Mercaptopropionic Acid-Capped Mn^{2+}: ZnSe/ZnO Quantum Dots with both Downconversion and Upconversion Emissions for Bioimaging Applications

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Supporting Information

Experimental Section

Chemicals: Selenium powder (99.9%), $NaBH_4$ (96%), $ZnCl_2$ (99.9%), $MnCl_2$•$4H_2O$, $Zn(NO_3)_2$•$6H_2O$, NaOH, Ethanol were purchased from Sinopharm Chemical Reagent Co., Ltd., China. Mercaptopropionic acid (MPA) (99+%) was purchased from Sigma Aldrich. Dulbecco's modified eagle's medium (DMEM), fetal bovine serum (FBS), penicillin, and streptomycin were purchased from Gibco and Sigma, respectively. 4',6-Diamidino-2-phenylin doledihydrochloride (DAPI), Cell Counting Kit-8 (CCK-8) were purchased from Sigma Aldrich.

Preparation of Precursor Solution: Sodium hydrogen selenide (NaHSe) was prepared as the literature described[21]. Typically, 0.88mmol $NaBH_4$ was loaded to a small flask, then 3mL deionized water was added, after which 0.44mmol selenium powder (Se:$NaBH_4$=1:2) was added to the flask. The flask was sealed quickly with a small pinhole exposed to atmosphere and was placed in the oven at 30℃. After the black selenium powder disappeared completely, the resulting clear aqueous solution was NaHSe solution. The MPA stabilizing reagent solution was prepared by dissolving $ZnCl_2$, $MnCl_2$•$4H_2O$ and MPA in deionized water. The pH value of the mixed solution was adjusted by dropwise addition of 1 M NaOH solution under vigorous stirring. The solution was then was treated by N_2-saturating for 30 min. All precursor solutions were prepared freshly.

Synthesis of Mn-Doped ZnSe D-Dots: The freshly prepared NaHSe solution was added into the N_2-degassed MPA stabilizing reagent solution under vigorous stirring. Finally, the precursor solution was transferred into a Teflon-lined stainless autoclave. The autoclave was maintained at 200℃ under vigorous stirring for a certain time, and then cooled to room temperature by a hydrocooling process. In a typical experiment, 97mL MPA stabilizing reagent solution were loaded into the flask and degassed by N_2 for 30min. The amounts of Zn, Mn, MPA were 0.88mmol, 0.0176mmol and 3.168mmol (Zn:Mn:MPA=1:0.02:3.6). The pH was adjusted to 10.5. Then fresh prepared NaHSe ($Zn:HSe^-$=1:0.5) was injected into the flask. Finally, 25mL of the precursor solution was transferred into a 50mL Teflon-lined stainless autoclave. The autoclave was maintained at 200℃ under vigorous stirring for 25min, then cooled to room temperature by a hydrocooling process.

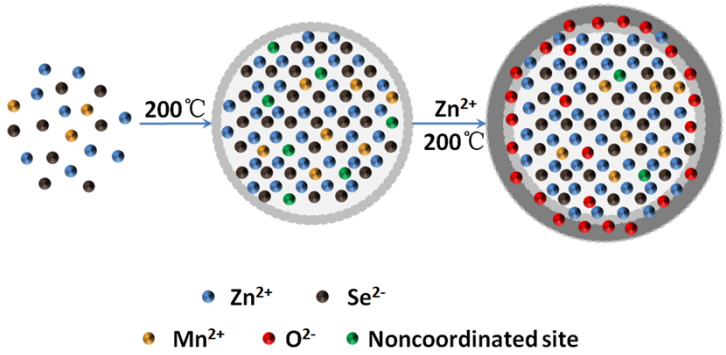
Deposition of ZnO Shell around Mn^{2+}:ZnSe D-Dots: The as-prepared Mn^{2+}:ZnSe d-dots was purified and washed by ethanol and re-dispersed in deionized water. MPA was added with a ratio of d-dots:MPA=1:3.6. The pH was adjusted to 10.5 by dropwise addition

of 1M NaOH. Then $Zn(NO_3)_2$ were added to the solutions with a d-dots:$Zn(NO_3)_2$ ratio from 1:0.3 to 1:1.2. The solution was transferred into a Teflon-linedstainless autoclave which maintained at 200℃ under vigorous stirring for a certain time, then cooled to room temperature by a hydrocooling process.

Characterization: UV–Vis absorbance and fluorescence data were acquired using UV–2550 Shimadzu UV–Vis spectrophotometer and RF-5301PC Shimadzu spectrofluorophotometer respectively at room temperature. All the aliquots were measured without any size sorting. The PLQY of the as-prepared nanocrystals was obtained by comparing their optical properties with those of Rhodamine B. Power X-ray diffraction (XRD) patterns were taken on a Bruker diffractometer using Cu Kα radiation. X-Ray photoelectron spectroscopy (XPS) was carried out on a AXIS ULTRA DLD with Al Kα excitation. Binding energy calibration was based on C1s at 284.6 eV. Electron paramagnetic resonance (EPR) spectra were recorded at room temperature on a Bruker BioSpin Corp., EMX-8 (Germany). Typical measurementconditions were microwave power 5mW, microwave freauency 9.85GHz, modulation frequency 100 kHz. ICP-MS was measured on iCAP 6000Radial. Transmission electron micrographs (TEM) were obtained on a JEOL-JEM 2100 electron microscope operating at an accelerating voltage of 200 kV. High-resolution TEM (HRTEM) images were obtained on a JEM-2100F. Bio-TEM were obtained on FEI Tecnai G2 Spirit Biotwin at an accelerating voltage of 120kV. Fluorescent decay curves were obtained by an FLS-920 Combined Steady State and Phosphorescence Lifetime Fluorimeter (Edinburgh Instruments).

Cell Viability Assay: CCK-8 (Cell Counting Kit-8) assay was performed to assess the metabolic activity of cells in the presence of d-dots. The assay was carried out in triplicate in the following manner. SKOV3, HeLa, and L02 cells were seeded into 96-well plates at the density of 10,000 cells per well in 100 μL of media, and the following experiments was conducted until 80% confluence. After removing the culture medium, the fresh medium (100μL) containing various concentrations of Mn^{2+}:ZnSe/ZnO d-dots were supplemented for 24h at 37°C under the humidified atmosphere (5% CO_2). Then, the CCK-8 was used to measure the cell viability according to the manufacturer's protocol. The absorbance at 450 nm was determined using POLARstar OPTIMA Multidetection Microplate Reader (BMG LABTECH).

SKOV3, HeLa, and L02 Cells Labeling and Fluorescent Imaging: SKOV3, HeLa, and L02 cells were propagated in Roswell Park Memorial Institute's medium (RMPI1640、DMEM) supplemented with FBS (10%) and penicillin/streptomycin (2%). Then the cultured cells were trypsinized and resuspended in the RMPI1640 medium at a concentration of about 2×10^4 /well. The cell suspension (200 μL) was transferred to each Petri dish (35 mm). After 24 h of incubation, the cells were carefully rinsed with PBS (0.01 mol/L, pH7.4). For each Petri dish, RMPI1640 medium (without fetal blood serum and FAs, 900 μL) and the Mn^{2+}:ZnSe/ZnO (100 μL, 1 mg/mL) were added and incubated for 24 h. The labeled cells were carefully rinsed with PBS to remove the unbonded d-dots. After wards the labeled cells in the dish were fixed with 4% paraformaldehyde for fifteen minutes and then the cells were washed three times with PBS. DAPI was used to stain the cell nucleus. The fluorescent imaging of the SKOV3, HeLa, and L02 cells was performed with a Leica TSC SP8 two photon confocal microscope and a Leica TSC SP5 confocal microscope.



Scheme S1. Schematic representation of the synthesis of MPA-Mn^{2+}:ZnSe/ZnO d-dots

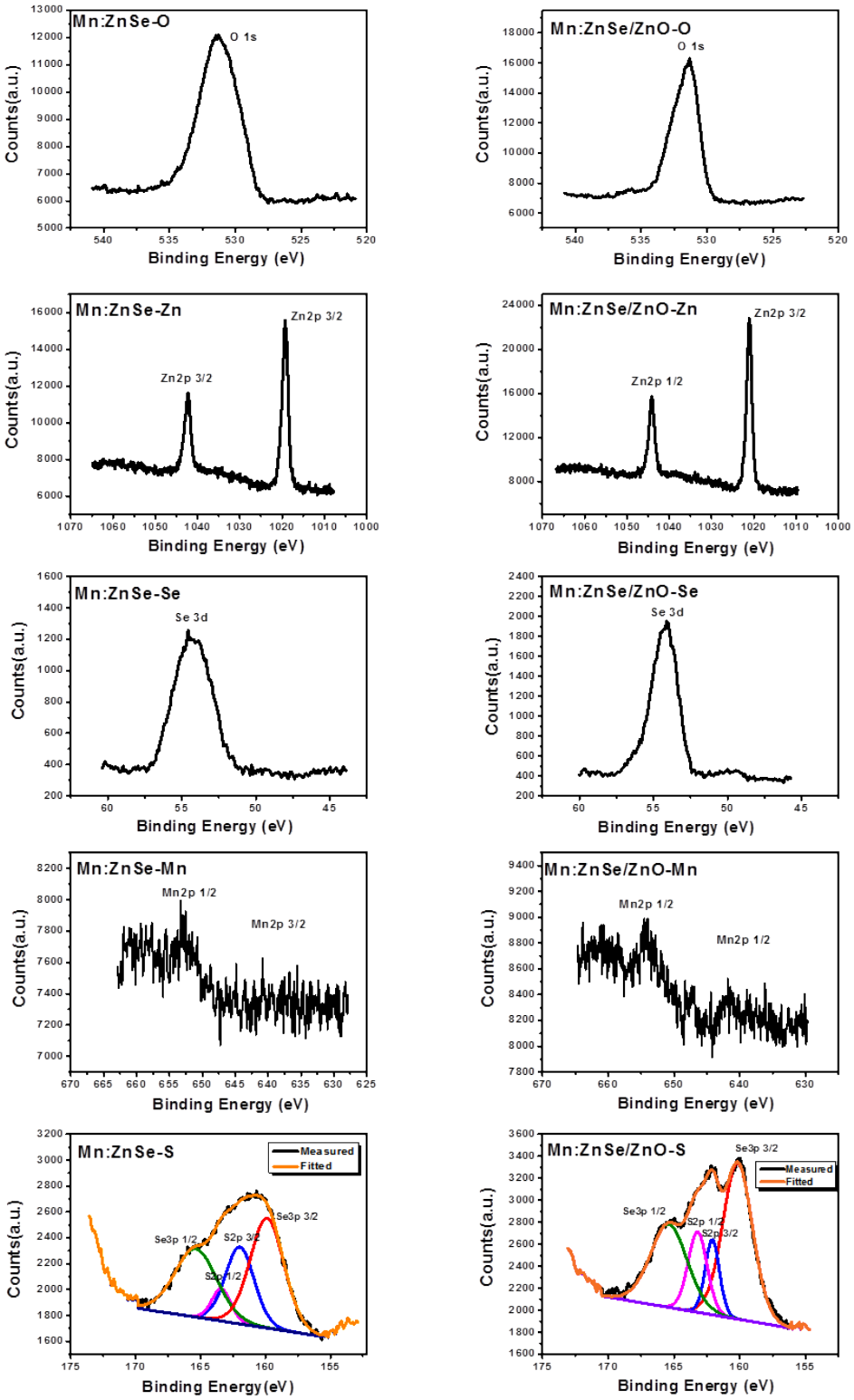


Figure S1. XPS spectra of MPA-Mn^{2+}:ZnSe and core/shell MPA-Mn^{2+}:ZnSe/ZnO d-dots.

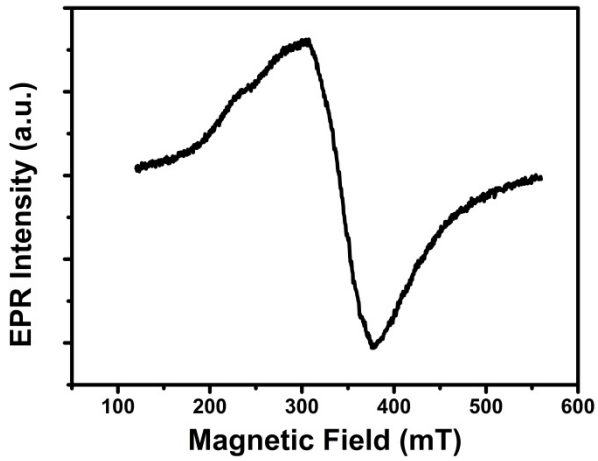


Figure S2. EPR spectrum of MPA-Mn^{2+}:ZnSe/ZnO d-dots.

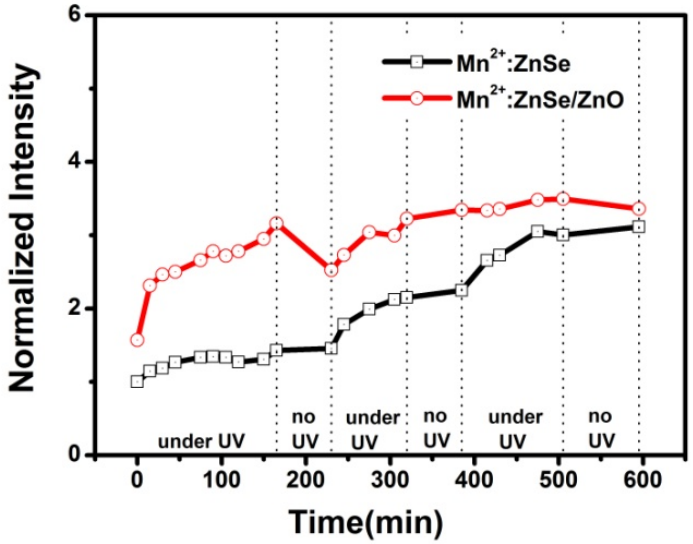


Figure S3. PL intensity evolution of MPA-Mn^{2+}:ZnSe and core/shell MPA-Mn^{2+}:ZnSe/ZnO d-dots under continuous 8W 365nm UV lamp irradiation.

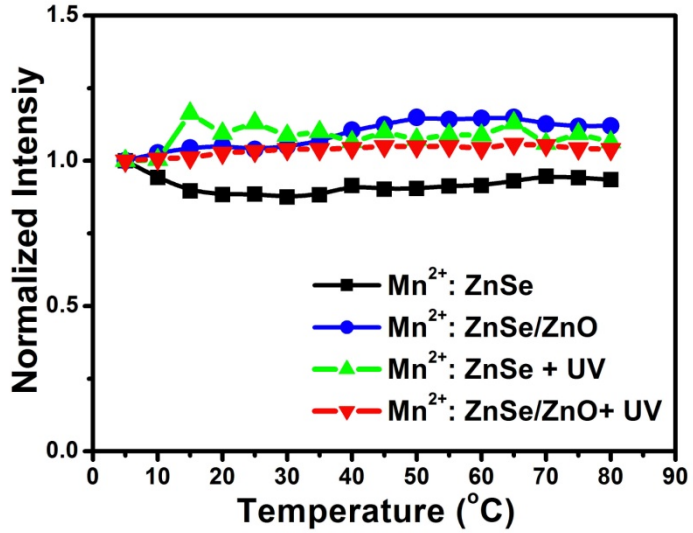


Figure S4. PL intensity variation of MPA-Mn^{2+}:ZnSe and core/shell MPA-Mn^{2+}:ZnSe/ZnO d-dots at different temperatures.

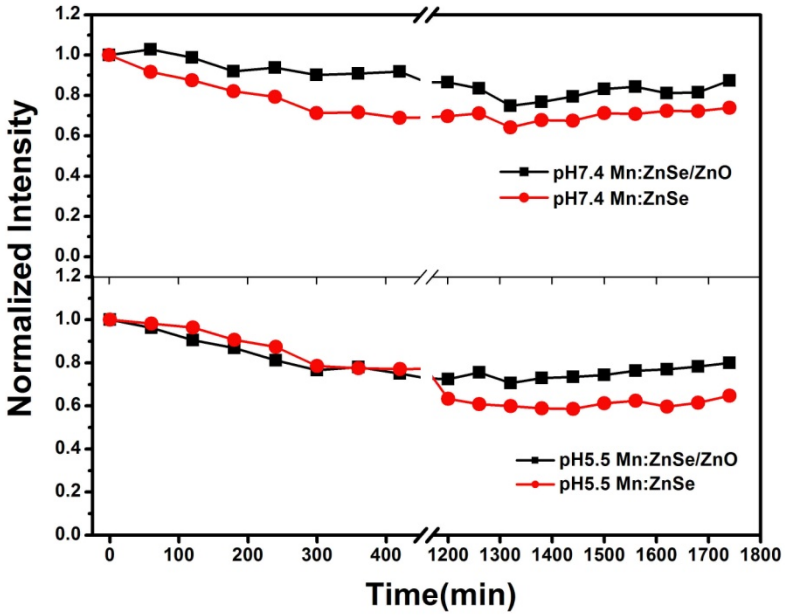


Figure S5. pH stability of MPA-Mn^{2+}:ZnSe and core/shell MPA-Mn^{2+}:ZnSe/ZnO d-dots

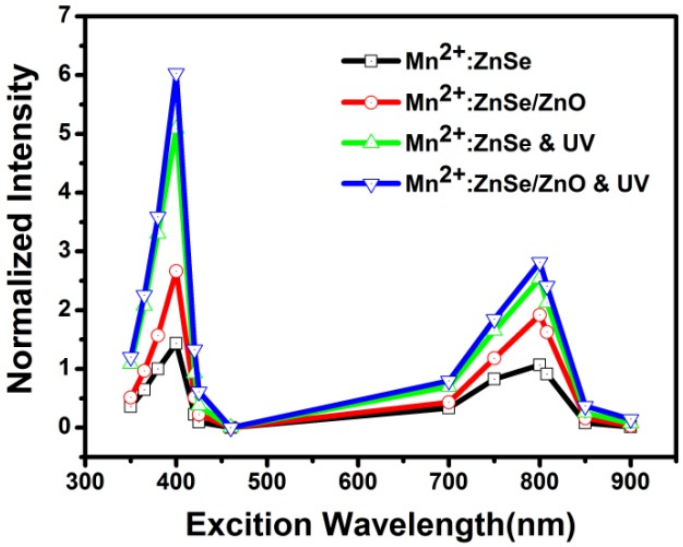


Figure S6. PL intensity variation of MPA-Mn^{2+}:ZnSe and core/shell MPA-Mn^{2+}:ZnSe/ZnO d-dots under different excitation wavelength

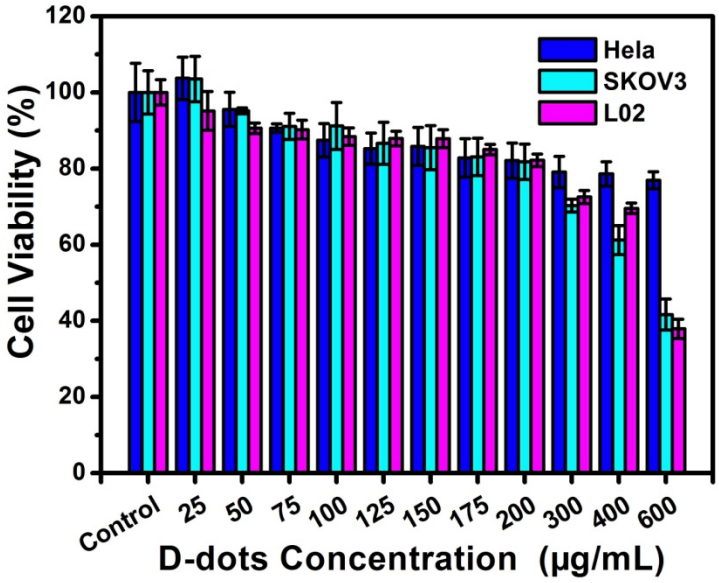


Figure S7. In vitro cell viability of SKOV, Hela and L02 cells treated with different concentrations of Mn^{2+}:ZnSe/ZnO d-dots after UV irradiation for 24h. Percentage cell viability of the treated cells is calculated relative to that of untreated cells (with arbitrarily assigned 100% viability).

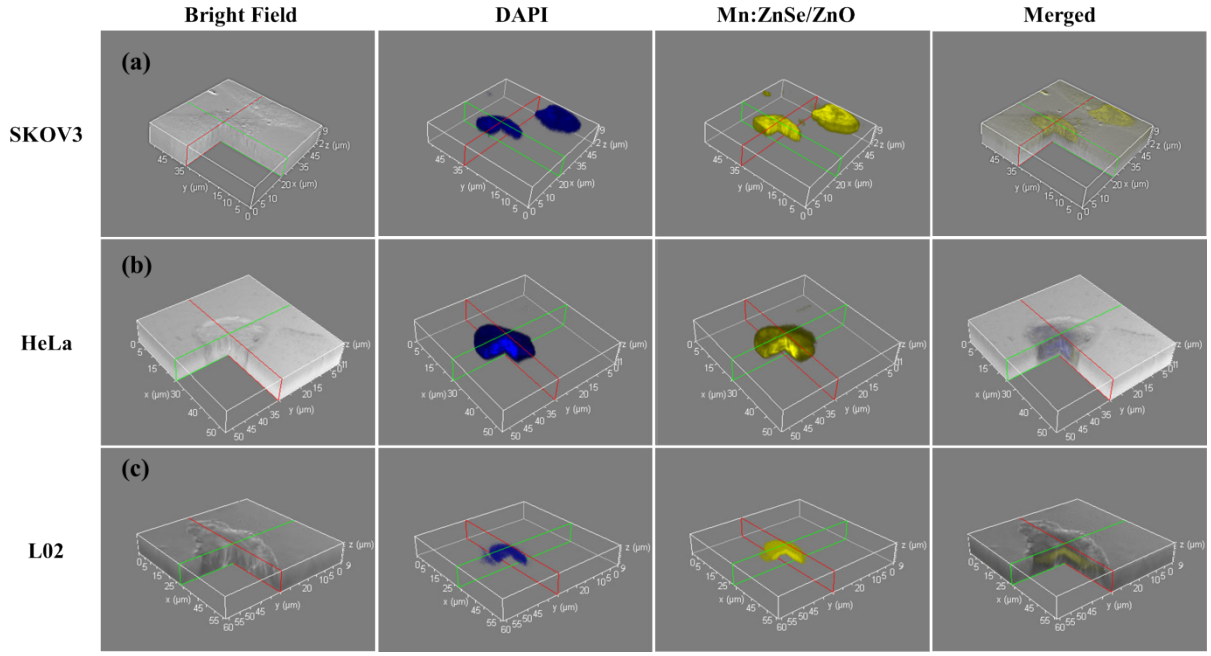


Figure S8. Reconstructed 3D images from Z-stack sections of 2D CLSM images of SKOV3 (a), HeLa (b) and L02 (c) cells. The left boxes correspond to bright field images, fluorescence images (DAPI and d-dots) are shown in the middle, and the right ones show overlays of the bright field and fluorescence images.

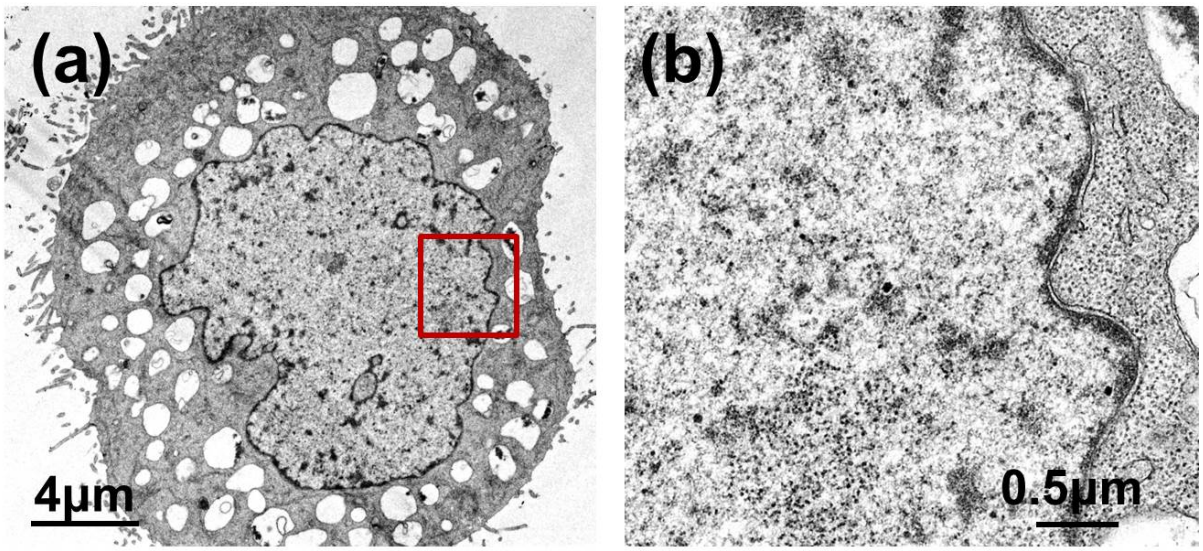


Figure S9. Transmission electron microscopy image of the MPA-Mn^{2+}:ZnSe/ZnO d-dots in HeLa cells(a) and the magnified image (b).

Table S1 Components ratios of MPA-Mn^{2+}: ZnSe and core/shell MPA-Mn^{2+}: ZnSe/ZnO d-dots measured by ICP-MS

Element	Molar Concentration (%)	
	MPA-Mn^{2+}:ZnSe	MPA-Mn^{2+}:ZnSe/ZnO
Zn	55.45	56.00
Se	23.38	20.17
S	19.44	21.91
Mn	1.71	1.92

Table S2. Time constants τ_1, τ_2 and τ_3 and average lifetime τ_{av} of Mn^{2+}: ZnSe and core/shell Mn^{2+}: ZnSe/ZnO d-dots

Sample (μs)	τ_1	τ_2	τ_{av}
Mn^{2+}:ZnSe	84.4	450	441
Mn^{2+}:ZnSe/ZnO	350	990	886
Mn^{2+}:ZnSe/ZnO &UV	340	1000	902
Mn^{2+}:ZnSe & UV	340	990	893

Movie S1 demonstrates that the yellow emitting MPA-Mn^{2+}:ZnSe/ZnO d-dots are accumulating in the nuclei and particularly in histone protein-enriched nucleoli of SKOV3 cells, showing sequential confocal optical slices illustrating a complex nucleolar structure.