Supplementary Information

In vitro and *in vivo* toxicity evaluation of halloysite nanotubes

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The stability of FITC-HNTs

UV-Vis spectra were used to investigate the stability of FITC-HNTs under all exposure conditions in the experiment. Briefly, for cell culture conditions, FITC-HNTs (50 μg/mL) were added into PBS (pH=5.4 and 7.4) and DMEM for 24 h, then the solution was centrifuged and then the supernate was measured with a UV-visible spectrophotometer (UV-2550, Shimadzu Instrument Ltd., Suzhou, China), PBS and DMEM were set as control groups, respectively. For zebrafish feeding conditions, FITC-HNTs (0.25 and 25 mg/mL) were added into egg water for 96 h, the supernate was measured with a UV–visible spectrophotometer after centrifuged treatment, respectively. Egg water was designed as control group.

As shown in **Fig. S1A,** no characteristic UV-Vis spectra peaks of FITC appear both in pH 5.4 and 7.4 PBS conditions. Similarly, the UV-Vis spectra of DMEM have no difference when FITC-HNTs for 24 h (**Fig. S1B**). The results demonstrate that FITC cannot break away from FITC-HNTs in cell culture conditions. **Fig. S1C** shows the UV-Vis spectra of egg water after different concentration of FITC-HNTs added for 96 h, both the low concentration and high concentration of FITC-HNTs processed egg water does not exhibit any obvious absorption peaks of FITC. This suggests that FITC-HNTs are stable in zebrafish feeding exposure conditions. In short, FITC-HNTs possess high stability under all exposure conditions in cell culture and zebrafish feeding exposure conditions.

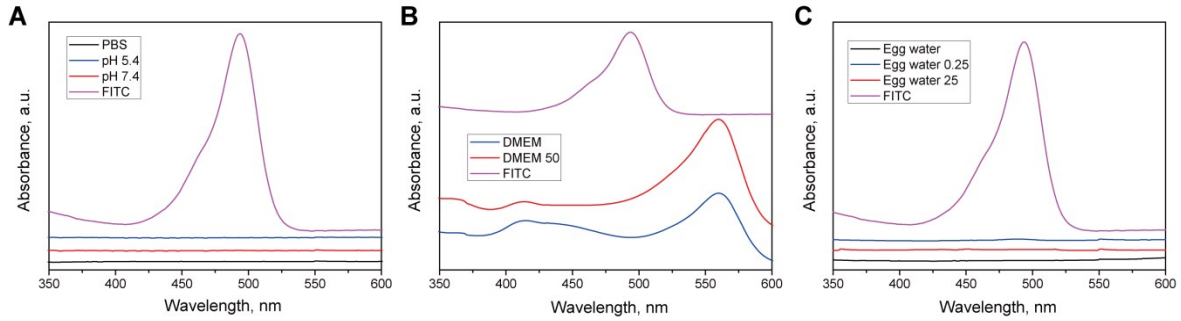

Fig. S1 UV-Vis spectra of FITC-HNTs (50 μg/mL) in (A) PBS, (B) DMEM for 24 h after centrifuged treatment. (C) UV–Vis spectra of FITC-HNTs (0.25 and 25 mg/mL) in egg water for 96 h after centrifuged treatment.

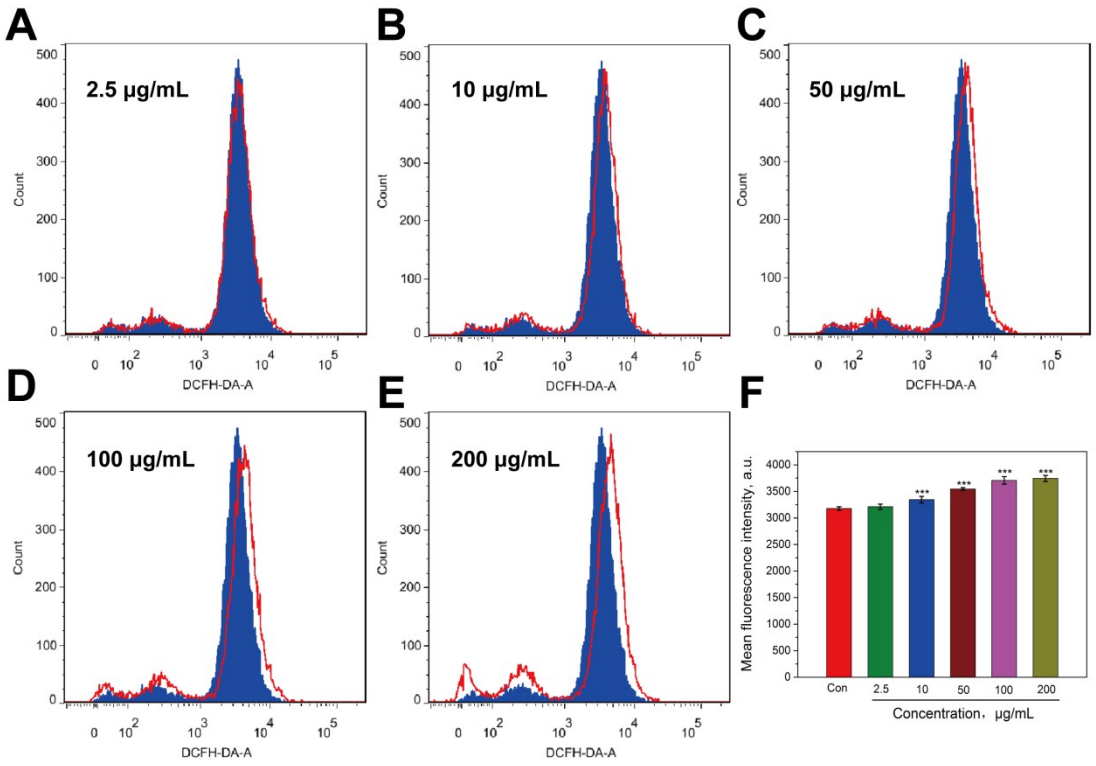

Fig. S2 ROS generation monitored by flow cytometry. HUVECs cells were treated with various concentrations of HNTs for 24 h, respectively. (A) 2.5 μg/mL, (B) 10 μg/mL, (C) 50 μg/mL, (D) 100 μg/mL, (E) 200 μg/mL. In these histogram plots, blue filled regions indicate control normal cells, unfilled regions indicate HNTs-treated cells. (F) Bar graph representation for ROS generation at various concentrations of HNTs. The values were represented as mean ± SD (n = 3). The data are performed by Graph Prim 6 for one way ANOVA and a Turkey post-hoc test. $^{***}P <$ 0.001 versus control group.

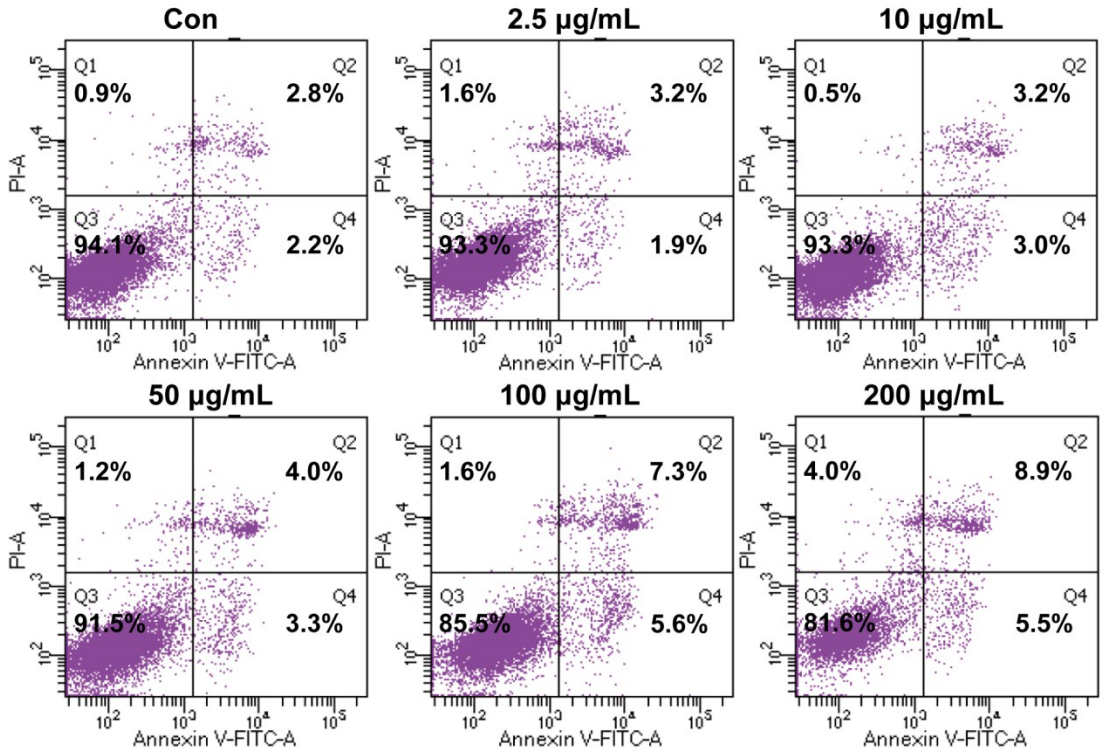

Fig. S3 Apoptosis detection by Annexin V-FITC/PI assay of HUVECs after treated with different concentrations of HNTs for 24 h, respectively.

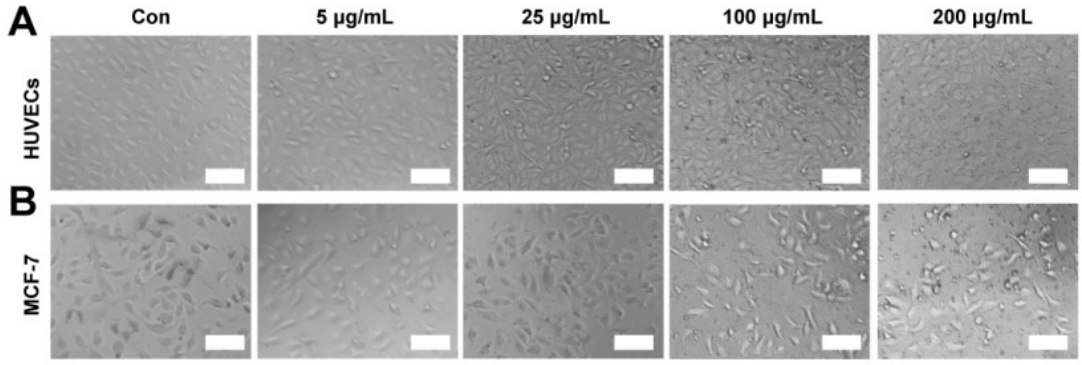

Fig. S4 Bright field images of (A) HUVECs and (B) MCF-7 cells after treated with different concentrations of HNTs for 24 h. Scale bar = 50 μm.

LD_{50} estimation

To estimate the LD_{50} values of HNTs towards HUVECs and MCF-7 cells, cells were cultivated in 96-wells at a density of 8×10^3 cells per well and incubated with different concentration of HNTs (10, 20, 40, 80, 160, 320, 640, 1280 μg/mL) for 24 h. Then the cells were washed with PBS twice and replaced with 100 fresh DMEM medium. 10 μL of CCK-8 reagents were added into the wells and incubated for another 4 h, the absorbance at 450 nm was measured using a microplate reader (Multiskan MK3, Thermo). The meanings of absorbance of control sample were considered as 100% viability, and the concentration of HNTs which led to

approximately 50% of cells was labeled as LD_{50}. The value was evaluated using GraphPad Prism 6 (GraphPad Software, La Jolla, CA).

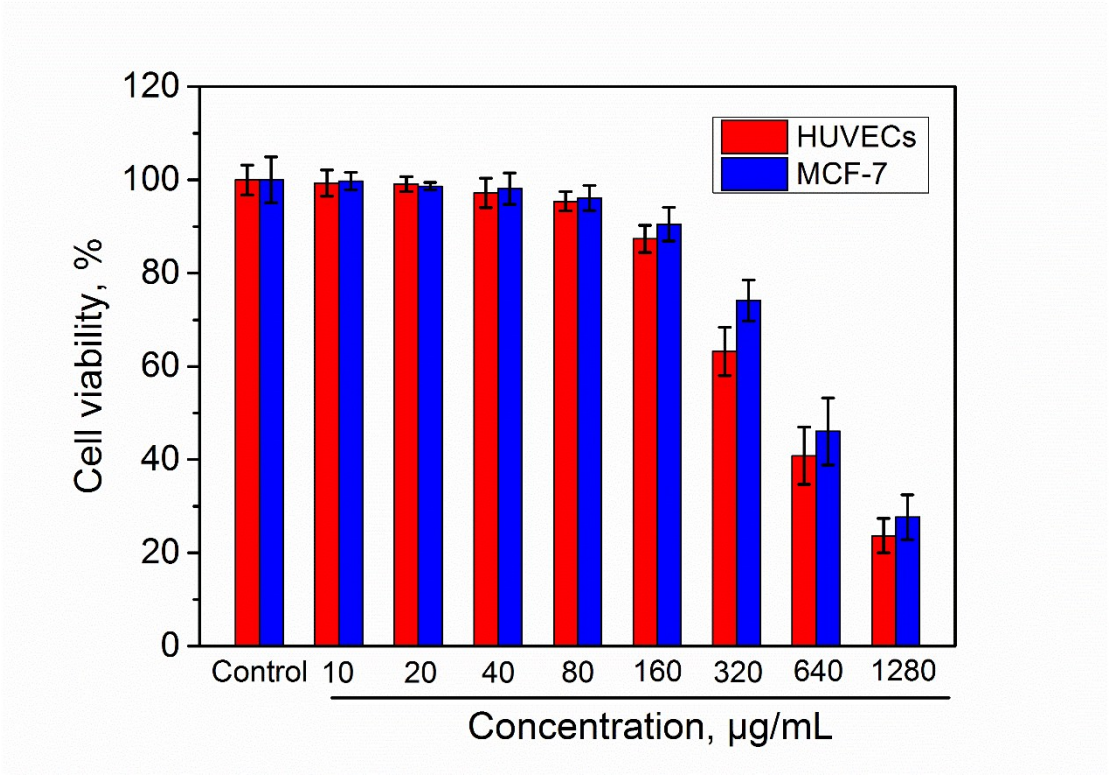

Fig. S5 LD_{50} estimation of HNT concentrations towards HUVECs and MCF-7 cells.