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Supporting Information

Targeting specific cell organelles with different-faceted nanocrystals that are selectively recognized by organelle-targeting peptides

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Experimental Section

Synthesis and characterization of Cd-based nanorods

The CdS nanorods (including high-energy (001) CdS and low-energy (101) CdS) were synthesized as described previously.[1] The CdSe nanorods ((001) CdSe and low-energy (110) CdSe) were synthesized using the methods reported in the literature.[2] The synthesized CdSe nanorods were characterized with field-emission scanning electron microscopy (FE-SEM, Nanosem 430, FEI) and X-ray diffraction (XRD, D/MAX2500, JAPAN SCIENCE). The zeta potentials of the CdS and CdSe nanorods in the culture medium (F-12K medium containing 10% FBS) were determined using a zeta potential analyzer (Brookhaven, ZETAPALS/BI-200SM).

The calculated surface energies of CdS-H and CdS-L are 0.627 J/m^2 and 0.451 J/m^2, respectively.[3] Theoretical calculations of CdSe surface energy were performed by employing first-principles projector augmented wave (PAW) method based on density functional theory (DFT) with Perdew–Burke–Ernzehof (PBE) functional as implemented in Vienna *ab initio* simulation package.[18] The kinetic cutoff was set to 520 eV, and Monkhors–Pack scheme *k*-point mesh was set to (9×9×1) and (5×4×1) for (001) and (110) surfaces of CdSe. A periodic slab model with a void region of 15 Å was used. During the structure optimization, both the atoms' position and the parameters of lattice were allowed to relax. The surface energy was defined as following, $E_{SE} = (E_{slab} - NE_{bulk}) / 2A$, where E_{slab} is the energy of the slab, E_{bulk} is the energy of per unit cell (Cd_2Se_2) of bulk CdSe, N is the number of Cd_2Se_2 unit in the slab, and A is the area of the slab. The slab models of (001) and (110) surface contain 19 and 9 atomic layers with chemical formula of $Cd_{19}Se_{19}$ and $Cd_{18}Se_{18}$, respectively. The calculated surface energies of (001) and (110) surface are 0.988 and 0.420 J/m^2, respectively.

Cell culture

The rat lung macrophage line NR8383 and the mouse kidney line NIH3T3 were obtained from the Cell Resource Center, Chinese Academy of Medical Science, Beijing, China. The macrophages were cultured in F-12K medium supplemented with 10% fetal bovine serum (FBS). The NIH3T3 cells were cultured in Dulbecco's Modified Eagle's medium (DMEM) medium supplemented with 10% FBS. The cells were incubated in 24-well flat-bottom polystyrene plates within a humidified incubator at 37 °C in 5% CO_2 for 24 h for further experiments.

Cellular uptake of CdS and CdSe nanorods

To determine the cellular uptake contents of CdS and CdSe nanorods, the cells were treated by 20 mg/L CdS-H, CdS-L, CdSe-H or CdSe-L for 48 h. The cells were then washed 3 times with phosphate buffer saline (PBS) to remove the nanorods outside of the cells, counted with hemocytometers and overnight digested with 30% HNO_3. Cd contents in the digestion liquid were determined with inductively coupled plasma–mass spectrometry (ICP–MS, PerkinElmer, ELAN DRC-e).

Electron microscopy

To examine the distribution of CdS and CdSe nanorods in intracellular ultrastructures, treated cells were fixed with 2% glutaraldehyde solution at 4 °C for 48 h, followed by post-fixing with 1% osmium tetroxide solution for 1 h. The samples were dehydrated with graded ethanol, embedded in LR White Resins (Sigma, USA) and sectioned. The ultrathin sections were stained with uranyl acetate and lead citrate, and observed with a transmission electron microscope (Tecnai G2 F-20, FEI, USA).

Adsorption experiments

Adsorption of the FITC-tagged mitochondrion-targeting peptide (FITC-Cha-D-Arg-Cha-Lys-Cha-D-Arg-Cha-Lys), the ER-targeting peptide (FITC-Met-Lys-Trp-Val-Thr-Phe-Ile-Ser-Leu-Leu-Phe-Leu-Phe-Ser-Ser-Ala-Tyr-Ser) and the actin-targeting peptide (FITC-Met-Gly-Val-Ala-Asp-Leu-Ile-Lys-Lys-Phe-Glu-Ser-Ile-Ser-Lys-Glu-Glu) to CdS and CdSe nanorods was carried out using a batch adsorption approach.[10,11] Briefly, 100 mg/L of CdS or CdSe nanorods were incubated with 50 mg/L of the peptides at 37 °C for 48 h. The mixtures were then centrifuged at 12,000 rpm for 10

min to thoroughly pellet the nanorods. To determine the concentration of the target peptide in the supernatant, the fluorescence density of the supernatant was analyzed using a microplate reader (EnSpire, PerkinElmer, USA). The adsorbed mass of the targeting peptides on the nanorods was calculated based on mass balance.

Protein extraction and Western blotting

To detect intracellular contents of Hspa5, HDAC1 and TryRs, the total proteins in the treated-cells were extracted with RIPA buffer containing the protease inhibitor cocktail. After separated with SDS-PAGE, these proteins were detected using corresponding mono-antibodies (Abcam, USA). To detect cytoplasmic cytochrome C (Cyt C) released from the mitochondria, the cells were broken with a Dounce homogenizer. After pre-centrifuged at 1,000 rpm for 5 min to remove the nuclei, the mitochondria were pelleted by centrifugation at 12,000 rpm for 10 min. Cyt C in the supernatant (Cyt $C_{(cyto)}$) and in the mitochondria (Cyt $C_{(mit)}$) were separated with SDS-PAGE and detected using the anti-Cyt C mono-antibodies (Abcam, USA).

Gene expression assays

To investigate transcription profiling under CdS or CdSe treatment, the macrophages were treated with 20 mg/L CdS-H, CdS-L, CdSe-H or CdSe-L for 48 h, and then harvested for RNA extraction. Total RNAs were extracted from the cells using the hot phenol method. The quality and quantity of the total RNA were analyzed using a NanoDrop 2000 spectrophotometer (Thermo Scientific, USA) and gel electrophoresis. RNAs were used to generate double-stranded cDNA using the SMARTTM cDNA Library Construction Kit (Clontech, USA). The obtained cDNAs were then used to construct a 454 library. Roche GS-FLX 454 pyrosequencing was conducted with Illumina HiSeq^{TM}2000 (NovoCompany, China). Gene annotations were retrieved from the rat genome browser (http://rgd.mcw.edu/nomen/nomen.shtml). Assignment of Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) terms was based on JGI annotations. Enrichment of differentially regulated genes in GO and KEGG were determined using GOSeq analysis.

To verify the transcription profiling data, total mRNAs of the macrophages treated by CdS or CdSe nanorods or receiving no treatment were extracted using the Trizol agents, and the corresponding cDNAs were prepared with Oligo (dT)-primed RT reagent Kit (Promega, USA). Analysis by qRT-PCR for expression of UPR genes was performed using the RealMaster Mix (SYBR Green) Kit (TransGen, China) with the qRT-PCR detection system (Realplex2, Eppendorf, USA). Transcription levels of the tested genes were normalized against the levels of actin. Each sample was analyzed in triplicate. The results were expressed as fold change compared with the untreated wild-type strain.

Assays of cell viability and apoptosis

The cells were treated with 20 mg/L of nanorods for 48 h. Cell viability (revealed by succinate dehydrogenase activity) of the treated cells was assessed using a CCK-8 cell viability Kit (Beyotime, China). Apoptosis and necrosis were assessed by an FITC-Annexin V/PI Kit (Sungene Biotech, China) and flow cytometry. The fluorescence density of the stained cells was analyzed using a flow cytometer (CaLibar, Beckton Dickson, USA).

Statistical Analysis

All the experiments were carried out in triplicates. Differences between groups were examined using Student's *t* test ($p < 0.05$). Statistical analyses were conducted using Statistical Packages for the Social Sciences (SPSS) Version 20.0.

Table S1. Selected characteristics of CdS and CdSe nanorods [a].

	CdS-H	CdS-L	CdSe-H	CdSe-L
Length (nm) [b]	110 ± 26	108 ± 11	115 ± 30	121 ± 28
Width (nm) [b]	25 ± 3	22 ± 4	38 ± 5	40 ± 4
ζ potential (mV) [c]	-11.95 ± 1.91	-9.82 ± 1.56	-14.09 ± 4.42	-10.16 ± 2.40
Surface energy (J/m^2)	0.627	0.451	0.988	0.420

[a] The suffixes "H" and "L" represent high-energy-faceted and low-energy-faceted nanorods, respectively.

[b] Values reported are average of 300 individual nanorods.

[c] ζ potential measured with a zeta potential analyzer (Brookhaven, ZETAPALS/BI-200SM) in F-12K medium containing 10% FBS. Values reported are average of triplicates.

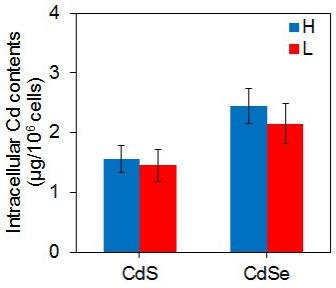

Fig. S1 Different faceted CdS and CdSe nanorods were intracellularly assimilated to similar extents in NR8383 cells. The suffixes "H" and "L" represent high-energy-faceted and low-energy-faceted nanorods, respectively. The cells were treated with 20 mg/L CdS-H, CdS-L, CdSe-H or CdSe-L for 48 h, harvested and washed three times to remove the nanorods outside of the cells. The cells were then digested with HNO_3 and the Cd contents were determined using inductively coupled plasma–mass spectrometry (ICP–MS). Error bars represent standard deviations of triplicate treatments.

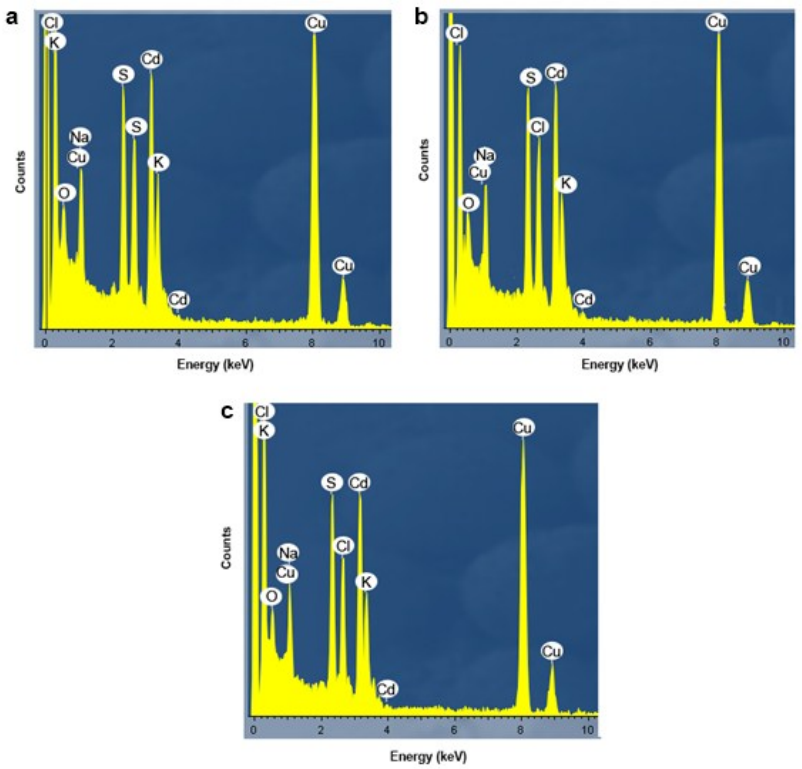

Fig. S2 Energy dispersive X-ray (EDX) spectra of CdS-L accumulating in the mitochondria (a), and CdS-H accumulating in the ER (b) and the nucleus (c) as shown in Figure 3a.

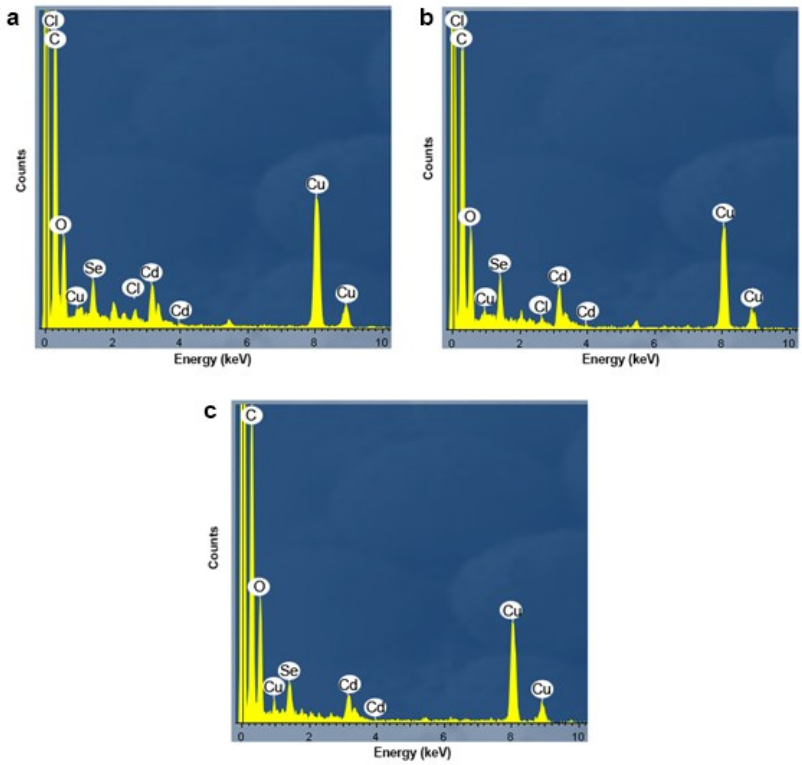

Fig. S3 EDX spectra of CdSe-L accumulating in the mitochondria (a), and CdSe-H accumulating in the ER (b) and the nucleus (c) as shown in Figure 3c.

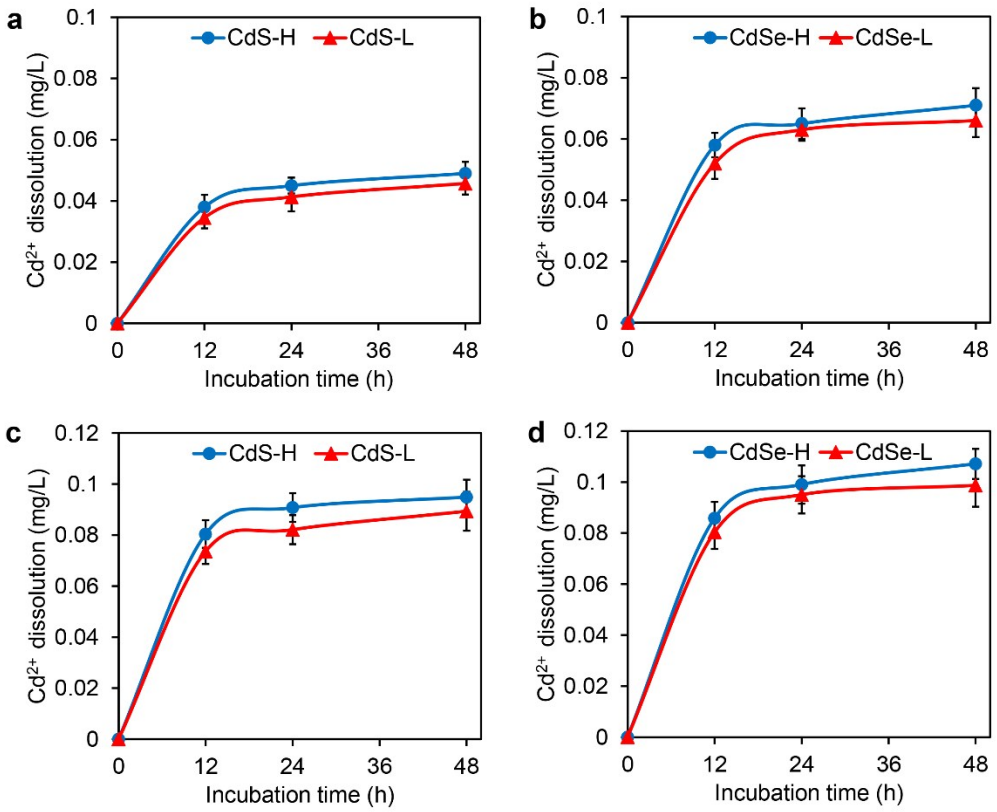

Fig. S4 Cd^{2+} dissolution from CdS/CdSe nanorods (20 mg/L) in the acid solution (pH = 5.5, a, b) and in the cells (c, d) at different incubation time.

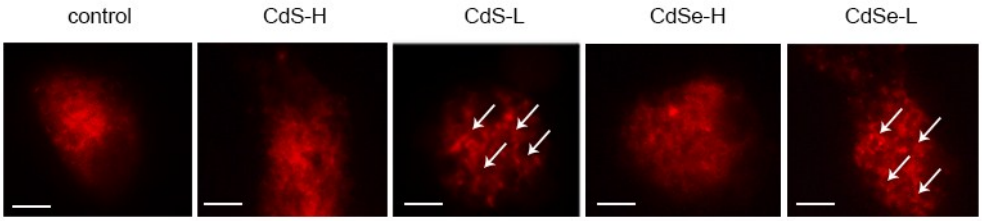

Fig. S5 Low-energy (L) nanorods cause more severe fragmentation of the mitochondria than high-energy (H) nanorods, for both CdS and CdSe (20 mg/L, 48-h exposure) in NR8383 cells. The nanorod-treated cells were stained by MitoTracker Red, and then examined by confocal microscopy. Scale bar = 5 μm. The white arrows indicate fragmented mitochondria.

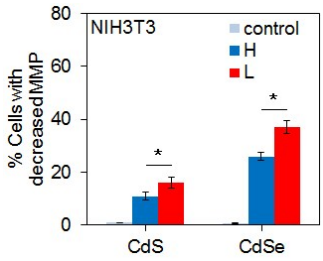

Fig. S6 Low-energy-faceted CdS and CdSe nanorods have higher impact on mitochondrial membrane potential (MMP) in NIH3T3 cells. The cells were treated by 20 mg/L CdS or CdSe nanorods for 48 h, harvested, stained by JC-1 and used for flow cytometry. * indicates significant difference between the CdS-H groups and the CdS-L groups (p < 0.05). Error bars represent standard deviations of triplicate treatments.

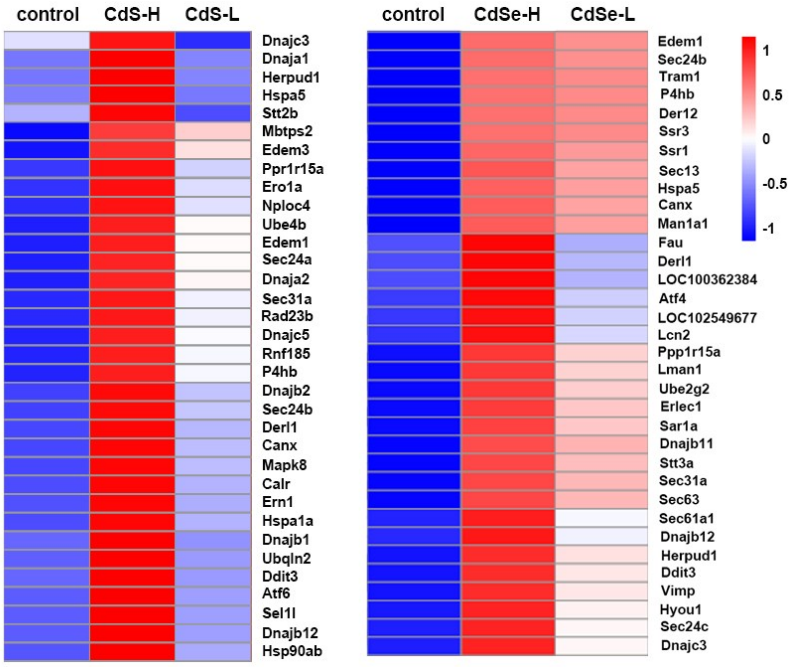

Fig. S7 High-energy-faceted CdS and CdSe nanorods induce higher expression levels of unfolded protein response (UPR) genes, revealed by transcription profiling analysis of NR8383 cells.

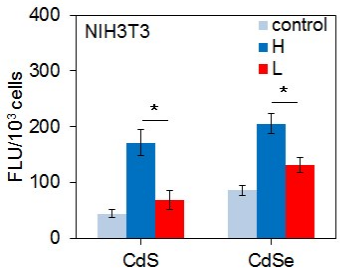

Fig. S8 High-energy-faceted nanorods induce higher cytoplasmic calcium levels than low-energy-faceted nanorods in NIH3T3 cells (20 mg/L of nanorods, 48-h exposure). * indicates significant difference in cytoplasmic calcium levels between the CdS(e)-H groups and the CdS(e)-L groups (p < 0.05) (n = 3).

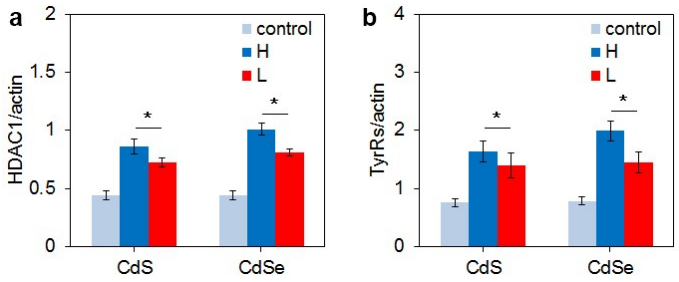

Fig. S9 High-energy (H) nanorods induce higher expression of nucleus damage-markers than low-energy (L) nanorods for both CdS and CdSe (20 mg/L, 48-h exposure). This is indicated by Western blotting assays for HDAC1 (a) and TyrRs (b) in the treated macrophages. * indicates significant difference between the low- and high-energy nanorods ($p < 0.05$) (n = 3).

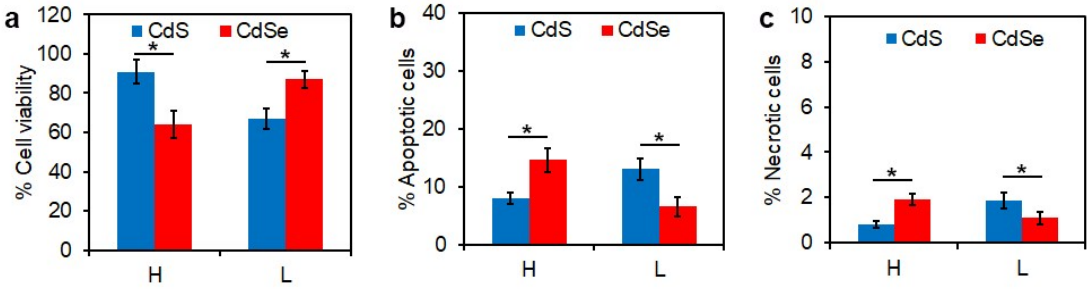

Fig. S10 Cd-based nanorods do not significantly compromise the overall cell viability (assessed as succinate dehydrogenase activity) in NR8383 cells as indicated by the high percentage of cell viability (a), low percentage of apoptotic cells (b) and low percentage of necrotic cells (c) after treated with Cd-based nanorods (20 mg/L, 48-h exposure). Asterisks (*) indicate significant difference between high energy (H) and low energy (L) nanorods ($p < 0.05$) (n = 3).

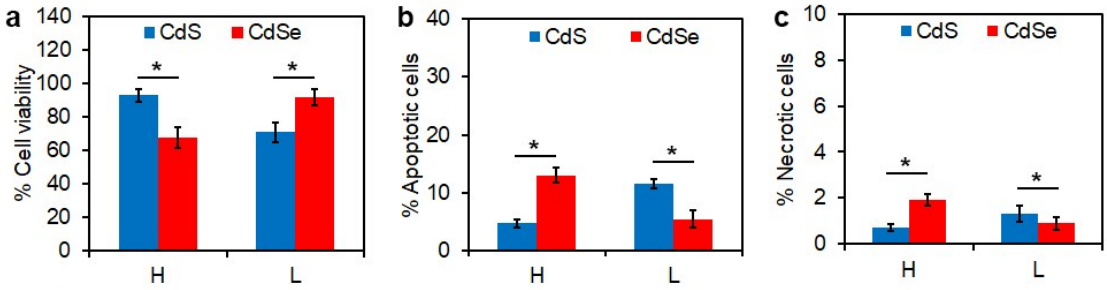

Fig. S11 Cd-based nanorods do not significantly compromise the overall cell viability (assessed as succinate dehydrogenase activity) in NIH3T3 cells as indicated by the high percentage of cell viability (a), low percentage of apoptotic cells (b) and low percentage of necrotic cells (c) after treated with Cd-based nanorods (20 mg/L, 48-h exposure). * indicates significant difference between high energy (H) and low energy (L) nanorods ($p < 0.05$) (n = 3).

References

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