Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Supporting Information

Cellular internalisation, bioimaging and dark and photodynamic cytotoxicity of silica nanoparticles doped by $\{Mo_6I_8\}^{4+}$ metal cluster

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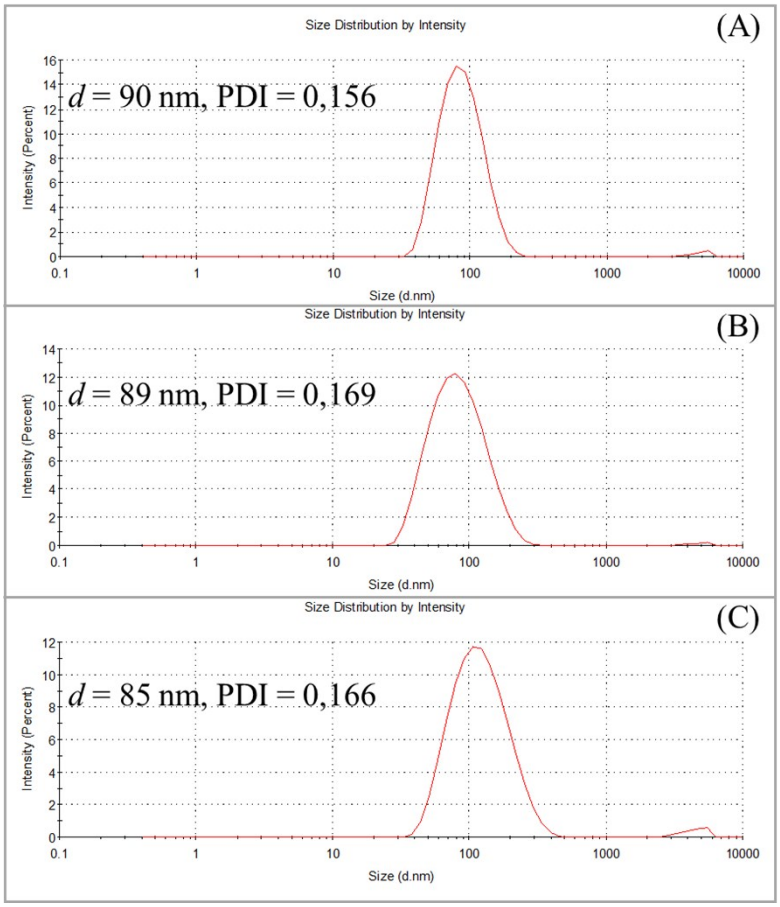


Figure S1. DLS data of neat SiO_2(A), $\{Mo_6I_8\}^{0.001}$@SiO_2 (B) and $\{Mo_6I_8\}^{0.01}$@SiO_2 (C)

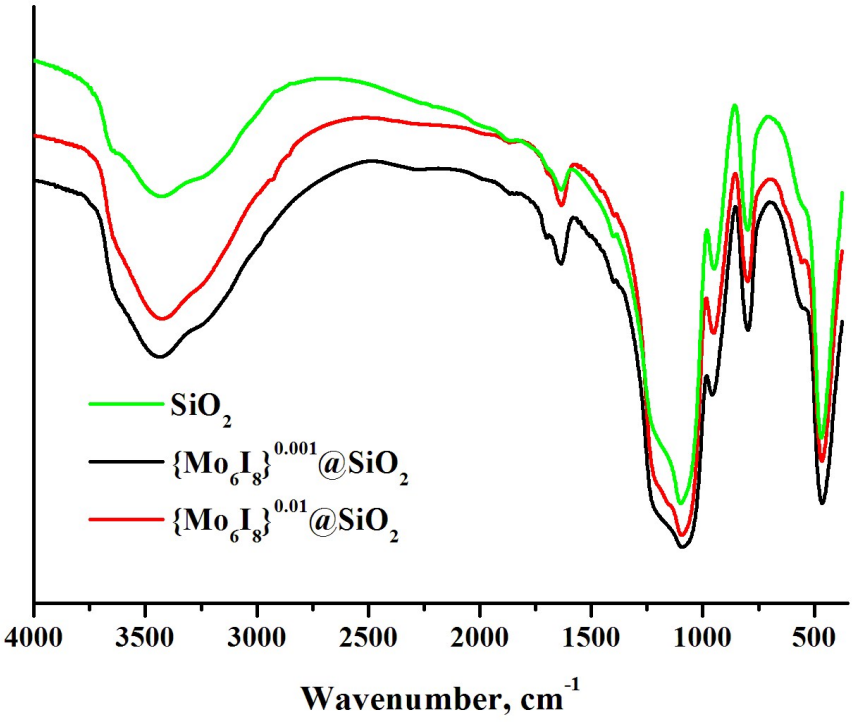


Figure S2. FTIR data of SNPs

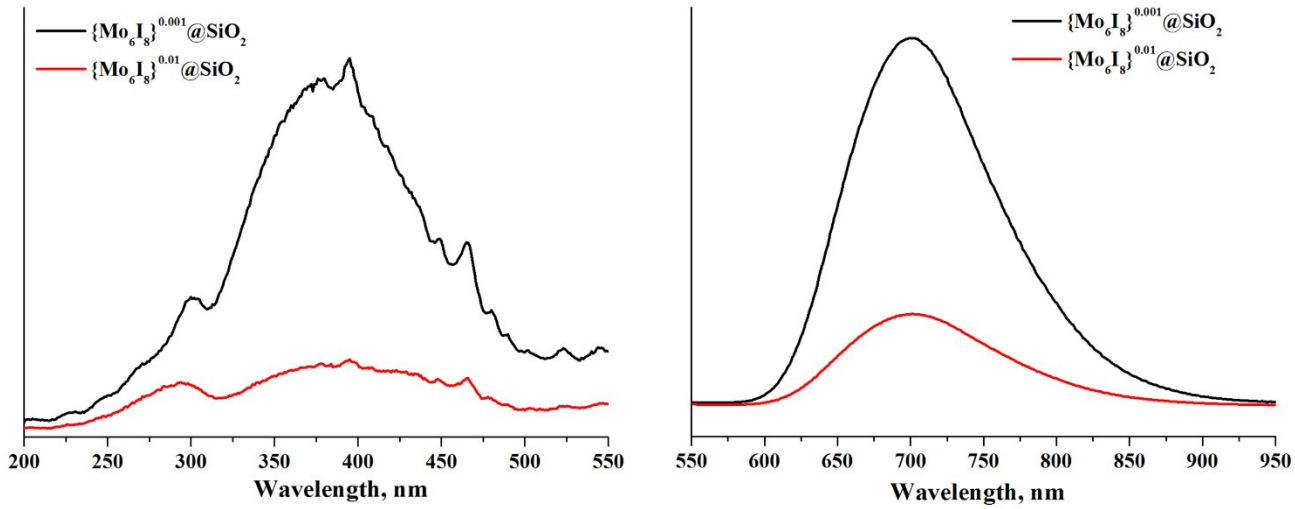


Figure S3. Excitation (left) and emission (right) spectra of SNPs

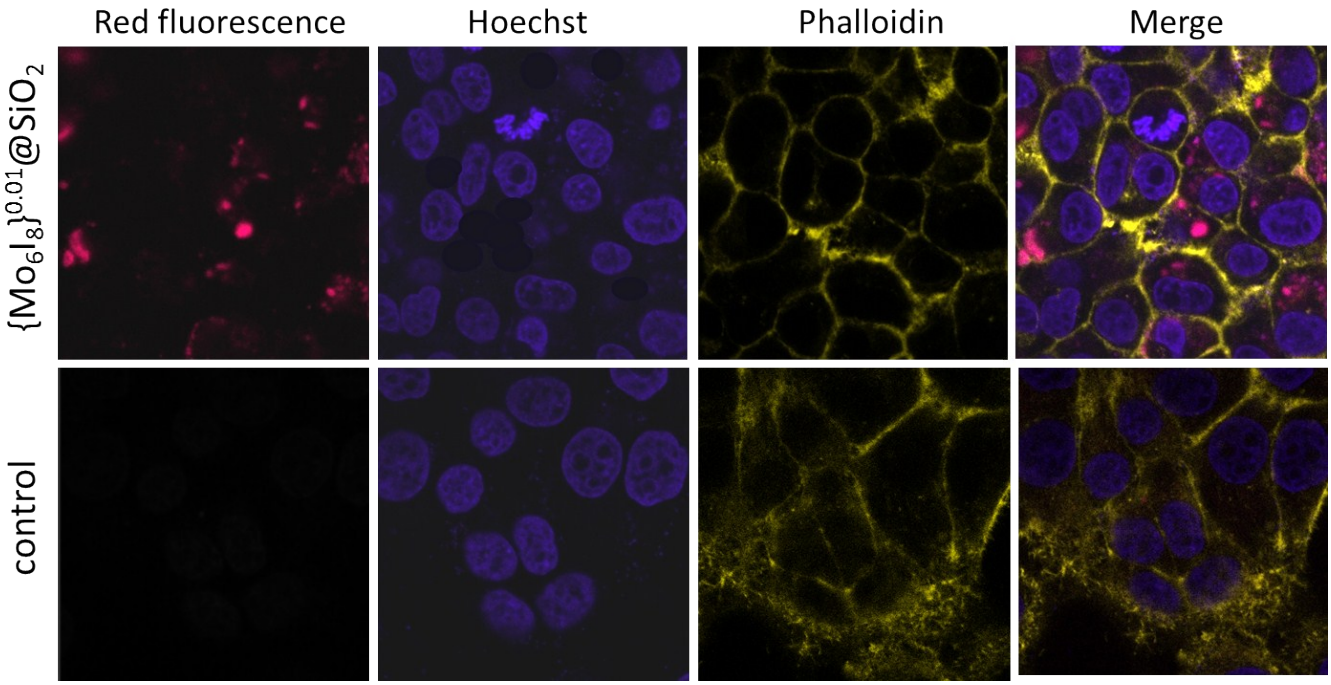


Figure S4. Fluorescent confocal images of cellular uptake and distribution of $\{Mo_6I_8\}^{0.01}@SiO_2$ in Hep-2 cells. Yellow colour show F-actin stained by Alexa Fluore-532 phalloidin. Cell nucleus were stained by DAPI. Hep-2 cells were seeded on slides (1.5×10^5 cells/slide) and incubated overnight at 37 °C under a 5% CO_2 atmosphere. The medium was then replaced with a fresh medium containing 0.1 mg/mL of $\{Mo_6I_8\}^{0.01}@SiO_2$ and incubated for 2 h. The cells incubated in the absence of SNPs were used as a control. Finally, the cells were washed twice with PBS, fixed in 4% paraformaldehyde, and washed thrice with PBS. Then the cells were permeablised with 0.1% Triton X-100 in PBS for 5 min at room temperature, and washed thrice with PBS. F-actin was then stained with Alexa Fluore-532 phalloidin for 15 min at room temperature, washed thrice with PBS. Coverslips were washed thrice with PBS and water, and sealed using mounting medium for fluorescence with DAPI. Cells was visualised by using a Zeiss LSM 510 confocal microscope (Carl Zeiss Inc., Jena, Germany) equipped with a laser diode (405 nm) for fluorescence and with a 100× oil immersion objective. The images were obtained and analysed with ZEN 2009 software. Each experiment was repeated three times on separate days.

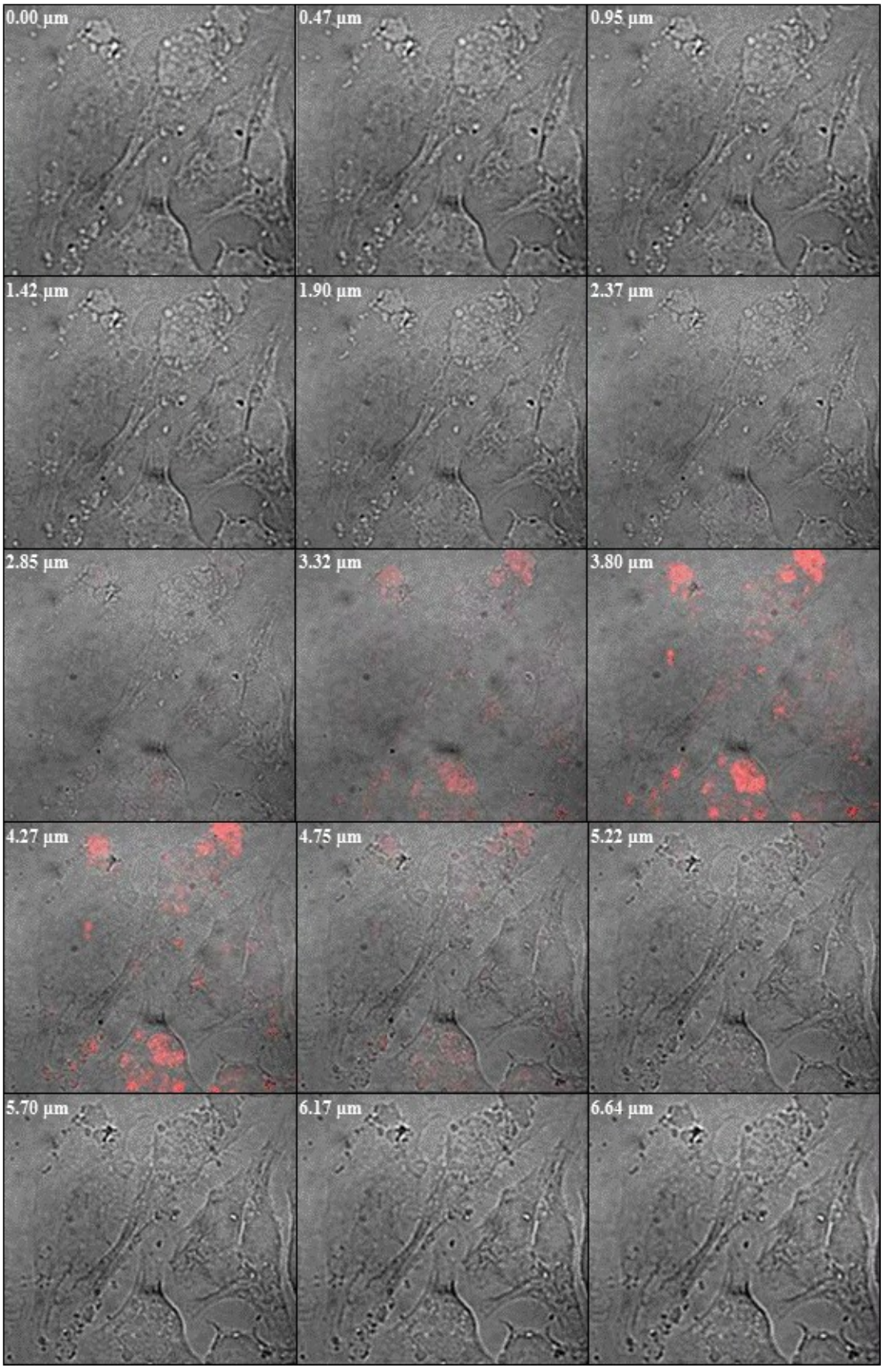


Figure S5.Z series of confocal images of Hep-2 cell incubated with 0.1 mg/mL of $\{Mo_6I_8\}^{0.001}$@SiO_2 for 24 h.

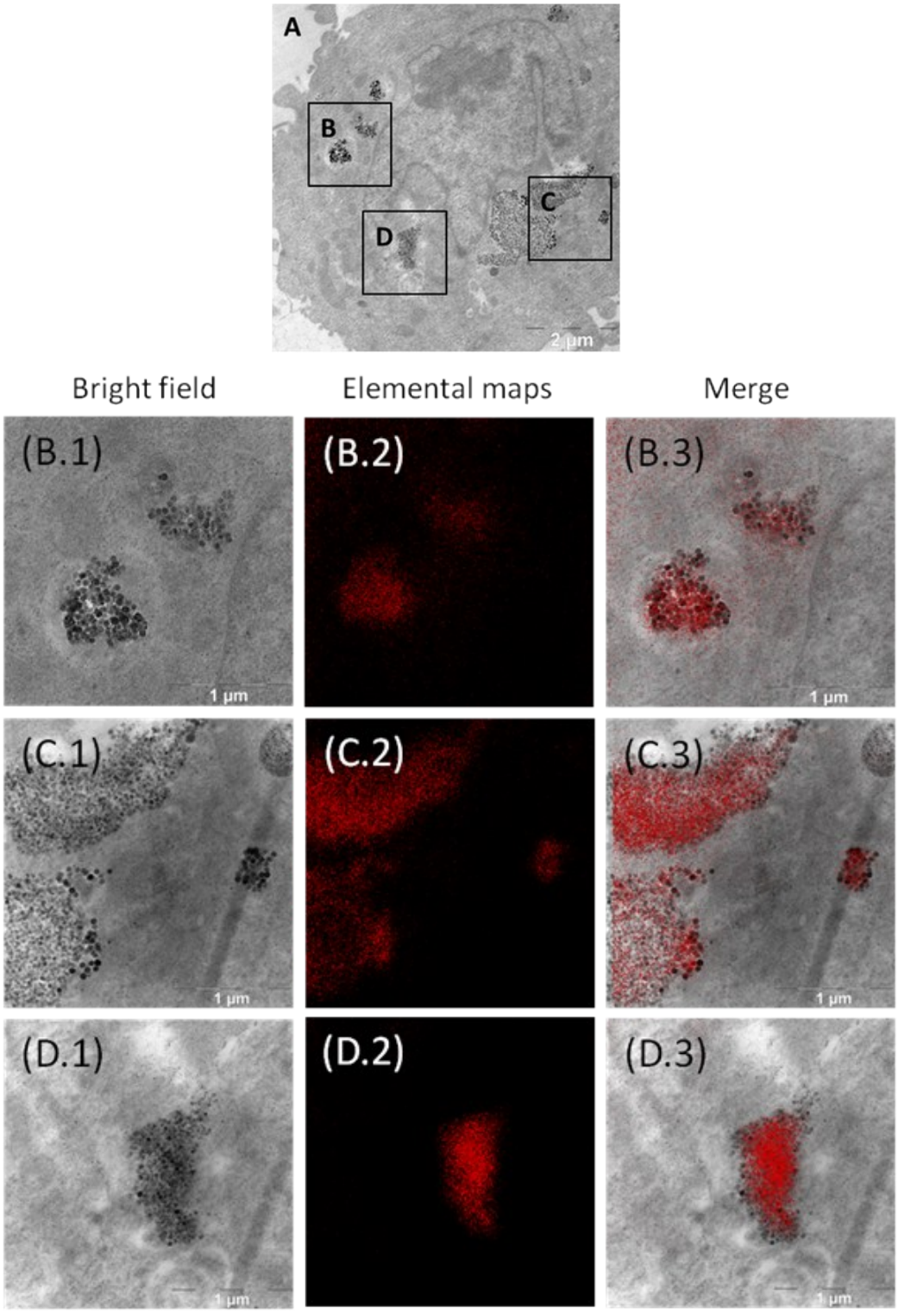


Figure S6. TEM images of a Hep-2 cell after incubation with 0.1 mg/mL neat SNPs for 24 hoursat 37°C.**A)** General view of Hep-2 cell. **B), C), D)** Higher magnification image of the area indicated by the box B, C, D in A, respectively. Neat SNPs in endosome are indicated by the asterisk, free SNPs in cytoplasm are indicated by the arrows. **B.2) C.2) D.2)** Si L2,3-edge, EF-TEM elemental map shows concentrated regions of silicon and **B.3) C.3) D.3)** demonstrates the localisation of SNPs in the cell.

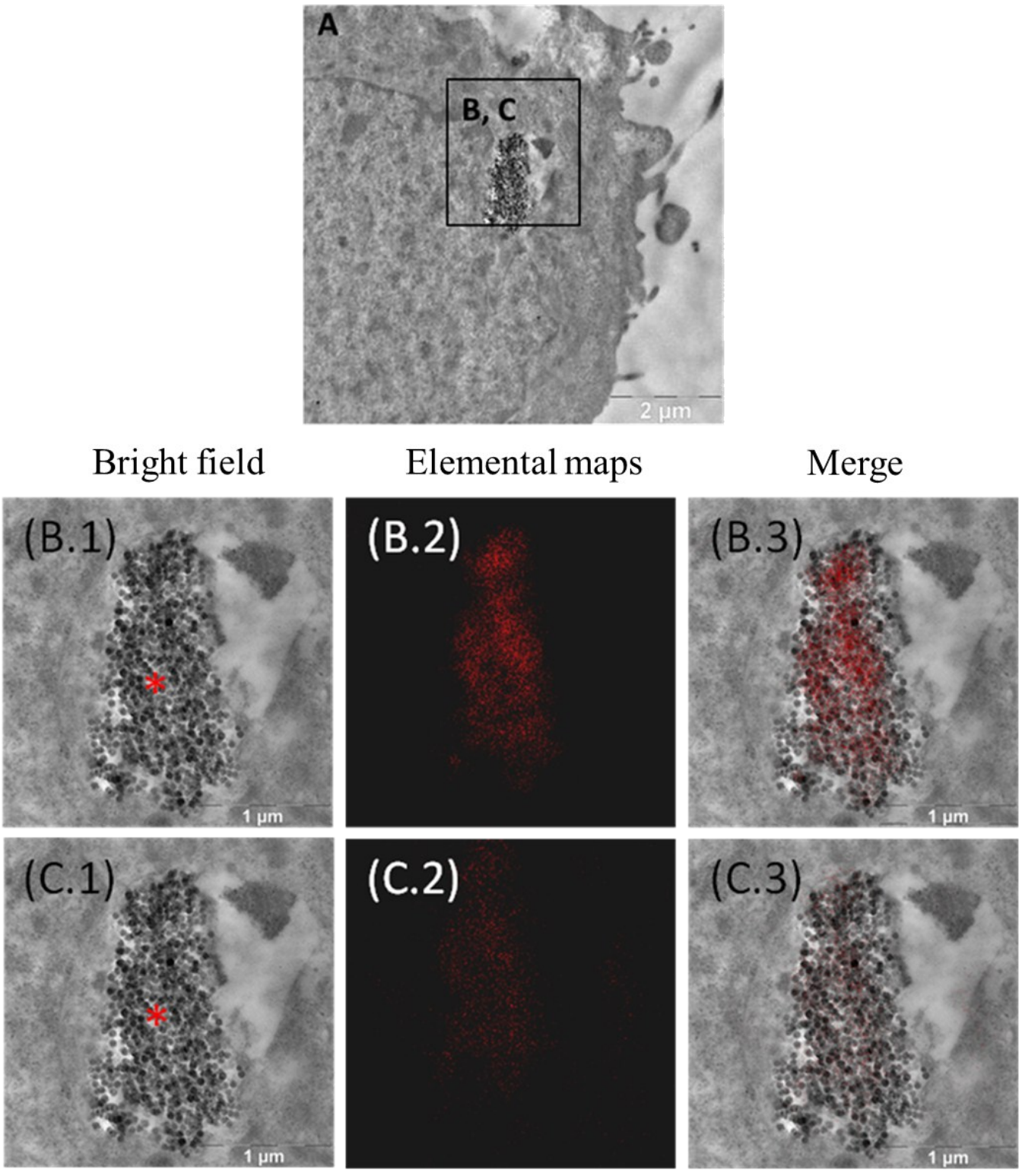
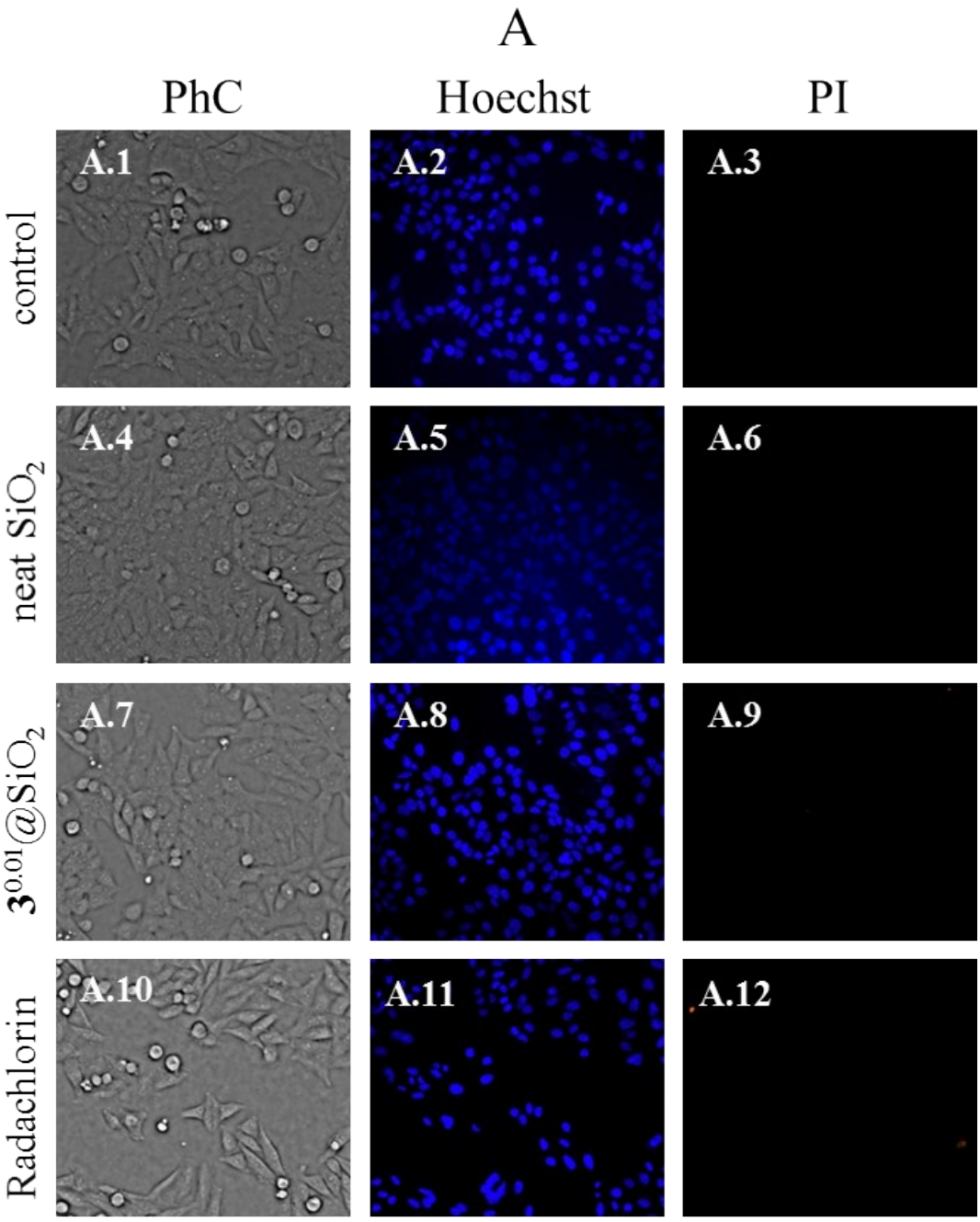


Figure S7.TEM image of a Hep-2 cell after incubation with 0.1 mg/mL $\{Mo_6I_8\}^{0.01}@SiO_2$ for 24 hours at 37°C.**A)** General view of Hep-2 cell.**B), C)** Higher magnification image of the area indicated by the box **B, C** in **A)**. SNPs in endosome are indicated by the asterisk. **B.2)** Si L2,3-edge, EF-TEM elemental map shows concentrated regions of silicon and **C.2)** Mo L2,3-edge, EF-TEM elemental map shows concentrated regions of molybdenum. **B.3) C.3)** clearly demonstrates the localisation of SNPs in the cell. Similarity between elemental maps for Si (**B.2**) and Mo (**C.2**) confirms that we visualised $\{Mo_6I_8\}^{0.01}@SiO_2$.


A
PhC
Hoechst
PI
control
neat SiO_2
$\mathbf{3}^{0.01}$@SiO_2
Radachlorin
A.1
A.2
A.3
A.4
A.5
A.6
A.7
A.8
A.9
A.10
A.11
A.12

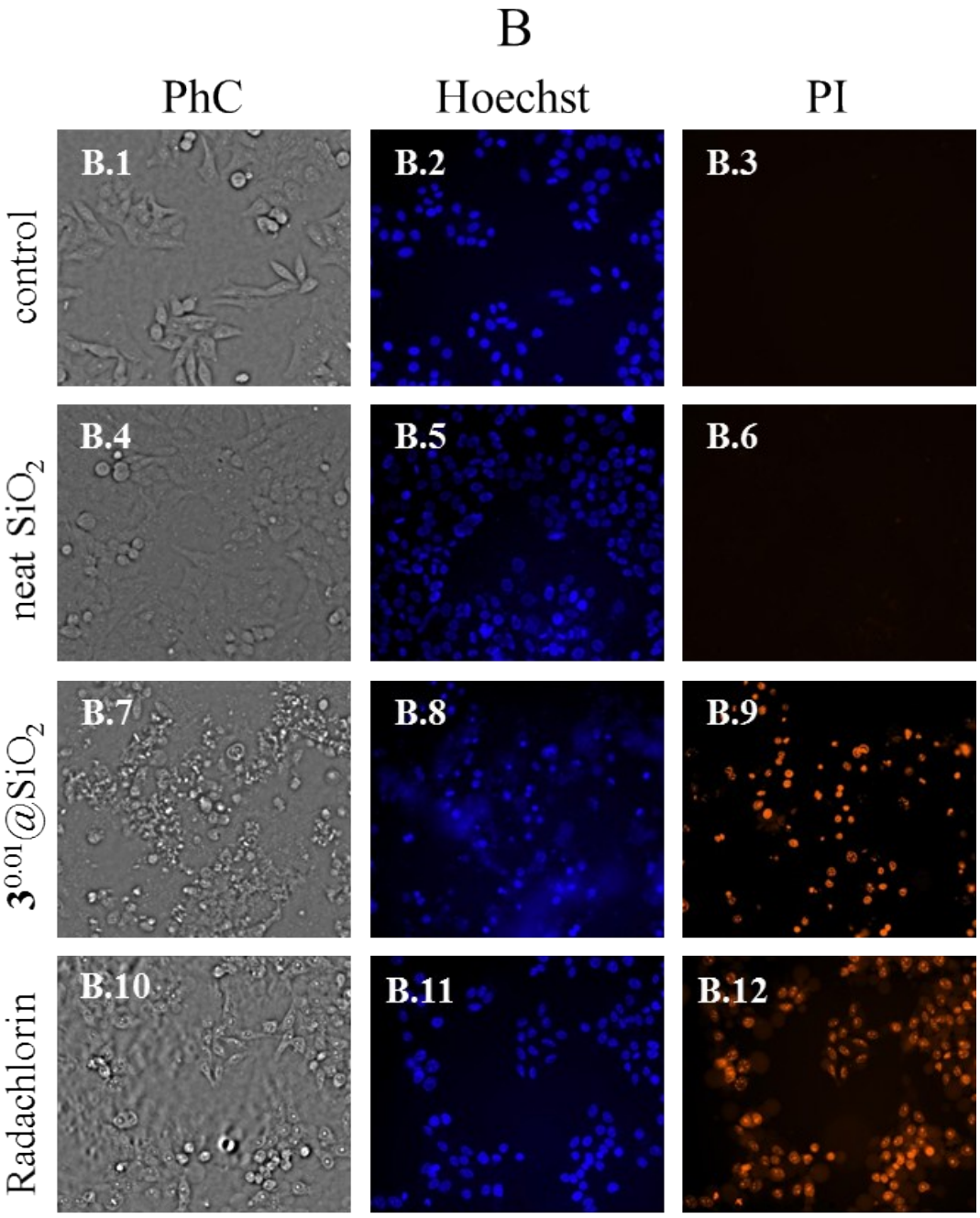


Figure S8. Effect of neat SiO_2, $\{Mo_6I_8\}^{0.01}@SiO_2$ in concentration 0.2 mg/ml and Radachlorinin concentration 61 μg/ml on Hep-2 cells before (A) and after (B) photoirradiation. Cells detected with dual staining of Hoechst 33342 and PI.

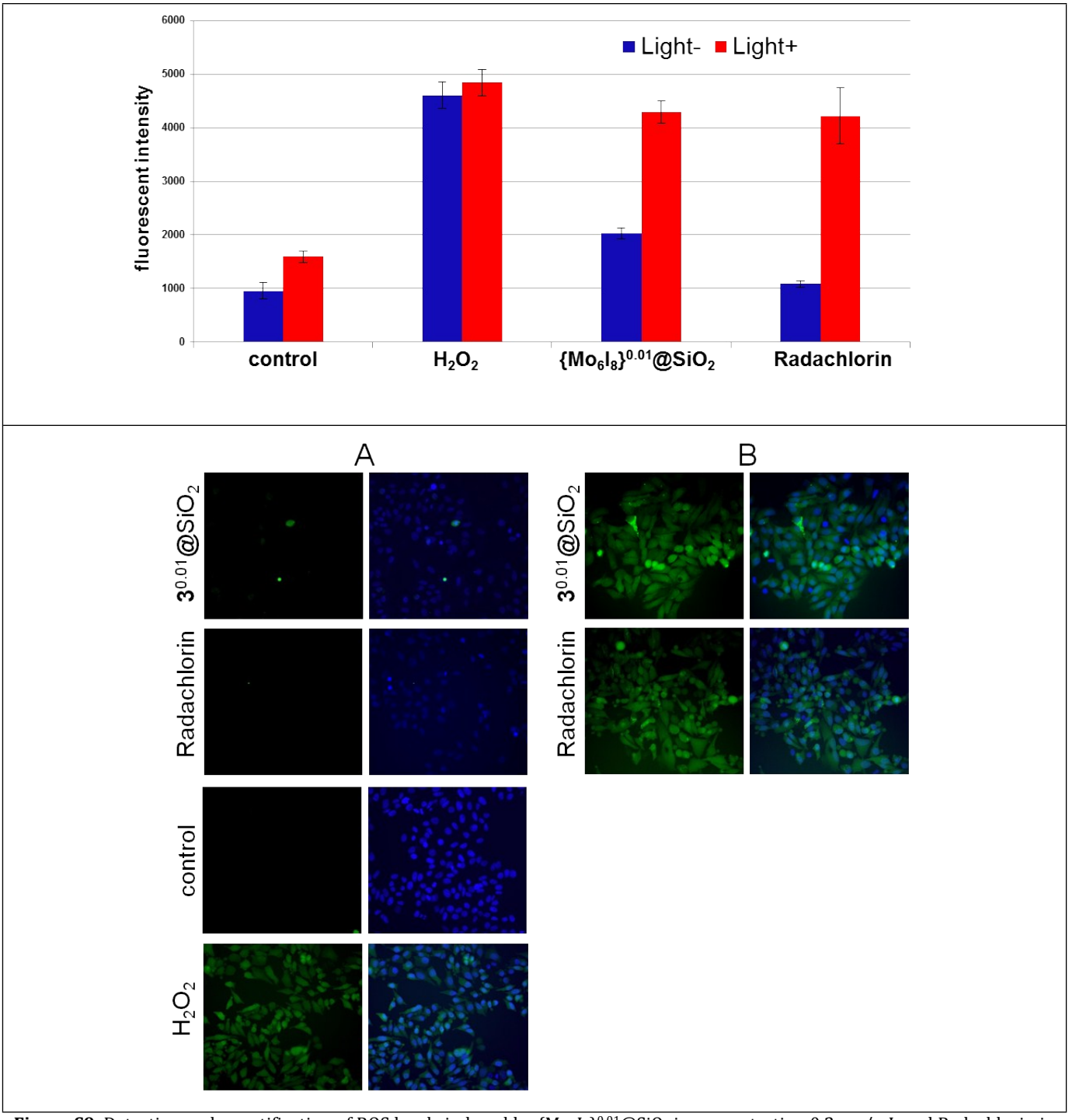


Figure S9. Detection and quantification of ROS levels induced by $\{Mo_6I_8\}^{0.01}@SiO_2$ in concentration 0.2 mg/mL and Radachlorin in concentration 0.03 mg/mL on Hep-2 cells before (I. blue columns, II. A) and after (I. red columns, II. B) photoirradiation. To monitor the intracellular ROS, we used cell-permeable oxidation sensitive fluorescent probes 5,6-carboxy-2',7'-dichlorofluoresceindiacetate (DCFH-DA) (Sigma-Aldrich). Hep-2 cells were seeded in 96- well plate (1×10^4 cells/well) and incubated overnight at 37 °C under a 5% CO_2 atmosphere. The medium was then replaced with a fresh medium containing 0.2 mg/mL of $\{Mo_6I_8\}^{0.01}@SiO_2$ and 0.03 mg/mL of Radachlorin and incubated for 2 h. The cells incubated in free media and with 100 μM H_2O_2 were used as a negative and positive control respectively. The cells were pre-incubated with 10 μM DCFH-DA in HEPES for 10 min at 37°C and then were irradiated with 500 W halogen lamp (λ≥400 nm) for 10 min. Cell nucleus were stained by DAPI. IN Cell Analyzer 2200 (GE Healthcare, UK) was used to perform automatic imaging of four fields per well under 200X magnification, in brightfield and fluorescence channels. The images produced were used to analyse DCF fluorescent intensity DCF positive cells among the whole population using the IN Cell Investigator software (GE Healthcare, UK). Data represent the mean DCF fluorescence intensity. Results are means ± SD.