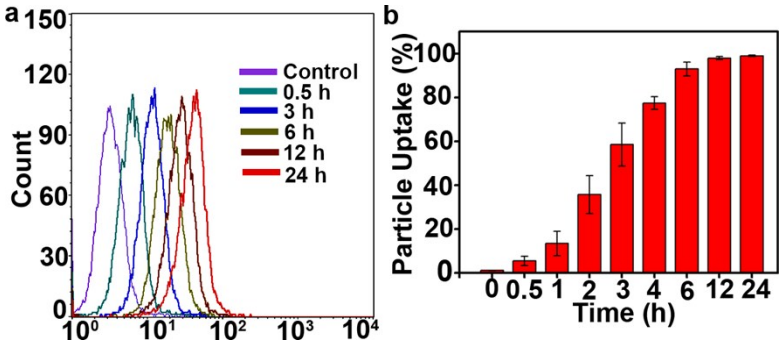

Figure S5. Time-dependent uptake of SiNPs by HeLa cells. (a) Flow cytometry histogram and (b) proportion analysis of particle uptake by flow cytometry.

The flow cytometry histogram shows the uptake of SiNPs increases during 24 h incubation, while above 93% cells have gained a strong fluorescence compared to control cells after incubation for only 6 h.

4. Energy-dependent uptake of SiNPs by flow cytometry

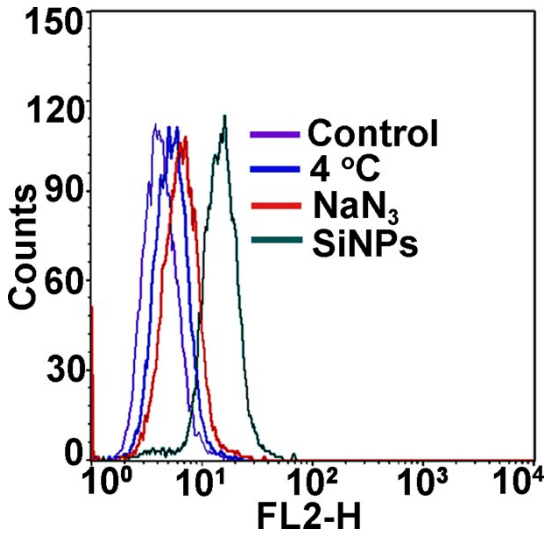

Figure S6. Flow cytometry histogram of cells with different treatments. Here, 'control' means cells are untreated with SiNPs, while '4 °C' and 'NaN_3' mean cells are treated with SiNPs performed at 4 °C or in the presence of NaN_3.

FCM analysis intuitively demonstrates that the uptake is inhibited by low temperature or NaN_3.

5. Uptake pathway of SiNPs

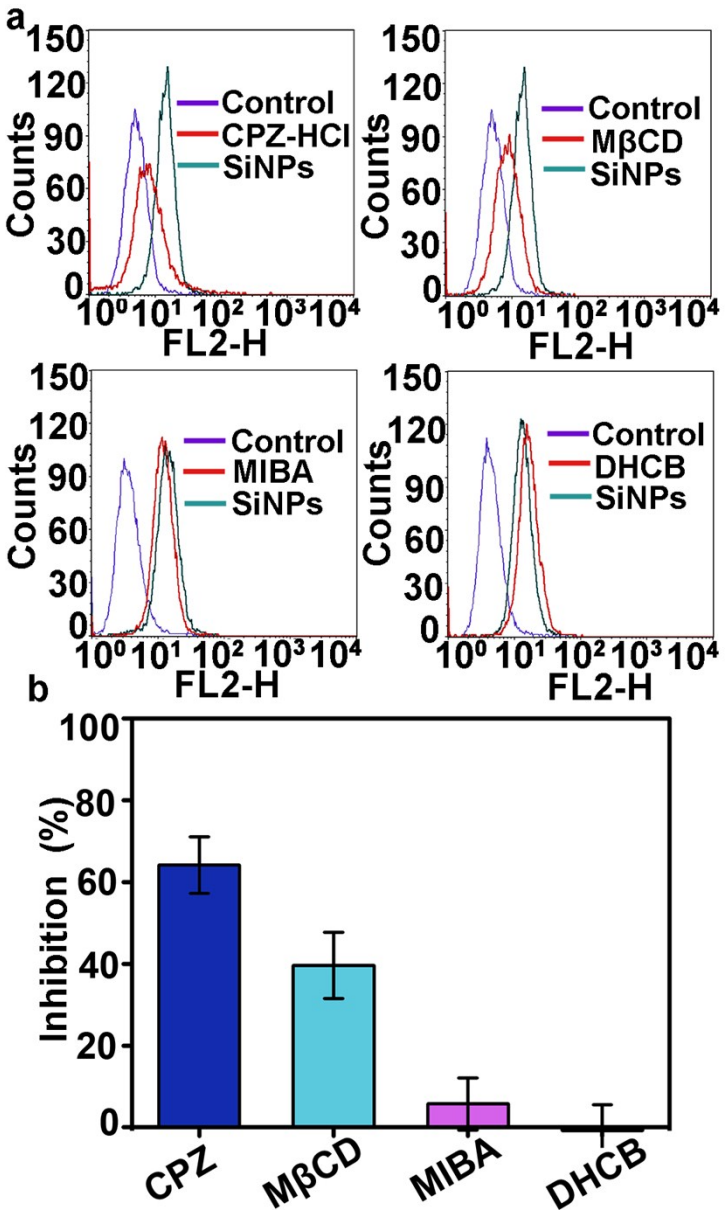

Figure S7. Uptake pathway of SiNPs. (a) Flow cytometry histogram for different inhibitors treated cells. (b) The inhibition percentages of SiNPs by different inhibitors.

The inhibition was calculated to vividly show the significance of various pathways on SiNPs uptake. Error bars represent the standard deviation of the mean from triplicate experiments.

6. 3D reconstruction images of cell incubated with SiNPs

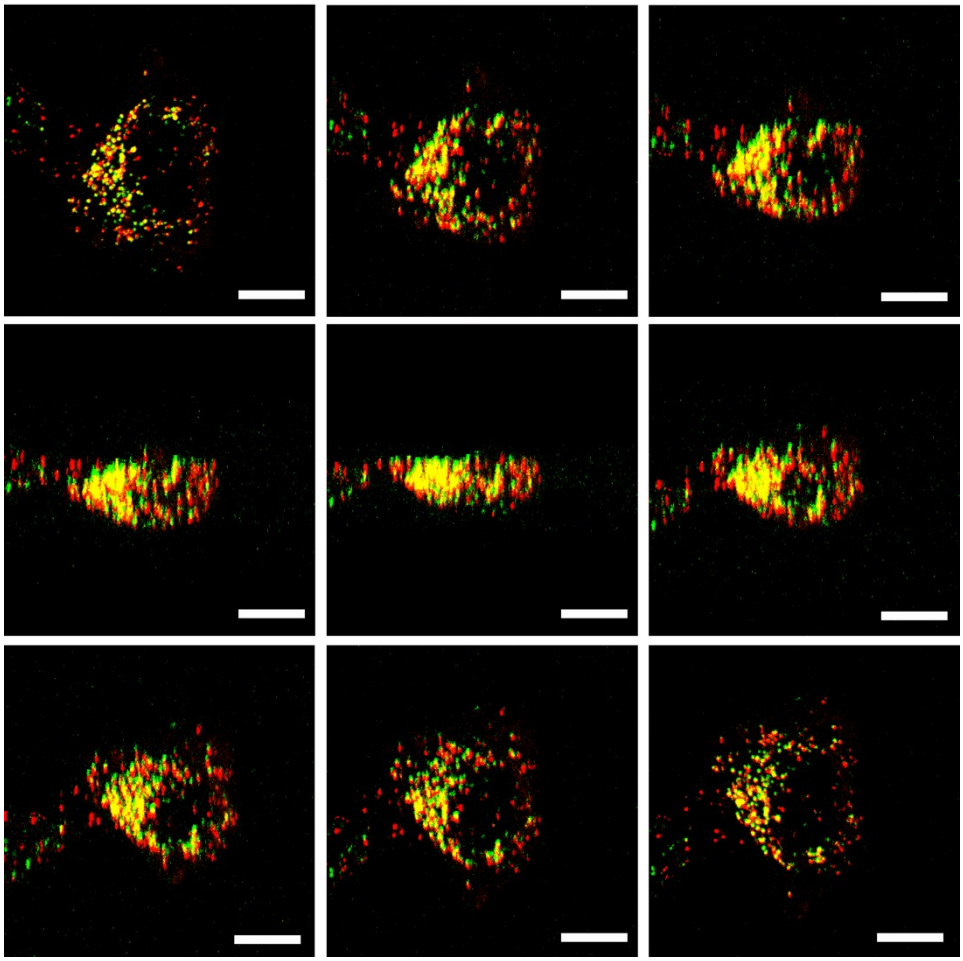

Figure S8. 3D reconstruction images of the SiNPs-incubated cell. 40 images are photographed every 0.3 μm. Snapshot images of HeLa cells incubated with SiNPs (green) for 24 h and stained with Lysotracker-Red DND-99 for lysosomes (red) labeling. Scale bar = 10 μm.

A stack of imaging planes was acquired in the z direction to cover the full height of cell. Three dimensional (3D) distribution of SiNPs within a single cell is shown. The green and red dots represent SiNPs and lysosomes, respectively. Colocalization of SiNPs and lysosomes is seen in yellow color in images. 3D images indicate that SiNPs are ultimately distributed in the perinuclear region of cell and colocalized with lysosomes after 24-h incubation.

7. Magnified images of colocalization of SiNPs with intracellular compartments

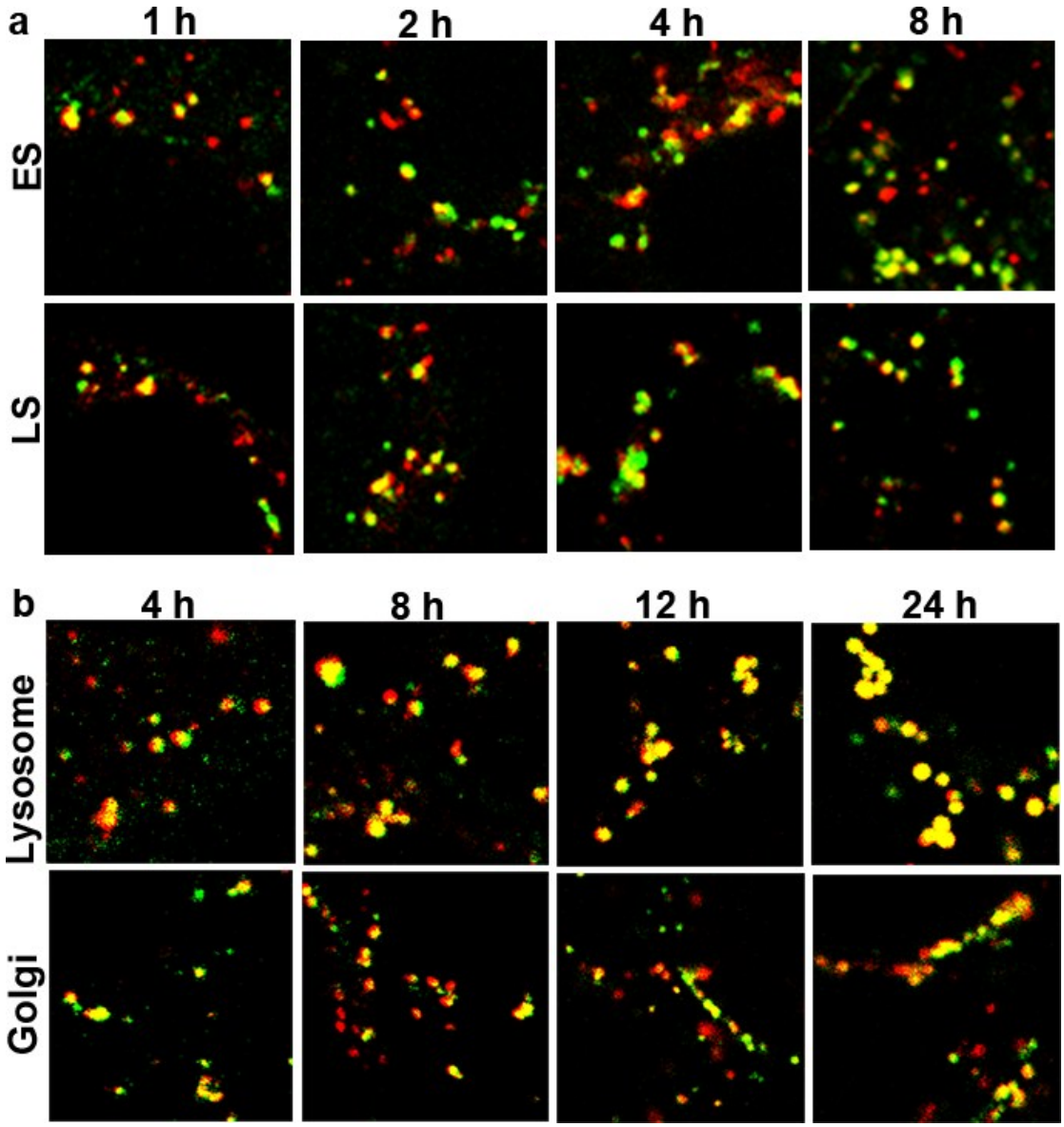

Figure S9. Magnified images in square in Figure 5 present colocalization of SiNPs with (a) ES and LS from 1 to 8 h incubation and (b) lysosome and Golgi from 4 to 24 h.

8. Colocalization of SiNPs with ER during 24 h incubation

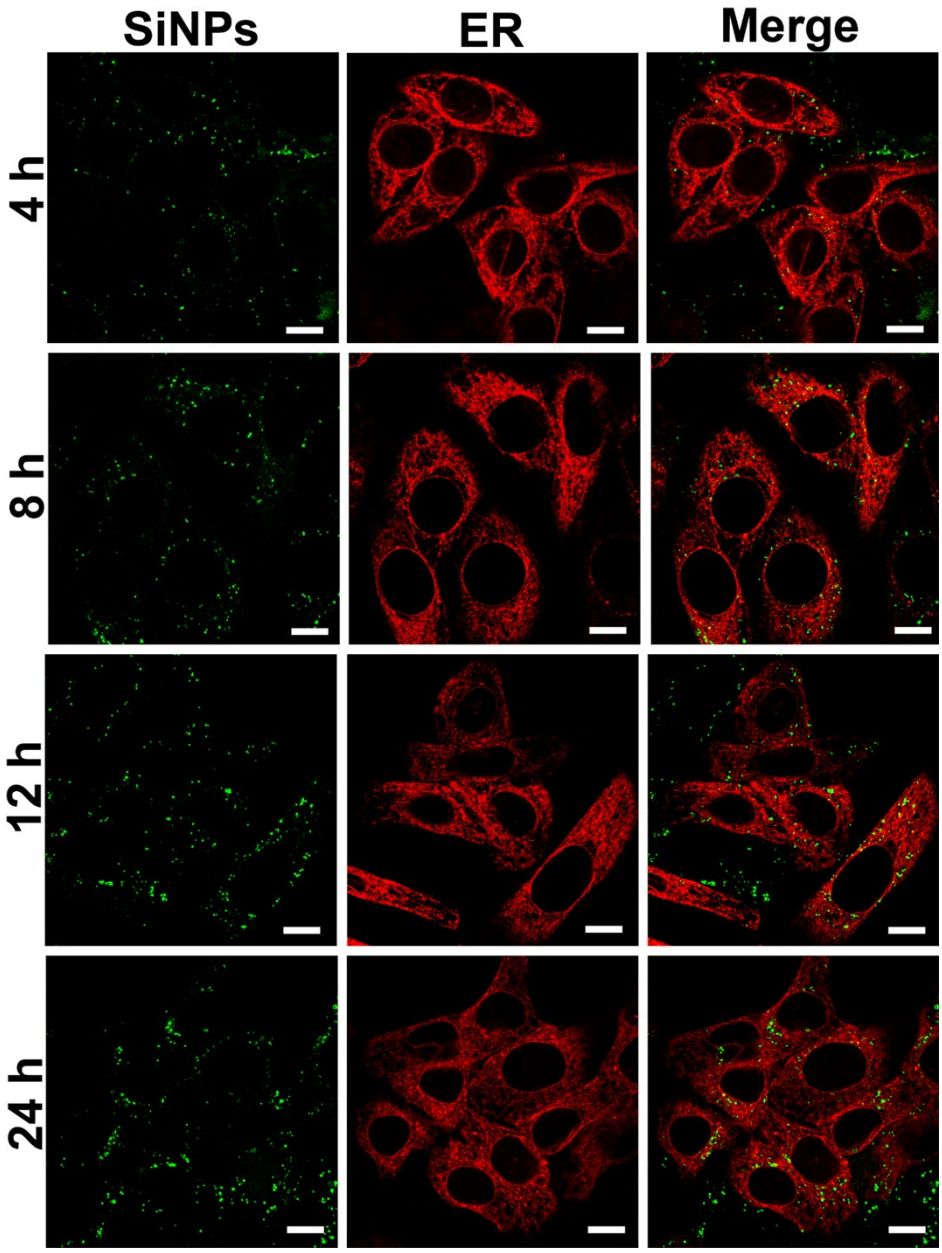

Figure S10. Colocalization analysis of SiNPs with ER during the 24 h incubation period, no colocalization can be seen over the entire incubation period. Scale bar = 10 μm.

9. References

1. M. P. Monopoli, C. Aberg, A. Salvati and K. A. Dawson, *Nat. Nanotechnol.*, 2012, **7**, 779-786.
2. Z. Zeng, J. Patel, S.-H. Lee, M. McCallum, A. Tyagi, M. Yan and K. J. Shea, *J. Am. Chem. Soc.,* 2012, **134**, 2681-2690.
3. M. Lundqvist, J. Stigler, T. Cedervall, T. Berggård, M. B. Flanagan, I. Lynch, G. Elia and K. Dawson, *ACS Nano*, 2011, **5**, 7503-7509.
4. F. Bertoli, G.-L. Davies, M. P. Monopoli, M. Moloney, Y. K. Gun'ko, A. Salvati and K. A. Dawson, *Small*, 2014, **10**, 3307-3315.