Supporting information for:

Photoluminescent Distinction of Lung Adenocarcinoma Cells A549 and Squamous Cells H520 Using Metallothionein Expression in Response to Cd-Doped $Mn_3[Co(CN)_6]_2$ Nanocubes

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Experimental Section

Materials. Potassium cobalticyanide ($K_3[Co(CN)_6]$), Manganese acetate ($Mn(CH_3COO)_2.4H_2O$), cadmium chlori ($CdCl_2$), depoly(vinylpyrrolidone) (PVP). All the chemicals were of analytic grade and used without further purification.

Synthesis of Cd-doped $Mn_3[Co(CN)_6]_2$ nanocubes. The $Mn_3[Co(CN)_6]_2$ nanocubes were prepared according to a literature procedure[1] with slight modifications. First, 0.15 mmol of $Mn(CH_3COO)_2{\cdot}4H_2O$ and 0.6 g PVP (K-30) were dissolved in a mixed solution (15 ml C_2H_5OH and 5 ml H_2O) to get a transparent solution. Then, 0.08 mmol $K_3[Co(CN)_6]$ dissolved in 6 mL distilled water was added dropwise into the above solution with stirring. After 10 min, 0.02 mmol $CdCl_2$ dissolved in 10 mL distilled water was added into the resulting white precipitation solution for Cd^{2+} doping. After continuing stirring for another 3 h, the reaction was aged at room temperature for 24 h without any interruption. At last, the products were filtered and washed with ethanol several times and then dried in an oven at 60 °C.

Cell Culture and Viability Tests. The in vitro cytotoxicity of $Mn_3[Co(CN)_6]_2$ nanocubes and Cd-doped $Mn_3[Co(CN)_6]_2$ nanocubes were assessed on human lung adenocarcinoma cells A549 and squamous cells H520 using the MTT method. Cells were cultured in a 96-well plate and maintained as subconfluent monolayers in Dulbecco's modified Eagle's medium (Invitrogen) with 10% fetal bovine serum (Hyclone, Logan, UT) and 100 units/ml penicillin plus 100 g/ml streptomycin (Invitrogen) at 37 °C with 8% CO_2. After cultured for 24 h, the $Mn_3[Co(CN)_6]_2$ nanocubes and Cd-doped $Mn_3[Co(CN)_6]_2$ nanocubes with different concentrations (25, 50, 100, 200 μg/mL) were added into the cultured medium separately. After cultured for another 24 h, MTT solution was put into each well. 4 h later, the medium was removed and DMSO was added to dissolve the formazan crystals.[2] Then an ELISA reader was used to measure the absorbance of each well.

Fluorescence imaging of cells cultured with Cd-doped $Mn_3[Co(CN)_6]_2$ nanocubes by confocal microscopy. Both A549 and H520 cells were employed for cell imaging experiment. Cells seeded onto Lab-Tek Chambered 1.0 Borosilicate Coverglass system (Nunc) and incubated with Cd-doped $Mn_3[Co(CN)_6]_2$ nanocubes at 37 °C

under the atmosphere of 5% CO_2 for 6h, 24 h and 48 h separately. Then, cells were rinsed three times in PBS to get rid of the nanocubes that did not enter the cells. After that, cells were maintained in CO_2-independent medium (Gibco) containing 10% (vol/vol) fetal bovine serum (Hyclone, Logan, UT). Images were taken at 37 °C with a laser scanning microscope (Zeiss L SM 710) using a 40×1.3 numerical aperture PlanApo objective. Figures were constructed using Adobe Photoshop.[3]

TEM study of subcellular localization of Cd-doped $Mn_3[Co(CN)_6]_2$ nanocubes in A549 cells and H520 cells. A549 cells and H520 cells were seeded onto sterile, acid-treated 6-well plates (Corning Glass Works) and maintained as subconfluent monolayers in DMEM separately. After that, Cd-doped $Mn_3[Co(CN)_6]_2$ nanocubes were added into the system and cultured with cells for 24 h. Then, cells were collected and fixed in PBS (pH=7.4) plus 2.5% glutaraldehyde (Sigma). After dehydration through an ethanol series twice (70% for 15 min, 90% for 15 min, and 100% for 15 min), cells were embedded in Epon-Araldite resin (polymerization at 65 °C for 15 h). Ultrathin sections containing the cells were then cut, placed on copper grids, as well as stained with 4% uranyl acetate and 0.2% Reynolds lead citrate (water). Finally, the samples were examined by a transmission electron microscope (TEM, Hitachi model H-800).

Detection of metallothioneins in A549 and H520 cells. The MTs generated in A549 or H520 cells in response to the stimulation of Cd-doped $Mn_3[Co(CN)_6]_2$ nanocubes was quantitatively analyzed by the Human MT1 (Metallothionein 1) ELISA Kit (Elabscience Biotechnology Co.,Ltd). A549 cells or H520 cells were seeded and cultured with Cd-doped $Mn_3[Co(CN)_6]_2$ nanocubes (50 μg/mL) for 24 h in the same manner as mentioned in 2.3. Cell culture and viability test. After that, the cell culture

supernate was collected and centrifuged to remove insoluble impurity and cell debris. Then the clear supernate, standard solution and reference standard solution & sample diluent were added into the bottom of micro ELISA plate wells, respectively. And then the plate was covered with sealer and incubated at 37 °C. 90 min later, the liquid of each well was removed and 100 μL of Biotinylated Detection Ab working solution was added instead. Mix the content thoroughly and incubate at 37 °C for another 1 h. Then, remove the liquid and wash the products in each well three times. 100 μL of HRP conjugate working solution was sequentially added to each well and kept the mixture being cultured for another 30 min at 37 °C. Subsequently, the wash process was repeated for five times as mentioned above. Finally, 90 μL of Substrate Solution was added to each well and the reaction was kept in the dark at 37 °C. Until apparent gradient appeared in standard wells, the reaction can be terminated by adding 50μL Stop Solution to each well. And a microplte reader was used to determine the optical density of each well immediately.

Characterization. The powder X-ray diffraction (XRD) patterns were collected on an X-ray diffractometer (Japan Rigaku D/MAX-cA X-ray) equipped with Cu-Kα radiation over the 2θ range of 10-70°. The morphology of the as-synthesized samples was observed a scanning electron microscope (SEM, JEOL JSM-6700M). X-ray photoelectron spectroscopy (XPS) was performed on an X-ray Photoelectron Spectrometer (ESCALAB 250) with Al Kα radiation to analyze the surface composition of the products. The distribution of elements in the products were characterized using a scanning transmission electron microscopy (STEM, JEM 2100F). The concentration of Mn, Co ,and Cd ions was measured using inductively coupled plasma-atomic emission spectroscopy (ICP, Atomscan Advantage).

Supporting Information Figures:

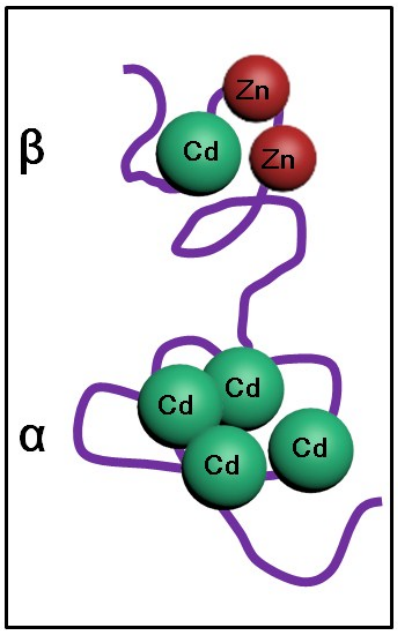


Figure S1. Structure of metallothioneins (MTs). The structure of MTs consist of two domains of polypeptide chain (α and β) which are linked by a lysine dimmer. Domain α can bind four cadmium (Cd) ions and domain β can bind one cadmium (Cd) ion and two zinc (Zn) ions.[4]

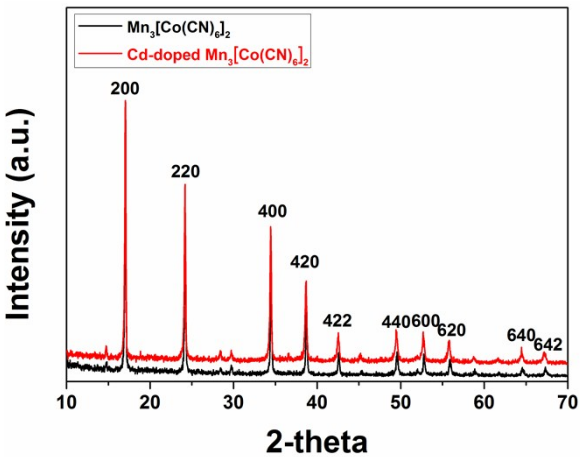


Figure S2. XRD patterns of $Mn_3[Co(CN)_6]_2$ and Cd-doped $Mn_3[Co(CN)_6]_2$ nanocubes.

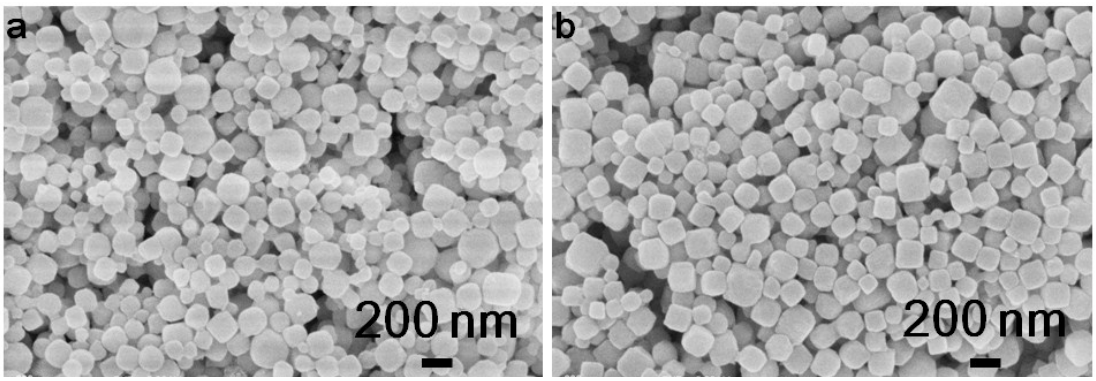


Figure S3. SEM images of (a) $Mn_3[Co(CN)_6]_2$ and (b) Cd-doped $Mn_3[Co(CN)_6]_2$ nanocubes.

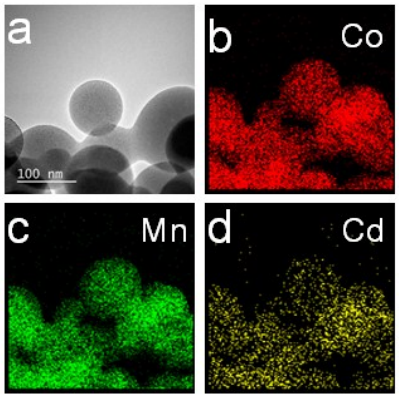


Figure S4. (a) Typical scanning transmission electron microscopy (STEM) image of Cd-doped $Mn_3[Co(CN)_6]_2$ nanocubes and the corresponding elemental mapping images of (b) cobalt, (c) manganese, and (d) cadmium.

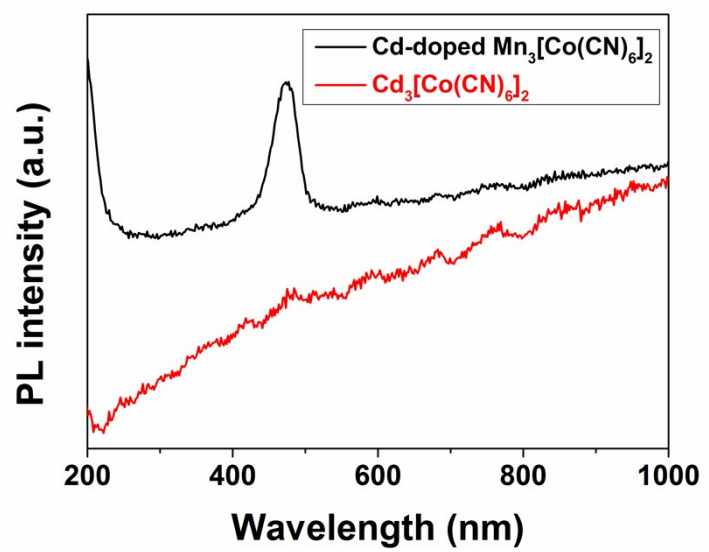
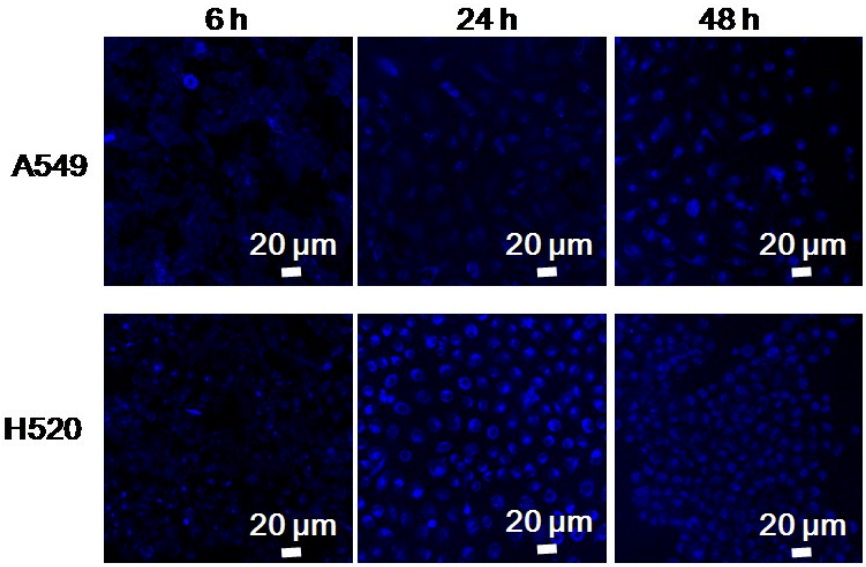


Figure S5. Emission spectra of Cd-doped $Mn_3[Co(CN)_6]_2$ nanocubes and $Cd_3[Co(CN)_6]_2$ nanocubes.



	Mean Intensity		
Culture Time	6 h	24 h	48 h
A549 Cell	32.48886	38.40098	39.11975
H520 Cell	24.96731	61.09226	58.50099

Figure S6. Confocal microscopy of fluorescence excited with 403 nm laser beams images for A549 cells or H520 cells after incubation with 50 μg/mL Cd-doped $Mn_3[Co(CN)_6]_2$ nanocubes for 6 h, 24 h and 48 h.

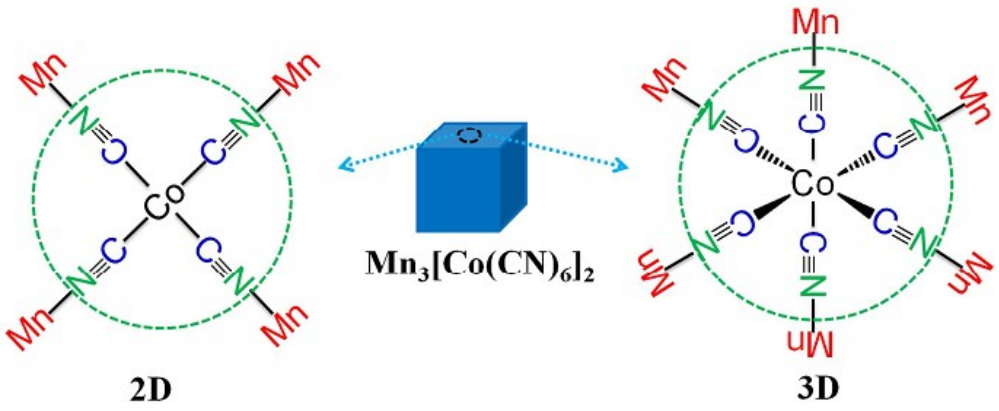
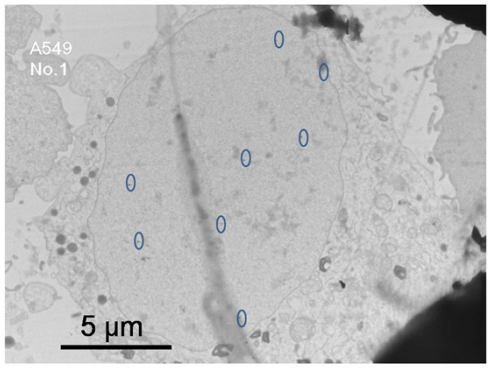
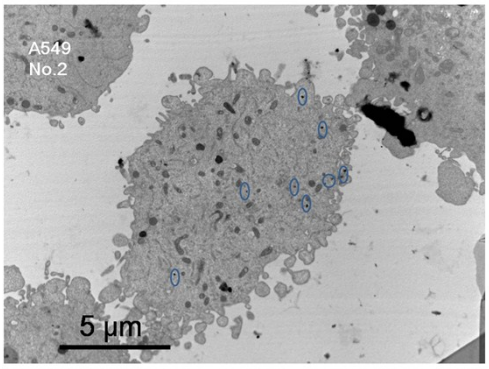


Figure S7. 2D and 3D micro-structure of carbon coordinated to cobalt within inner $Mn_3[Co(CN)_6]_2$ core.[5]


A549
No.1
5 μm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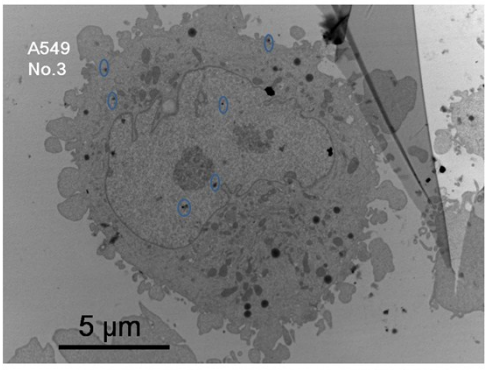
No.2
5 μm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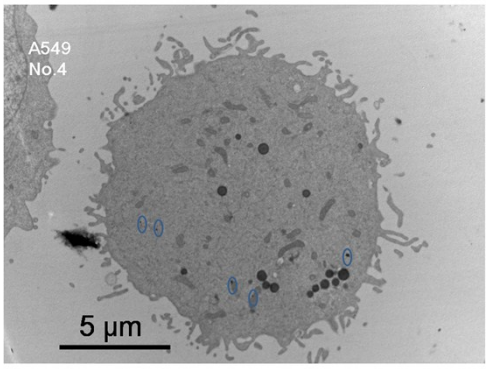
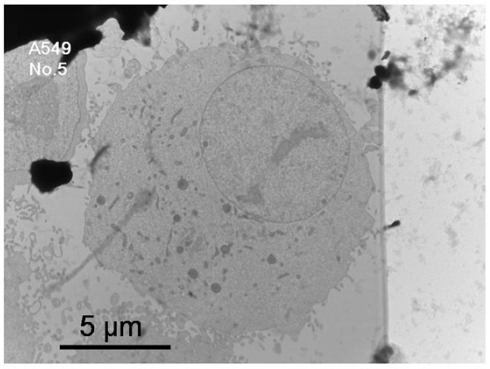

A549
No.4
5 μm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No.5
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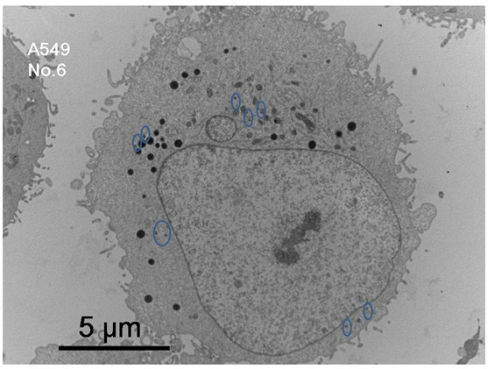
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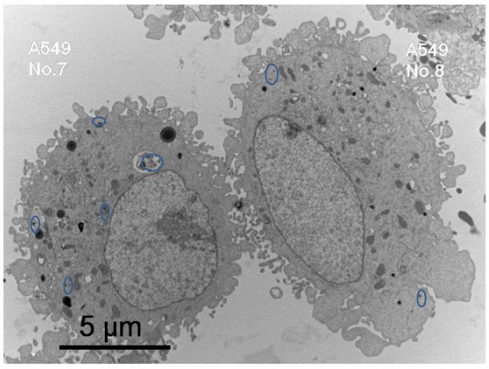
A549
No.7
A549
No.8
5 μm

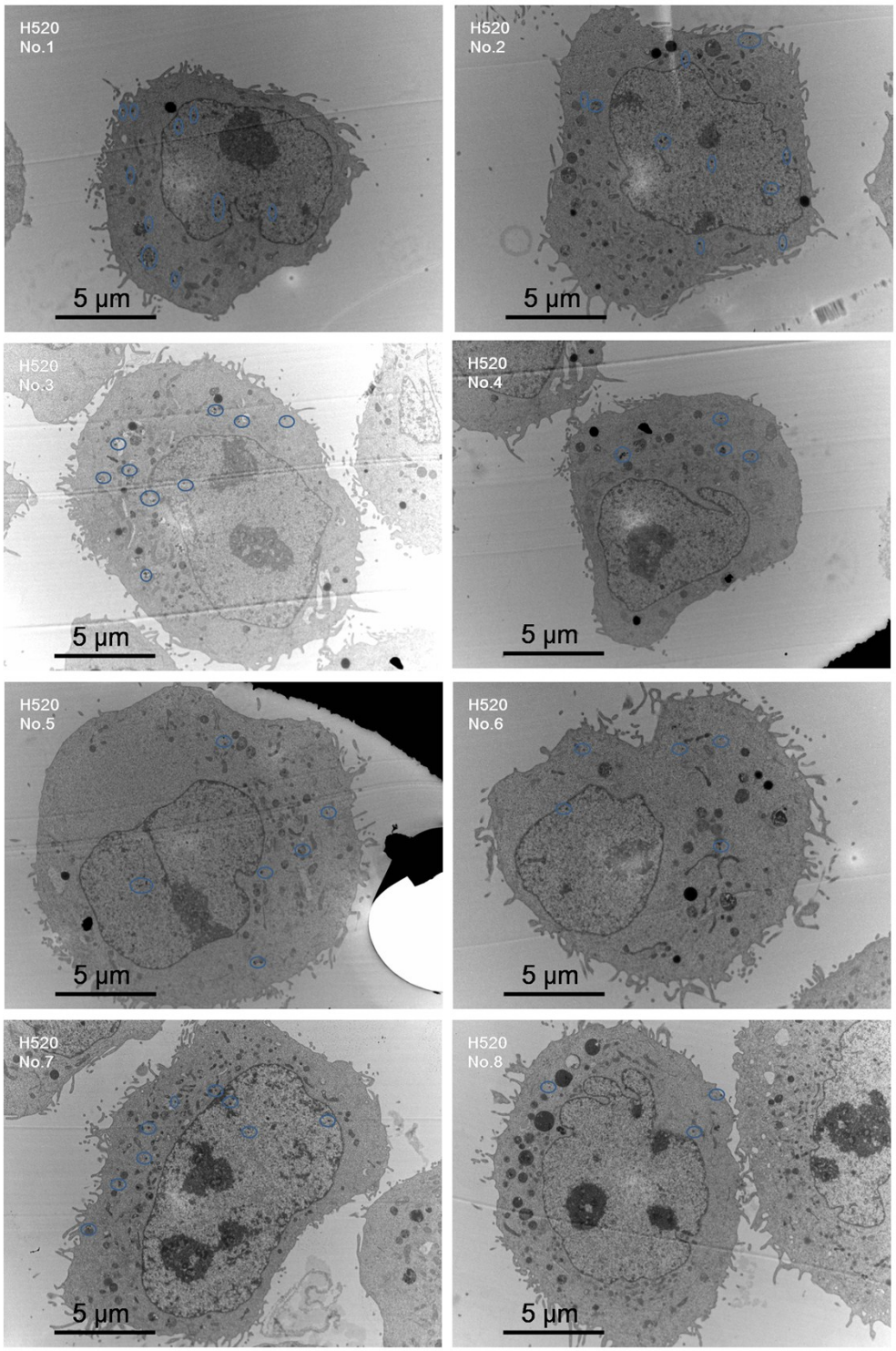

Figure S8. Bio-TEM images of A549 cells and H520 cells taken up Cd-doped $Mn_3[Co(CN)_6]_2$ nanocubes. The black dots circled with blue solid line indicate the existence of Cd-doped $Mn_3[Co(CN)_6]_2$ nanocubes in different cancer cells.

Supporting Information Table

	Average Quantity of Cd-doped $Mn_3[Co(CN)_6]_2$ Nanocubes in Every Cell							
	No.1	No.2	No.3	No.4	No.5	No.6	No.7	No.8
A549	8	10	7	5	0	9	10	3
H520	14	14	10	11	14	6	10	3

Table S1. The average quantity of Cd-doped $Mn_3[Co(CN)_6]_2$ nanocubes in A549 cells and H520 cells (per cell) according to the Bio-TEM images in Figure S5.

REFERENCES

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