Liposomal Co-delivery of Resveratrol and Coumarin-6 Enhances Radiosensitization across Microbial and Melanoma Systems

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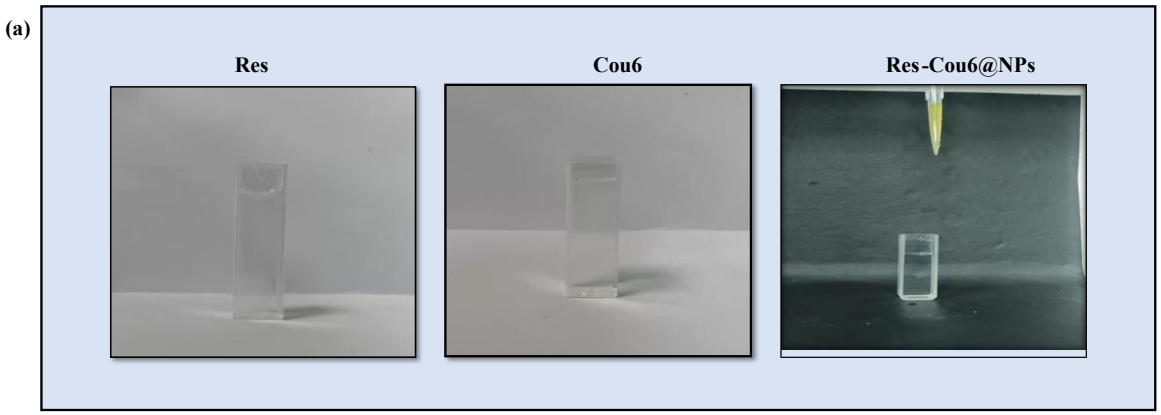
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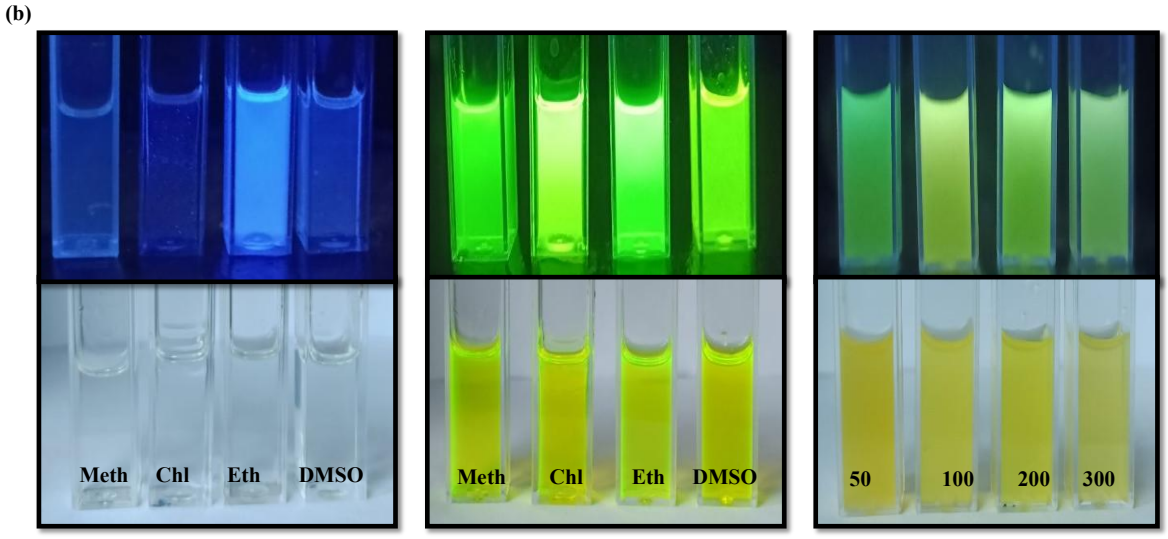
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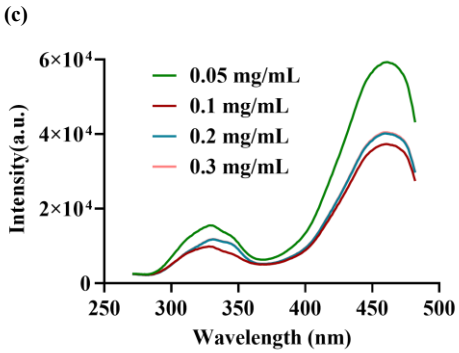
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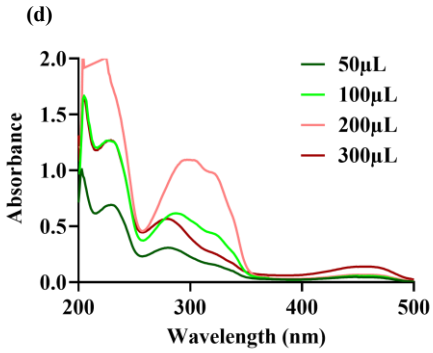
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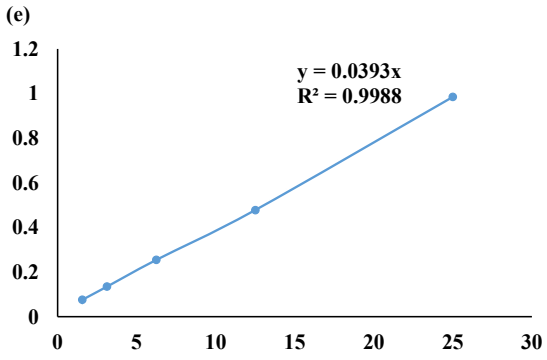
Thaninki Lakshmi Srujana (bm24resch11005@iith.ac.in)


(a)
Res
Cou6
Res-Cou6@NPs


(b)
Meth
Chl
Eth
DMSO
Meth
Chl
Eth
DMSO
50
100
200
300


(c)
Intensity(a.u.)
6×10⁴
4×10⁴
2×10⁴
0
0.05 mg/mL
0.1 mg/mL
0.2 mg/mL
0.3 mg/mL
250
300
350
400
450
500
Wavelength (nm)


(d)
Absorbance
2.0
1.5
1.0
0.5
0.0
50µL
100µL
200µL
300µL
200
300
400
500
Wavelength (nm)


(e)
1.2
1
0.8
0.6
0.4
0.2
0
y = 0.0393x
R² = 0.9988
0
5
10
15
20
25
30

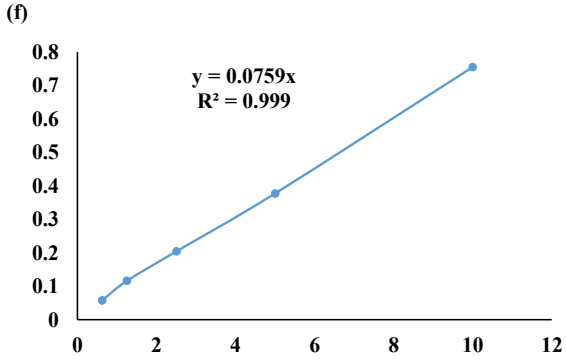

(f)
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
y = 0.0759x
R² = 0.999
0
2
4
6
8
10
12

Fig. S1. (a) Videos while adding Resveratrol alone in water, Coumarin-6 alone in water, and resveratrol and coumarin 6 loaded nanoparticles loaded into water (b) Bright field and UV images of Res and Cou 6 dissolved in the different organic solvents (Methanol, Chloroform, Ethanol, and DMSO), and different concentration of coumarin 6 in the nanoformulation (0.5, 0.1, 0.2, and 0.3 µg/mL (c) Fluorescence graph of all these concentrations of coumarin 6 in the nanoformulation (d) UV graph of all these concentrations of coumarin 6 in the nanoformulation. (e, f) Standard graph of Resveratrol and Coumarin-6.

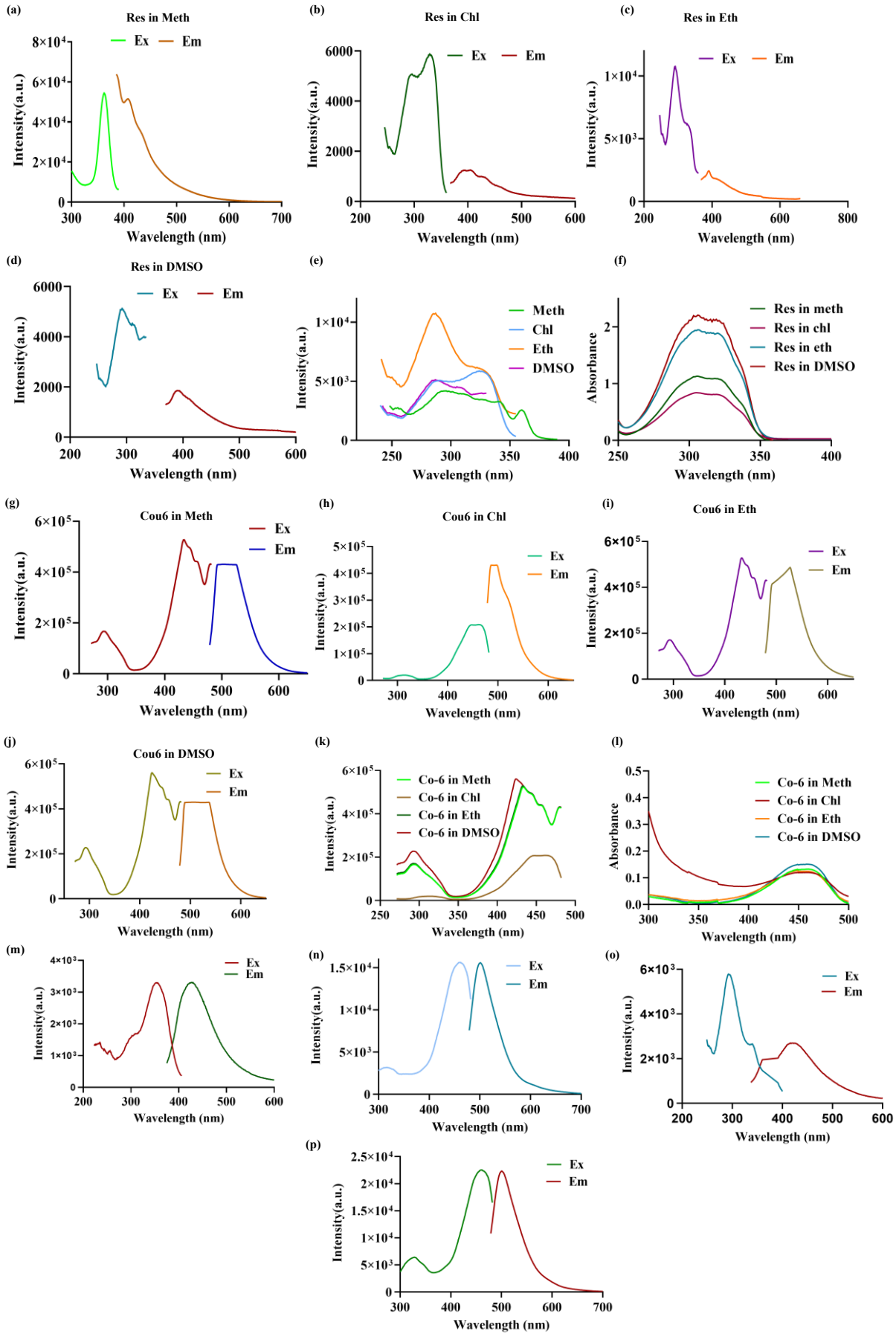

(a)
Res in Meth
Ex
Em
Intensity(a.u.)
Wavelength (nm)
(b)
Res in Chl
Ex
Em
Intensity(a.u.)
Wavelength (nm)
(c)
Res in Eth
Ex
Em
Intensity(a.u.)
Wavelength (nm)
(d)
Res in DMSO
Ex
Em
Intensity(a.u.)
Wavelength (nm)
(e)
Meth
Chl
Eth
DMSO
Intensity(a.u.)
Wavelength (nm)
(f)
Res in meth
Res in chl
Res in eth
Res in DMSO
Absorbance
Wavelength (nm)
(g)
Cou6 in Meth
Ex
Em
Intensity(a.u.)
Wavelength (nm)
(h)
Cou6 in Chl
Ex
Em
Intensity(a.u.)
Wavelength (nm)
(i)
Cou6 in Eth
Ex
Em
Intensity(a.u.)
Wavelength (nm)
(j)
Cou6 in DMSO
Ex
Em
Intensity(a.u.)
Wavelength (nm)
(k)
Co-6 in Meth
Co-6 in Chl
Co-6 in Eth
Co-6 in DMSO
Intensity(a.u.)
Wavelength (nm)
(l)
Co-6 in Meth
Co-6 in Chl
Co-6 in Eth
Co-6 in DMSO
Absorbance
Wavelength (nm)
(m)
Ex
Em
Intensity(a.u.)
Wavelength (nm)
(n)
Ex
Em
Intensity(a.u.)
Wavelength (nm)
(o)
Ex
Em
Intensity(a.u.)
Wavelength (nm)
(p)
Ex
Em
Intensity(a.u.)
Wavelength (nm)

Fig. S2. (a-d) Fluorescence data of Res in different organic solvents (e) Merged graph of Res fluorescence data in all organic solvents (f) UV data of Res in different organic solvents (g-j) Fluorescence data of Cou 6 in different organic solvents (k) Merged graph of Cou 6 fluorescence data in all organic solvents (l) UV data of Cou 6 in different organic solvents (m) Fluorescence spectra of Plain NPs (n) Fluorescence spectra of Res@NPs (o) Fluorescence spectra of Cou6@NPs (p) Fluorescence spectra of Res-Cou6@NPs.

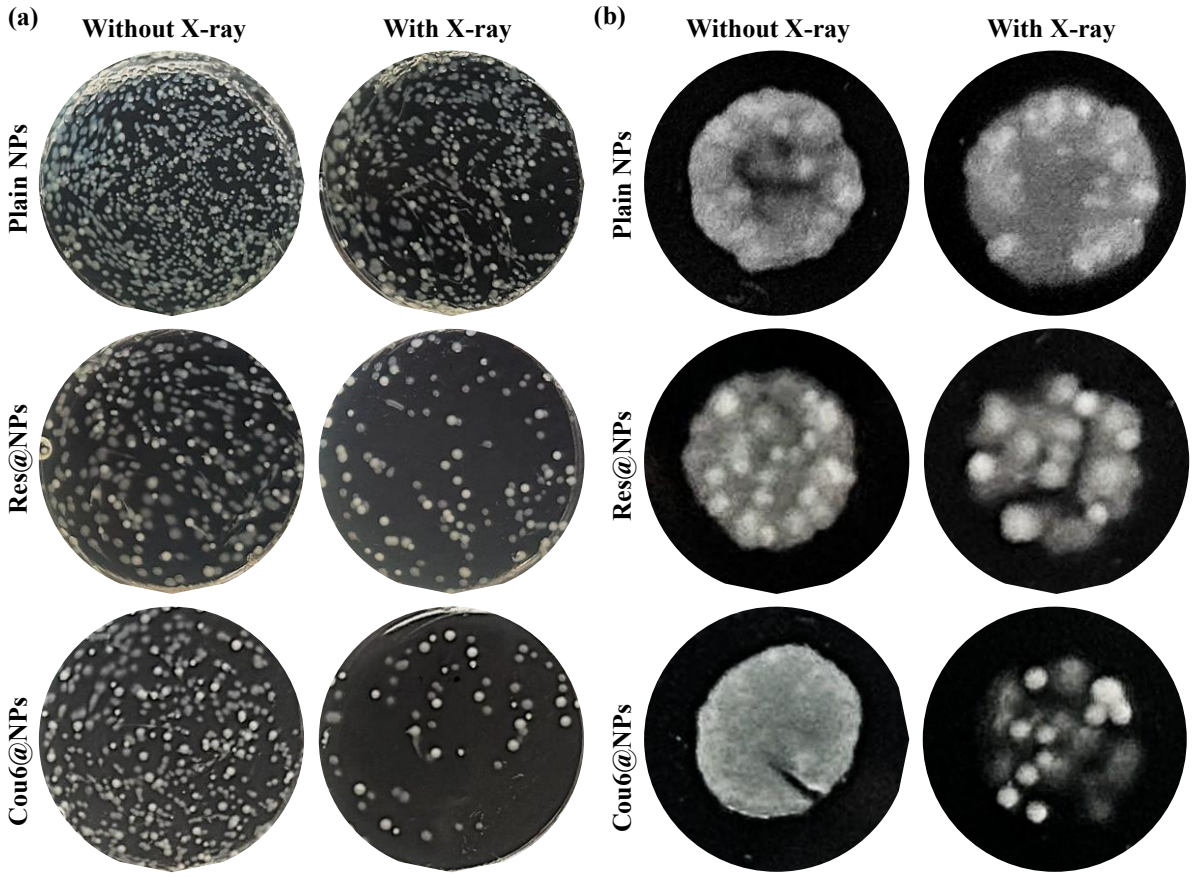


Fig. S3. (a) Spread assay of Plain NPs, Res@NPs, and Cou6@NPs on *Deinococcus radiodurans* with and without radiation. (b) Spot assay of Plain NPs, Res@NPs, and Cou6@NPs on *Deinococcus radiodurans* with and without radiation.

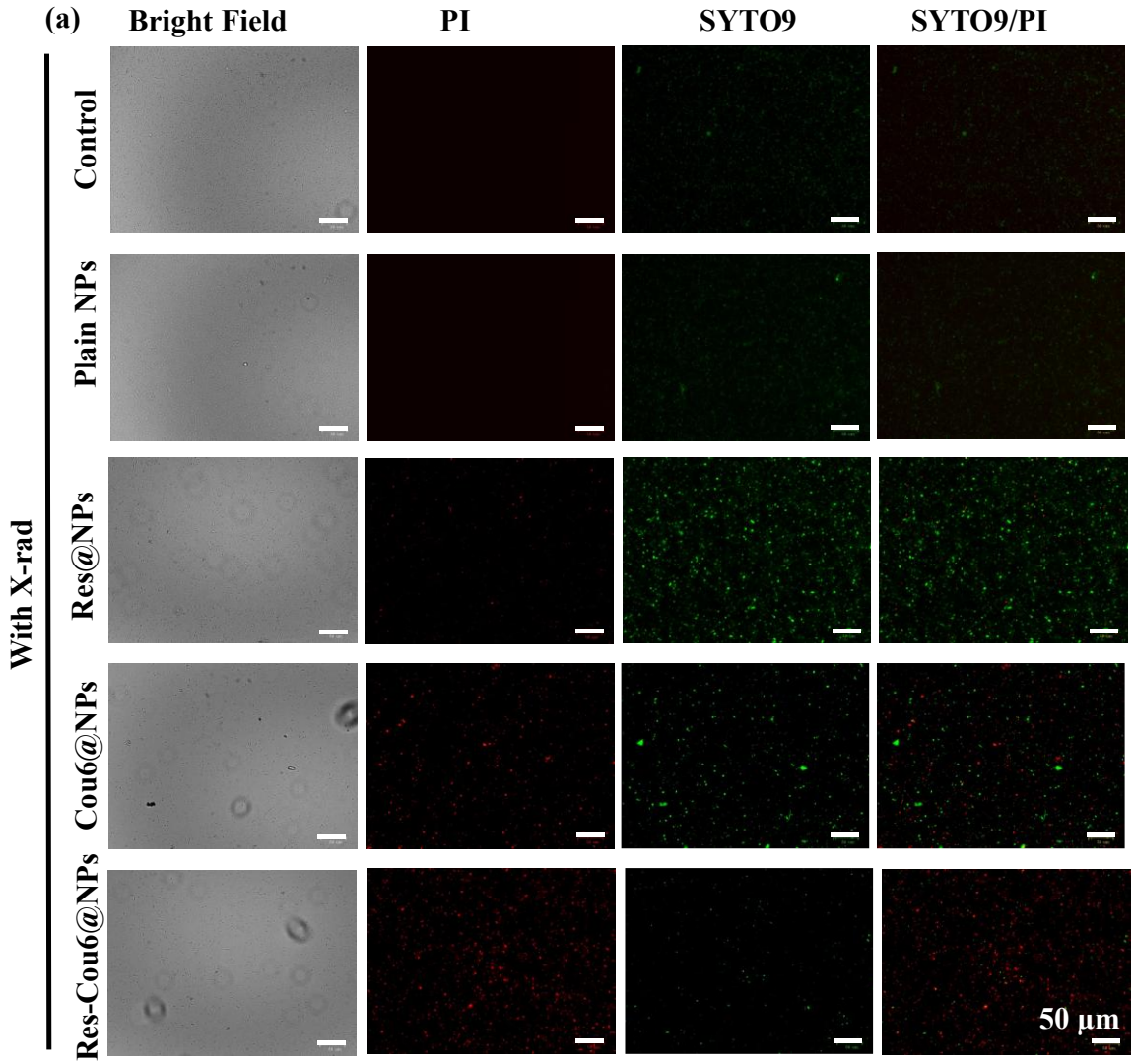


Fig. S4. (a) Live/Dead assay of control, Plain NPs, Res@NPs, Cou6@NPs and Res-Cou6@NPs on *Deinococcus radiodurans* with radiation.

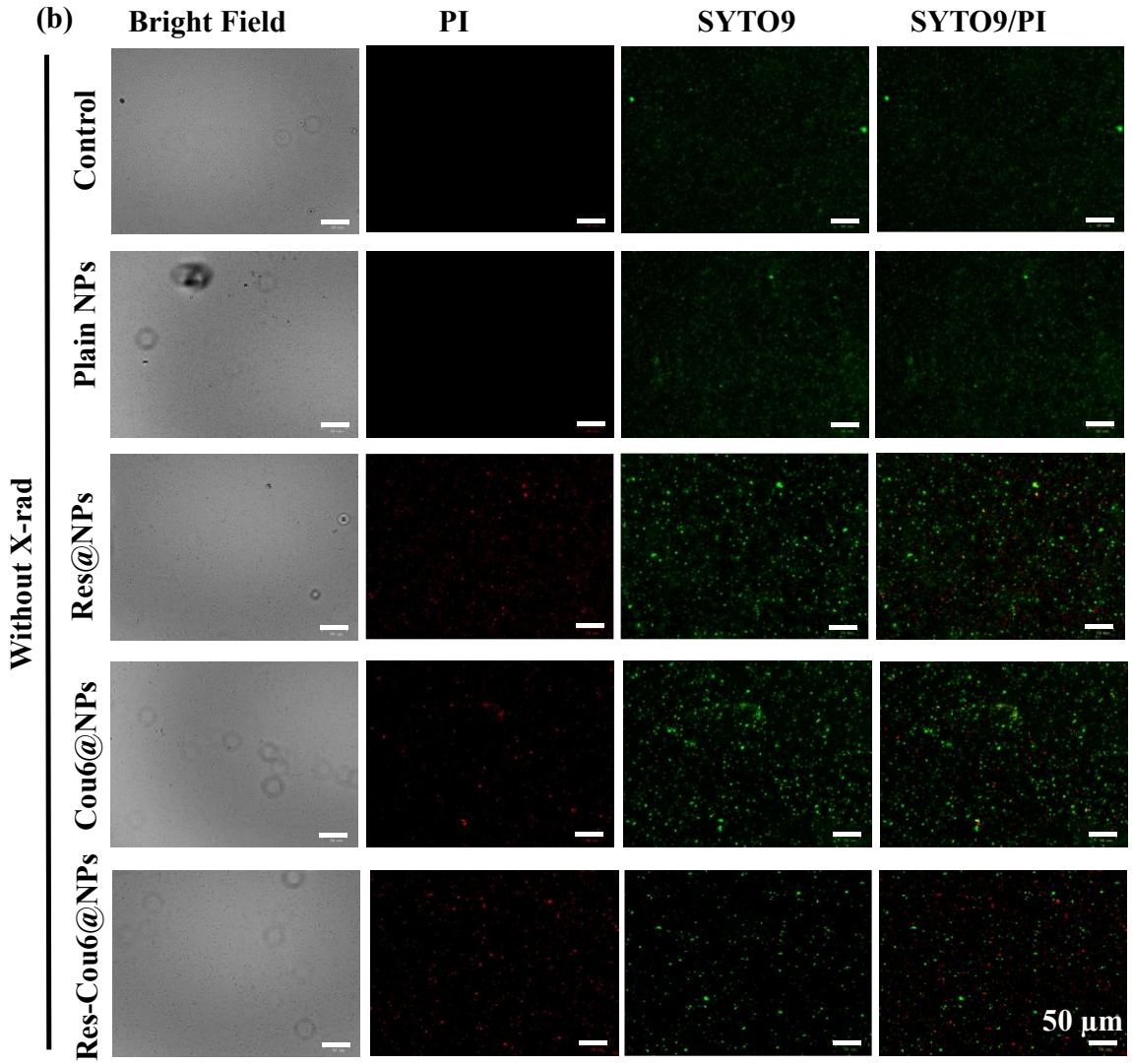


Fig. S5. (a) Live/Dead assay of control, Plain NPs, Res@NPs, Cou6@NPs and Res-Cou6@NPs on *Deinococcus radiodurans* without radiation.

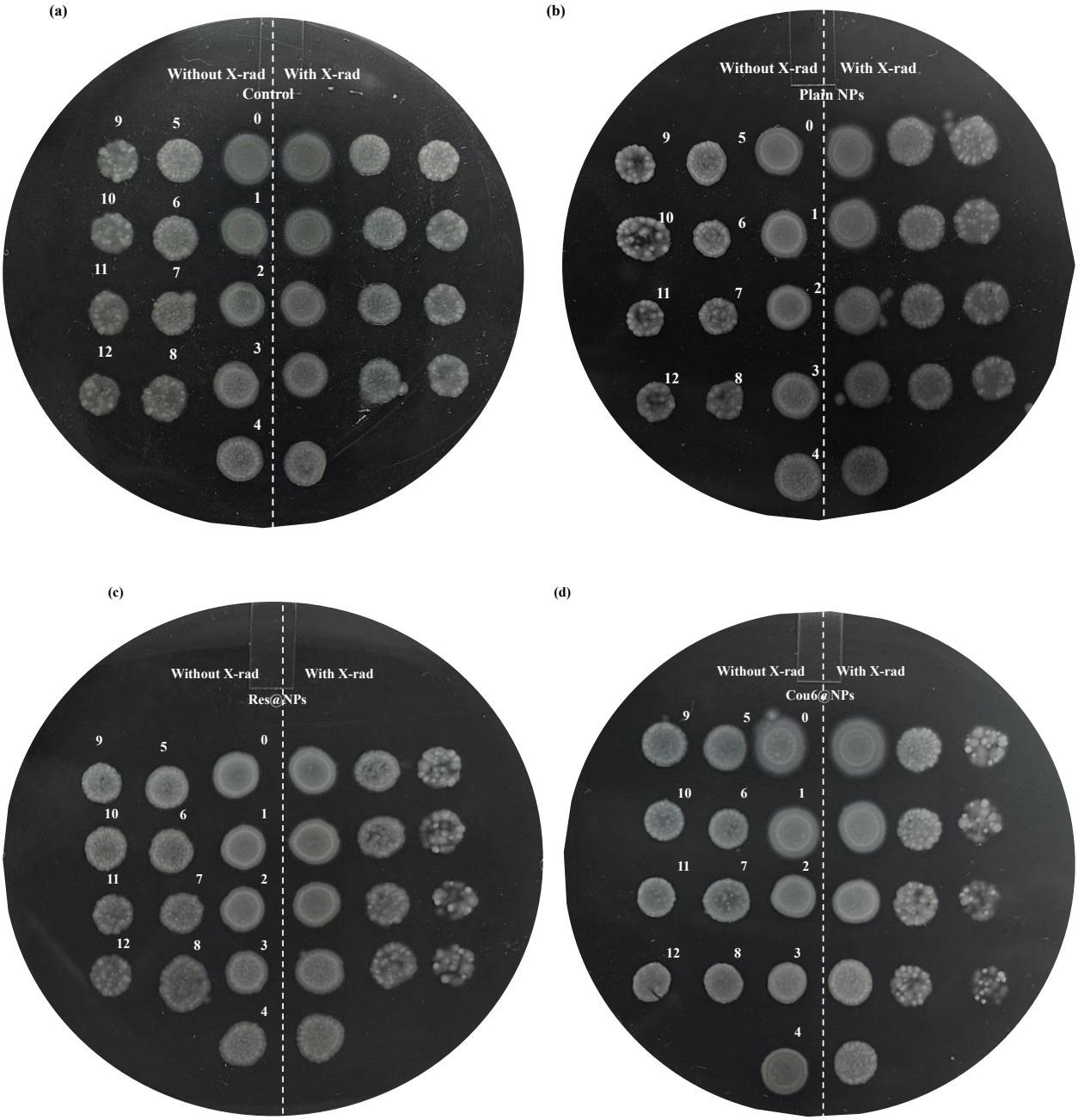


Fig. S6. (a) Spot assay raw plate image of control on *Deinococcus radiodurans* with and without radiation. (b) Spot assay raw plate image of Plain NPs on *Deinococcus radiodurans* with and without radiation. (c) Spot assay raw plate image of Res@NPs on *Deinococcus radiodurans* with and without radiation. (d) Spot assay raw plate image of Cou6@NPs on *Deinococcus radiodurans* with and without radiation.

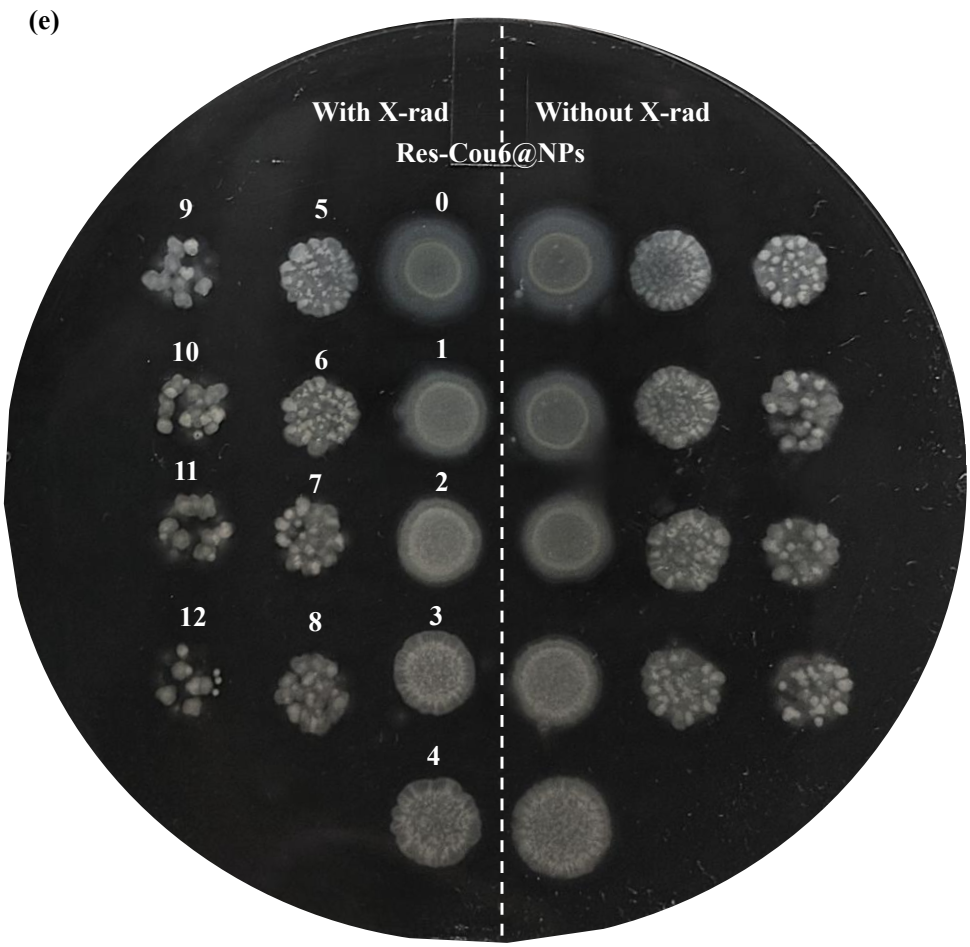


Fig. S7. (e) Spot assay raw plate image of Res-Cou6@NPs on *Deinococcus radiodurans* with and without radiation.

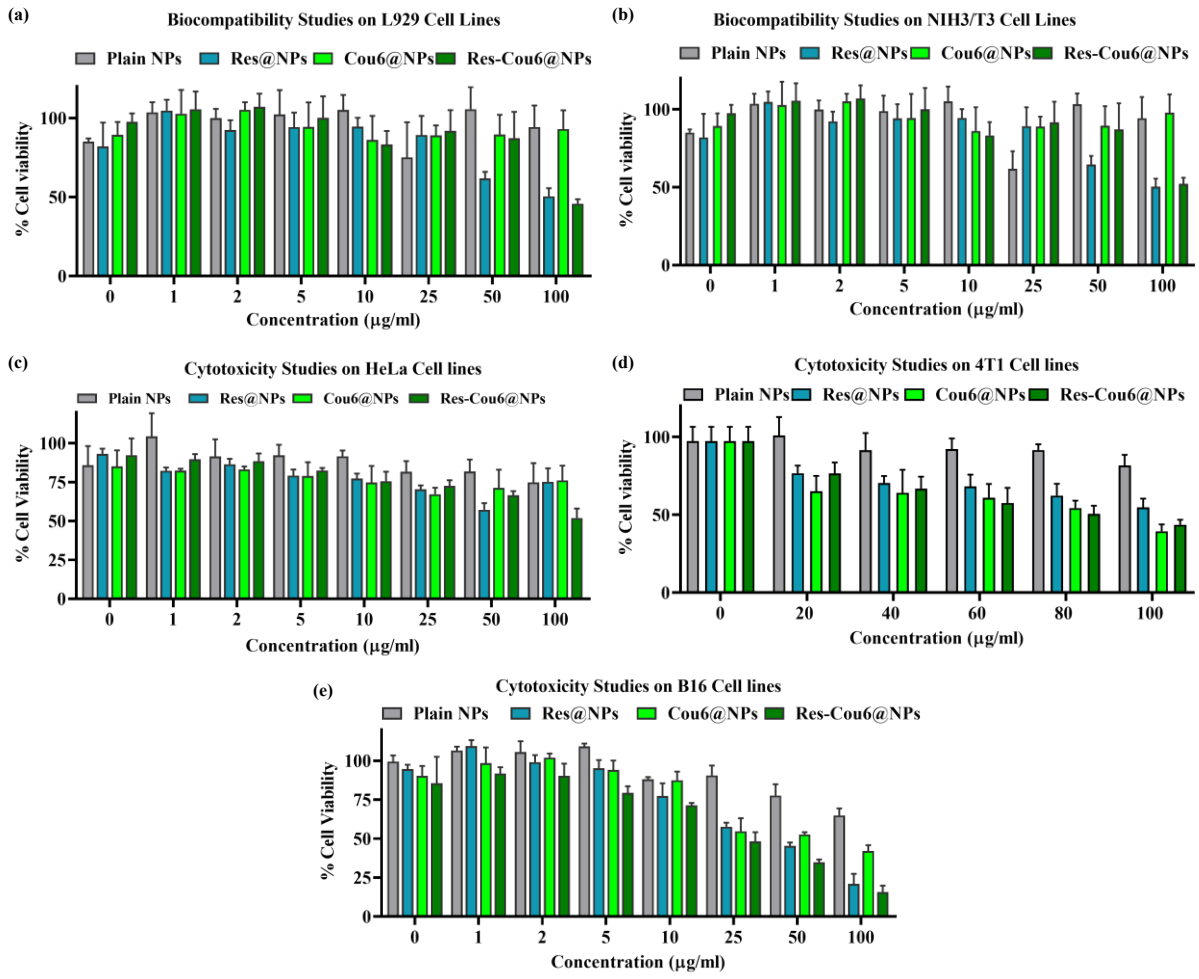


Fig. S8. (a, b) Biocompatibility bar graphs of Plain NPs, Res@NPs, Cou6@NPs, and Res-Cou6@NPs in L929 cells, and, NIH3/T3 cells (c-e) Cytotoxicity studies lines graphs of different nanoparticles in HeLa cells, 4T1 cells, and B16 cells.

S Table 1. SI of Res-Cou6@NPs in different cancer cell lines.

Normal cells/Cancer cells	Selectivity Index (SI)
L929/HeLa	1
L929/4T1	1.25
L929/B16F10	4.54

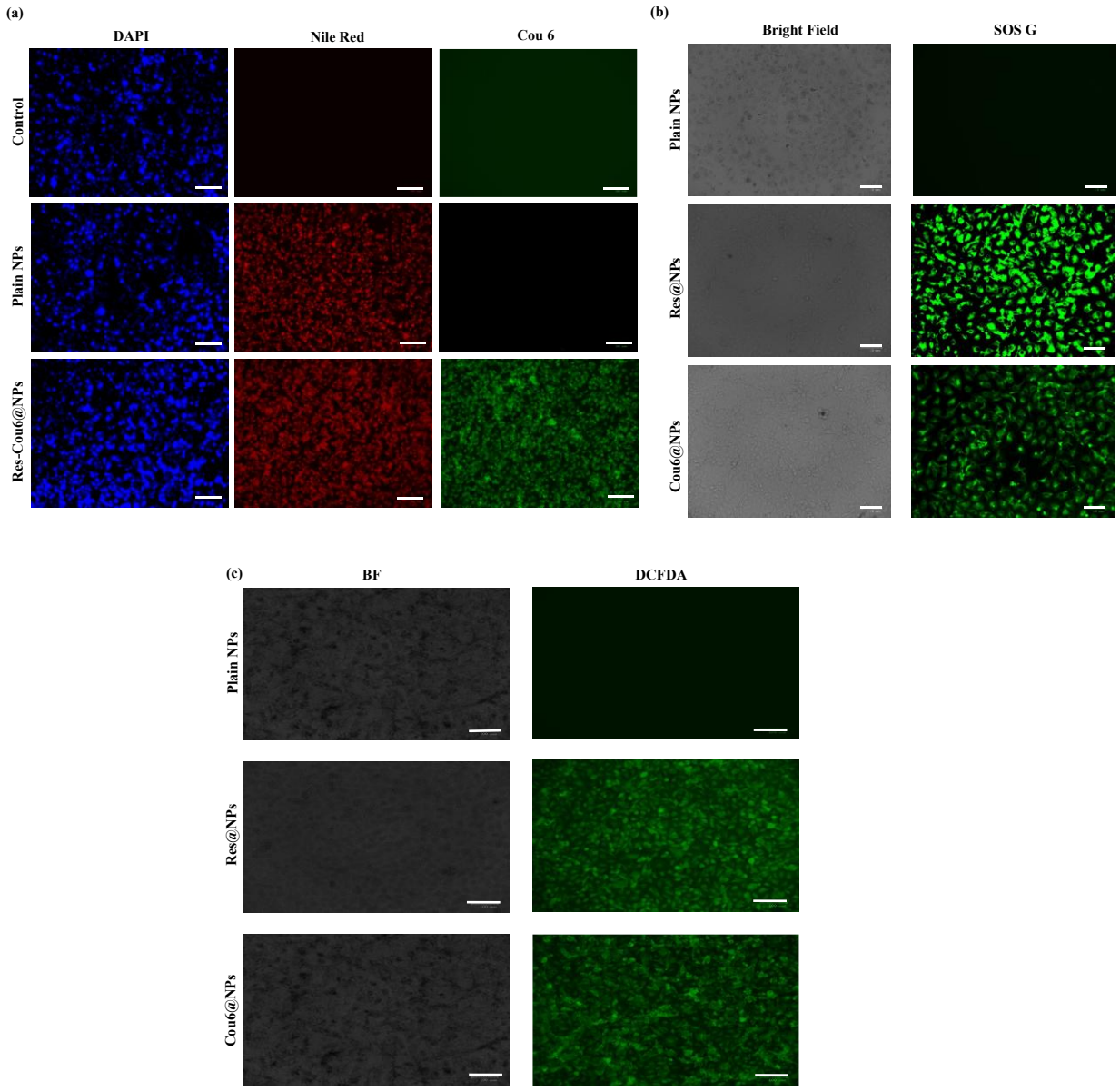


Fig. S9. (a) Uptake studies of Plain NPs, and Res-Cou6@NPs (scale bar: 100 µm) (b) Singlet oxygen species generation study of internal control groups, Plain NPs, Res@NPs, and Cou6@NPs (scale bar: 100 µm) (c) ROS generation study of internal control groups, Plain NPs, Res@NPs, and Cou6@NPs (scale bar: 100 µm).

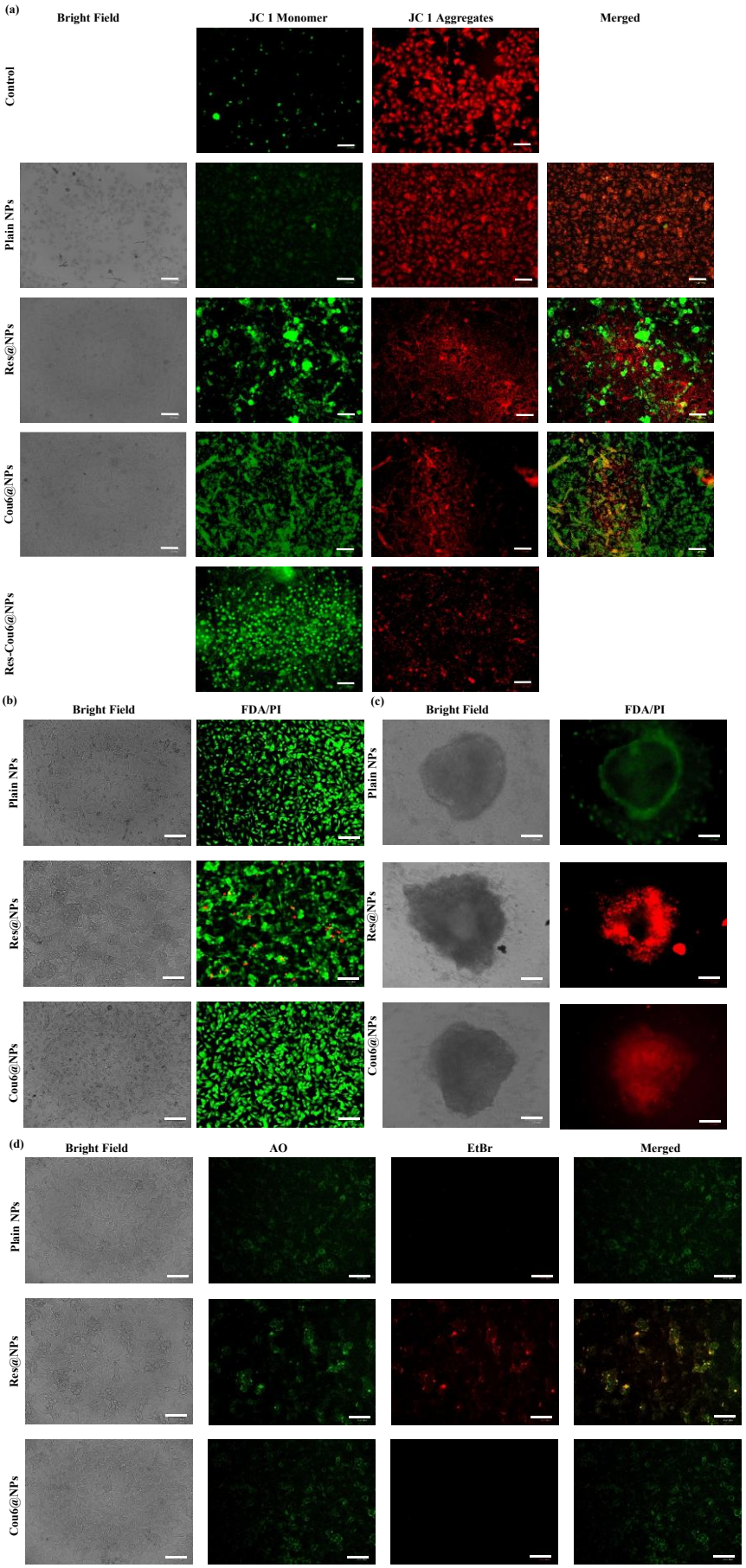

(a)
Bright Field
JC 1 Monomer
JC 1 Aggregates
Merged
Control
Plain NPs
Res@NPs
Cou6@NPs
Res-Cou6@NPs
(b)
Bright Field
FDA/PI
Plain NPs
Res@NPs
Cou6@NPs
(c)
Bright Field
FDA/PI
Plain NPs
Res@NPs
Cou6@NPs
(d)
Bright Field
AO
EtBr
Merged
Plain NPs
Res@NPs
Cou6@NPs

Fig. S10. (a) JC-1 assay of Control, Plain NPs, Res@NPs, Cou6@NPs, and Res-Cou6@NPs (scale bar: 100 µm) (b) Live/Dead assay of internal control groups, Plain NPs, Res@NPs, and Cou6@NPs (scale bar: 100 µm) (c) Spheroid assay of internal control groups, Plain NPs, Res@NPs, and Cou6@NPs (scale bar: 100 µm) (d) AO/EtBr assay of internal control groups, Plain NPs, Res@NPs, and Cou6@NPs (scale bar: 100 µm)

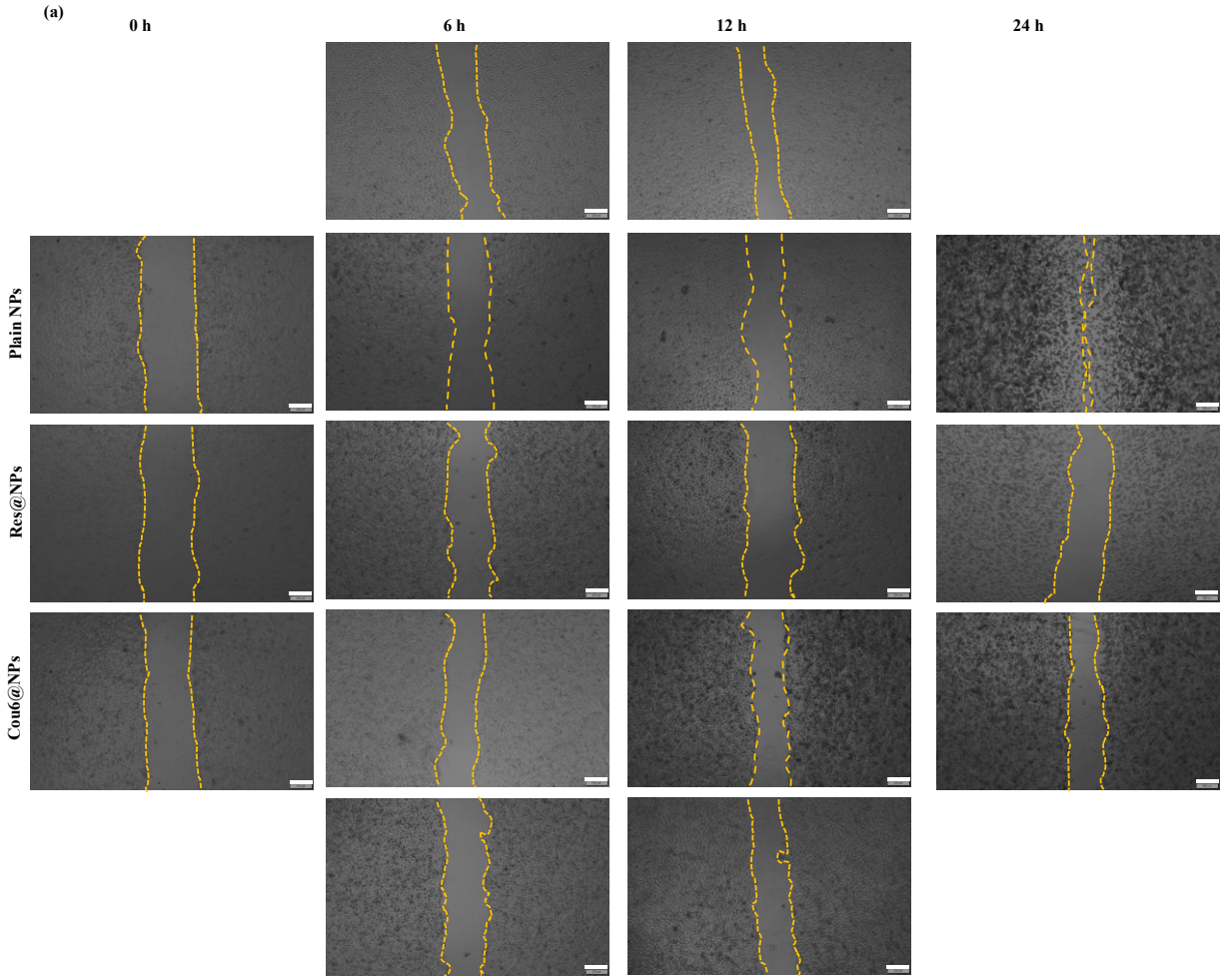


Fig. S11. (a) Scratch assay of internal control groups, Plain NPs, Res@NPs, and Cou6@NPs (Scale bar: 200 µm).

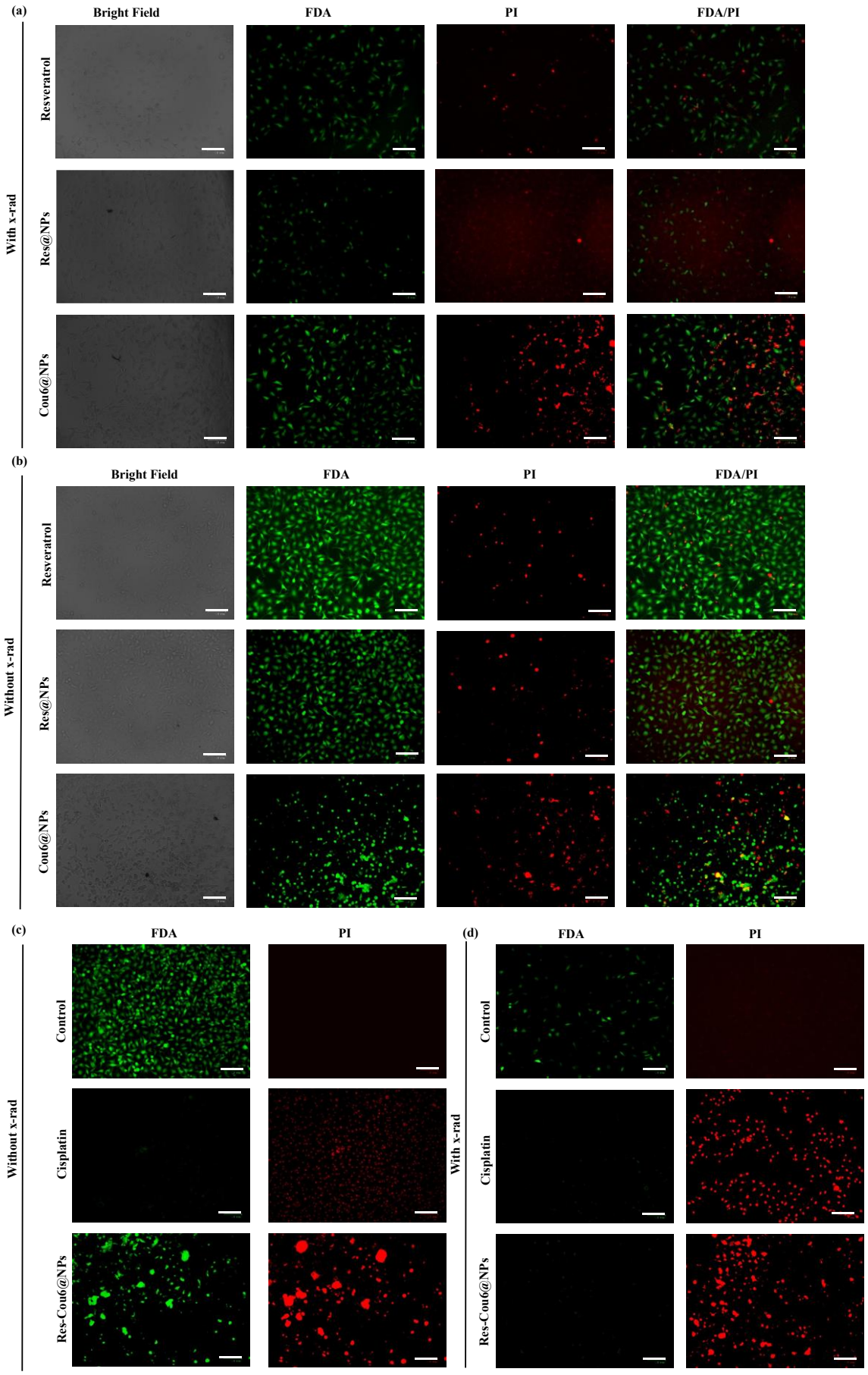

(a)
Bright Field
FDA
PI
FDA/PI
With x-rad
Resveratrol
Res@NPs
Cou6@NPs
(b)
Bright Field
FDA
PI
FDA/PI
Without x-rad
Resveratrol
Res@NPs
Cou6@NPs
(c)
FDA
PI
Without x-rad
Control
Cisplatin
Res-Cou6@NPs
(d)
FDA
PI
With x-rad
Control
Cisplatin
Res-Cou6@NPs

Fig. S12. (a) Live/Dead assay of internal control groups, Resveratrol, Res@NPs, and Cou6@NPs with radiation (scale bar: 100 µm) (b) Live/Dead assay of internal control groups, Resveratrol, Res@NPs, and Cou6@NPs without radiation (scale bar: 100 µm) (c, d) FDA/PI separate images of control, Cisplatin, and Res-Cou6@NPs with and without radiation (scale bar: 100 µm).

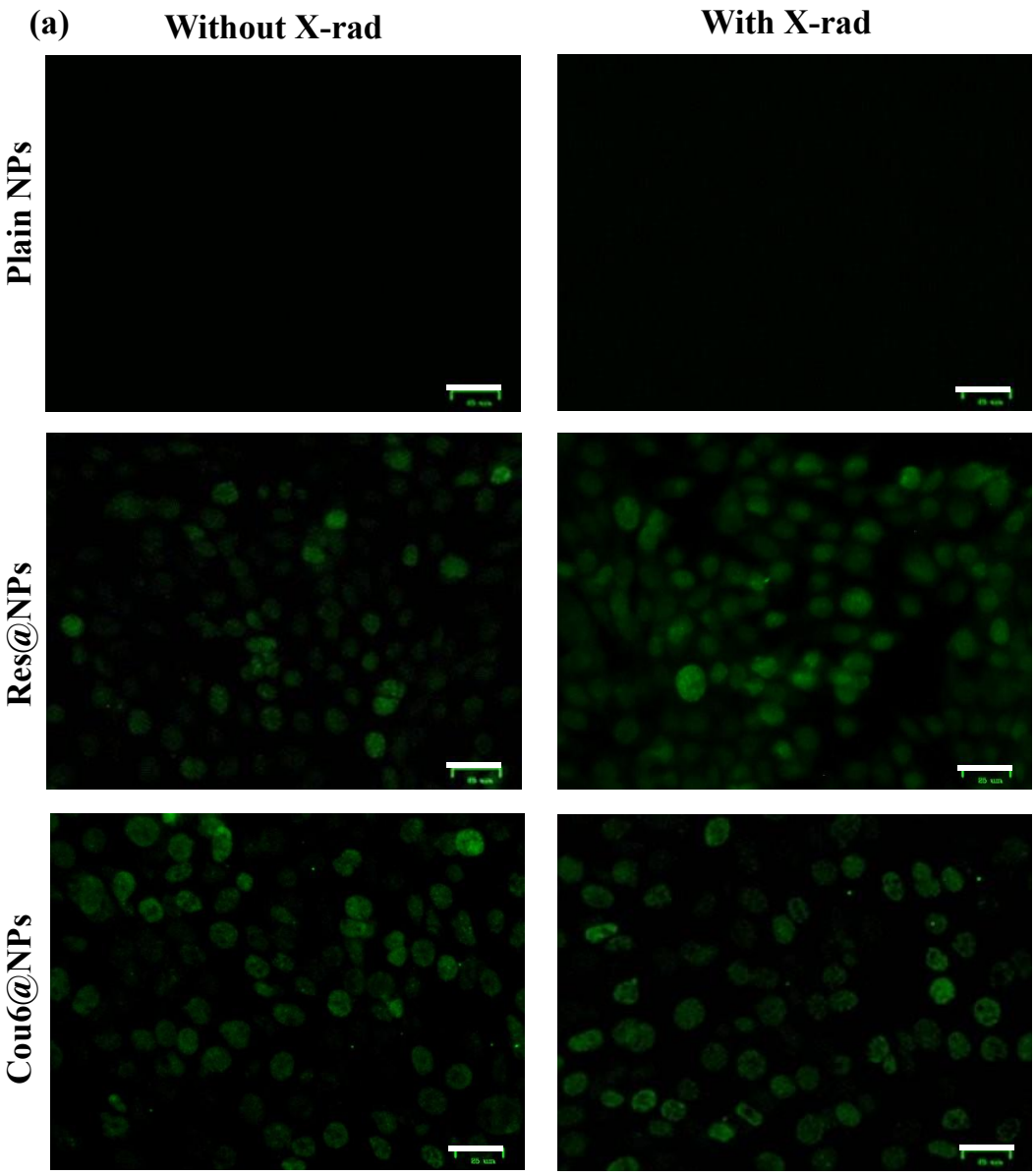


Fig. S13. (a) γ-H2AX assay of internal control groups, Plain NPs, Res@NPs, and Cou6@NPs without and with radiation (scale bar: 25 µm).