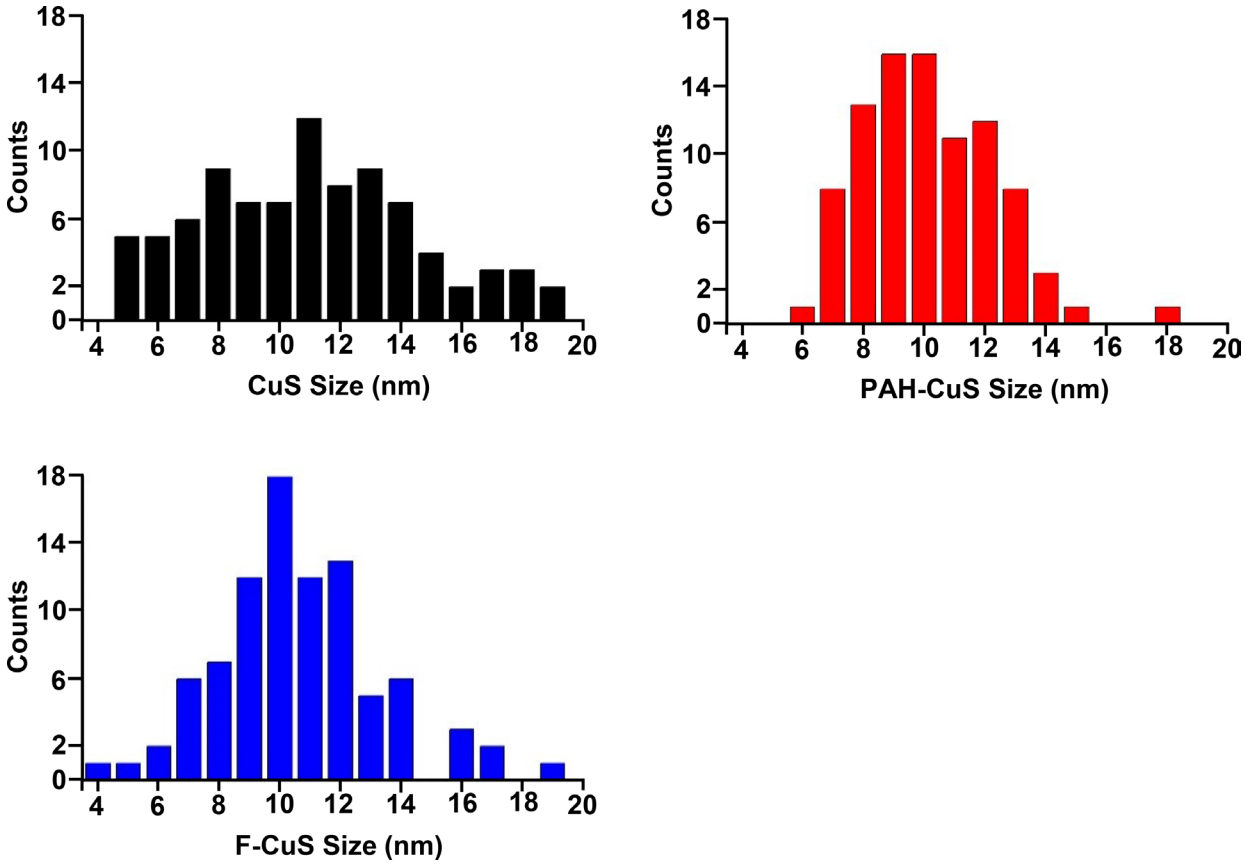
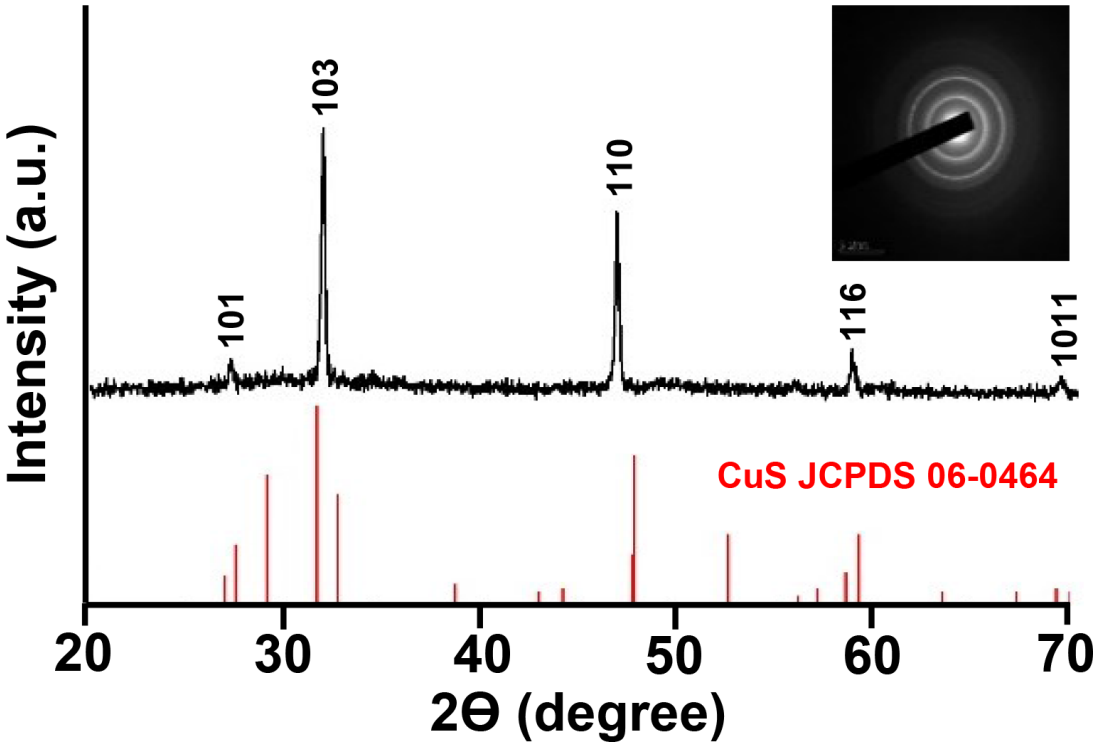
# Fucoidan-coated CuS nanoparticles for chemo- and photothermal therapy against cancer

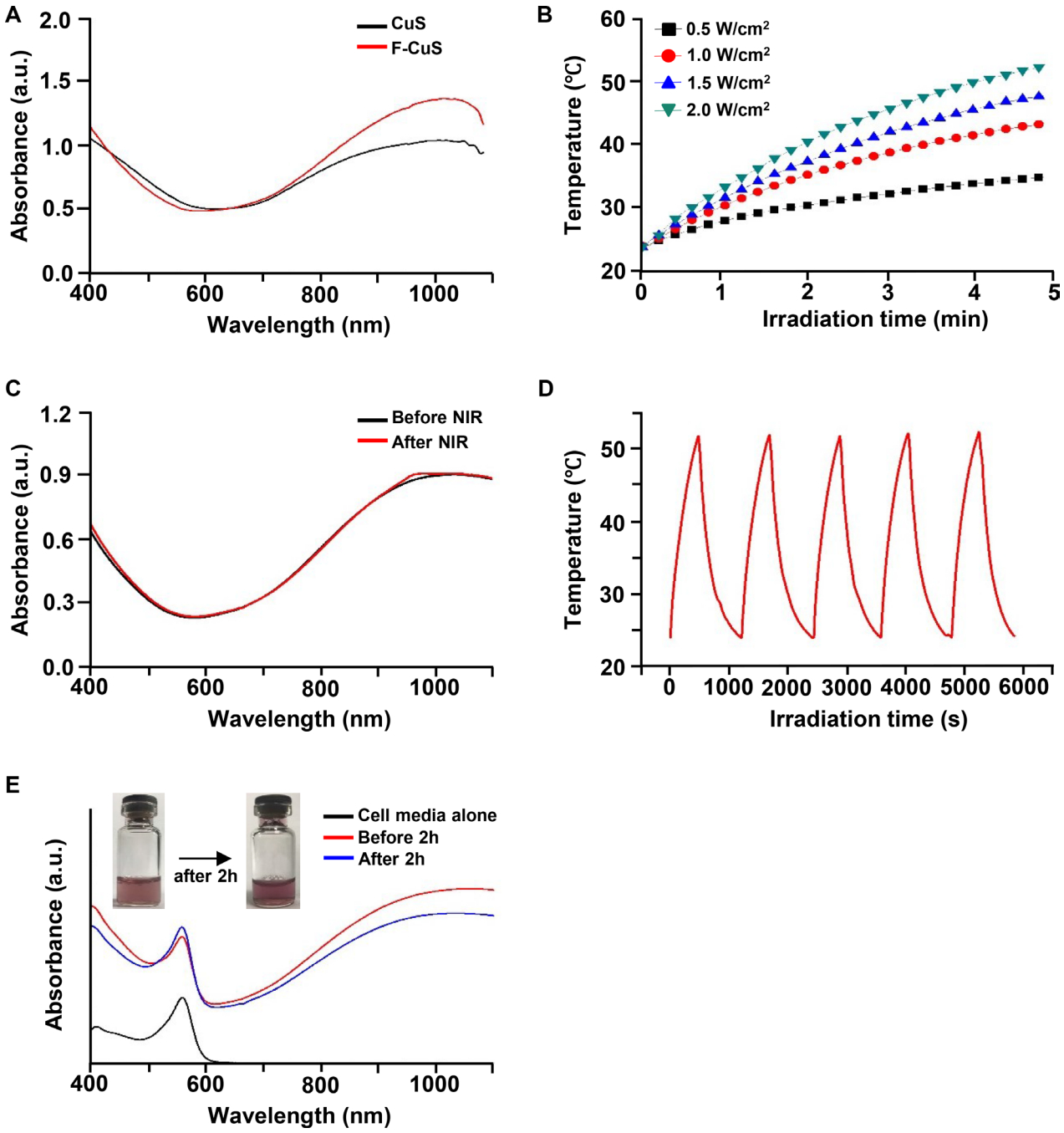
## SUPPLEMENTARY MATERIALS



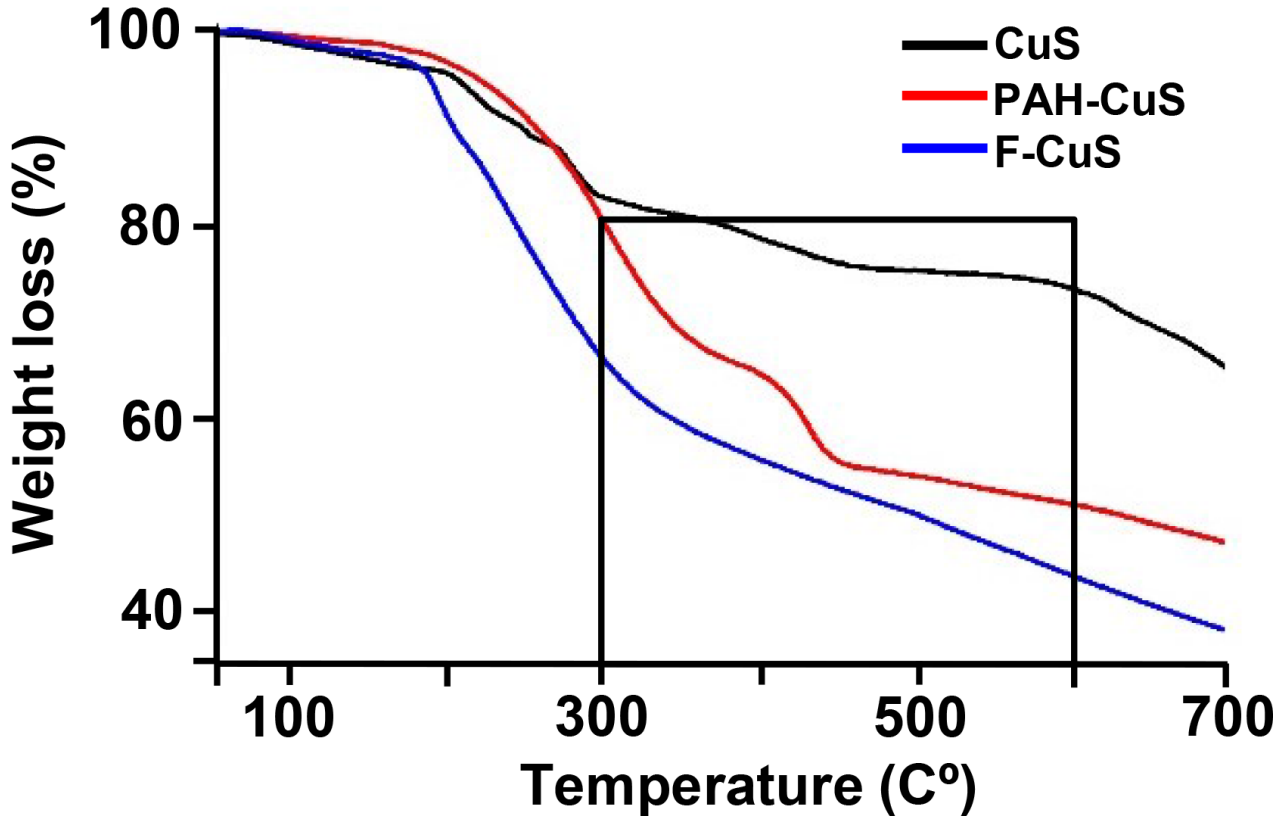

**Supplementary Figure 1: TEM corresponding size distribution histogram of each coated nanoparticles: CuS (black), PAH-CuS (red), F-CuS (blue).**



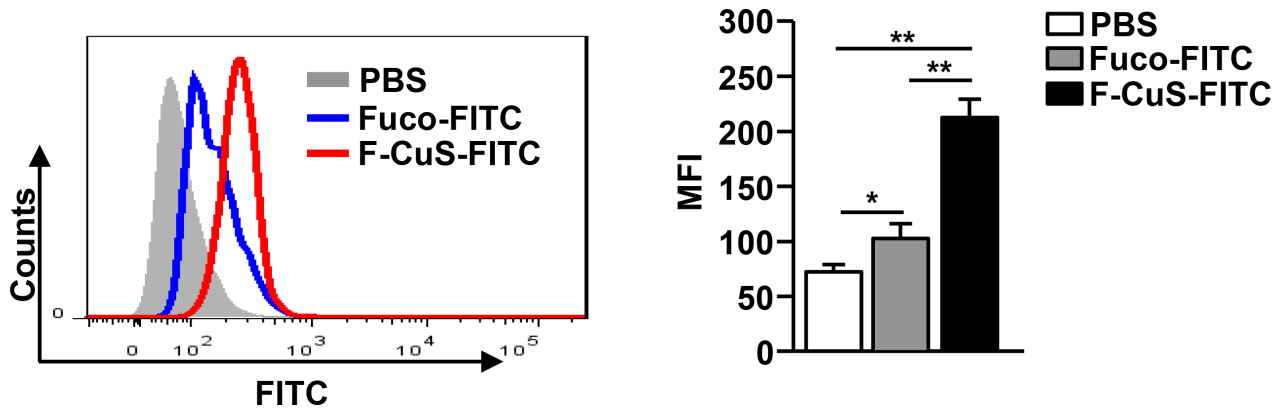

**Supplementary Figure 2: XRD pattern and ED pattern(inset) of CuS with the standard JCPDS card 06-0464 of CuS.**



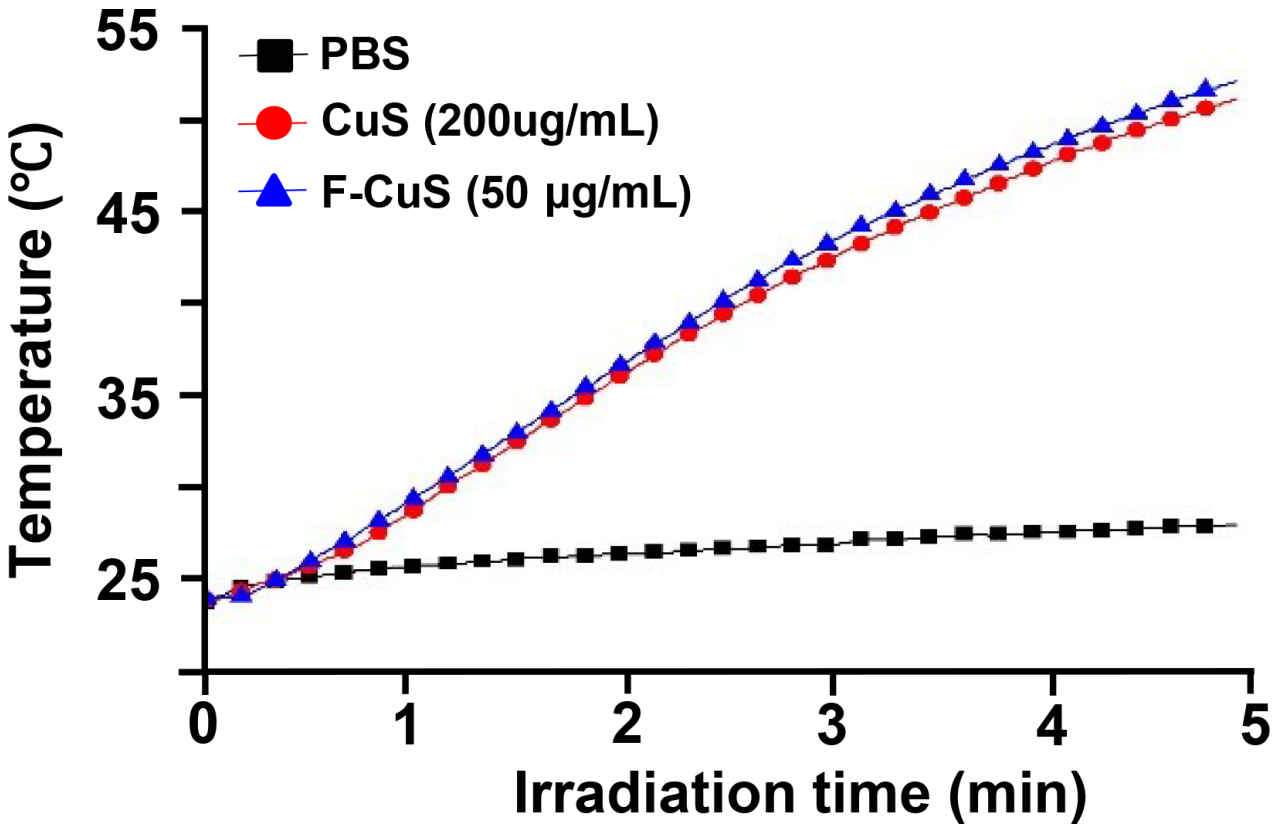

**Supplementary Figure 3:** (**A**) UV-vis absorption of CuS (black) and F-CuS (red). (**B**) Photothermal heating curves of F-CuS (50 µg/ml) dissolved in water, irradiated for 5 min with an 808 nm laser with indicated power density. (**C**) UV-vis absorption of F-CuS before (black) and after (red) NIR-irradiation. (**D**) Photothermal heating curves of F-CuS (50 µg/mL) during the successful 5 cycles of the laser ON/OFF mode (808 nm laser, 2W/cm$^2$). (**E**) UV-vis absorption of F-CuS dissoveled in cell media before and after 2 h.



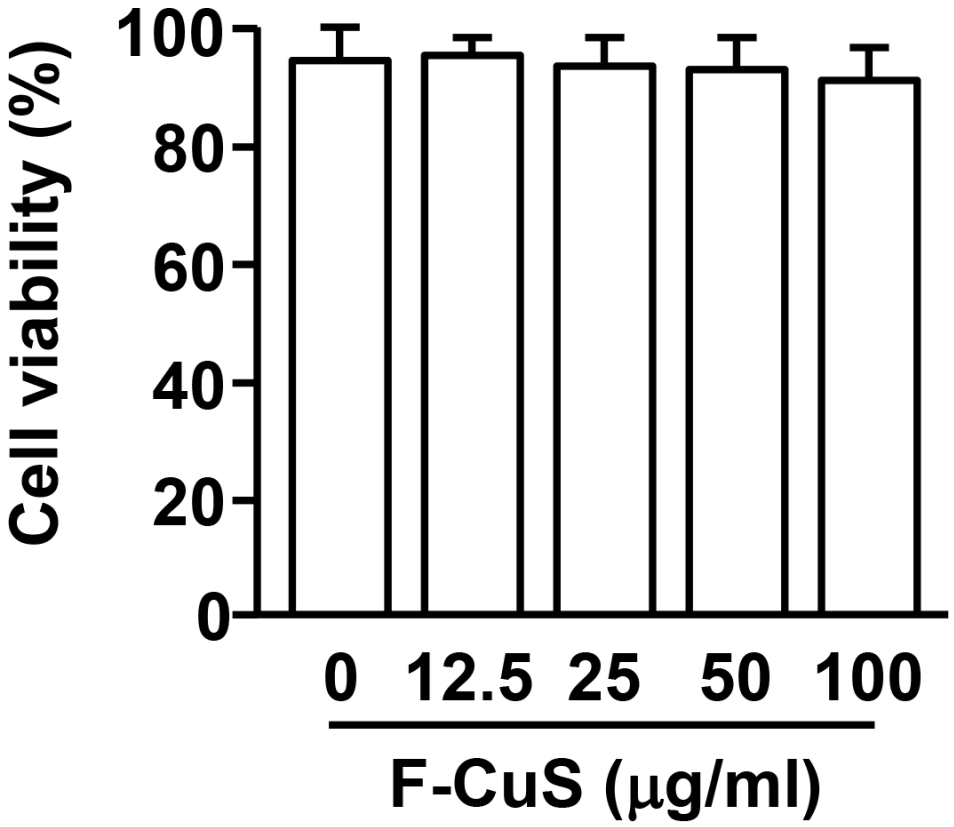

**Supplementary Figure 4: TGA curves of each coated nanoparticles: CuS (black), PAH-CuS (red), F-CuS (blue).**



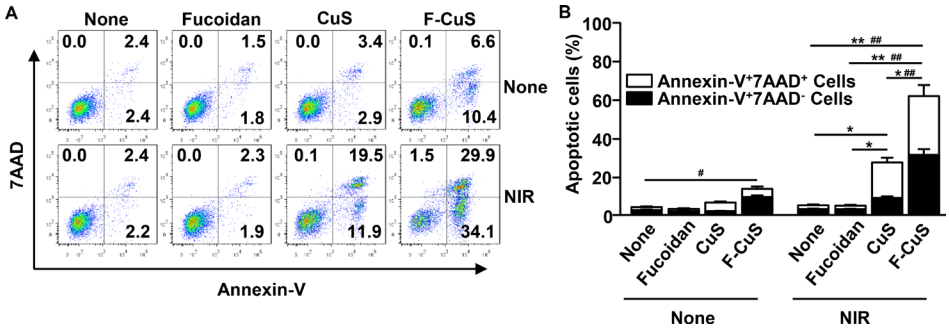

**Supplementary Figure 5: Intracellular uptake of fucoidan in HeLa cells by F-CuS.** HeLa cells ($2 \times 10^5$) were incubated with fucoidan-FITC (Fuco-FITC) and F-CuS-FITC for 2 h. The intracellular uptake of fucoidan was analyzed on a flow cytometry (left panel). Mean fluorescence intensity (MFI) of FITC positive cells was shown (right panel), $^{*}p < 0.05$, $^{**}p < 0.01$. Data are representative of or the average of analyses of 6 independent samples (2 samples per experiment, 3 independent experiments).



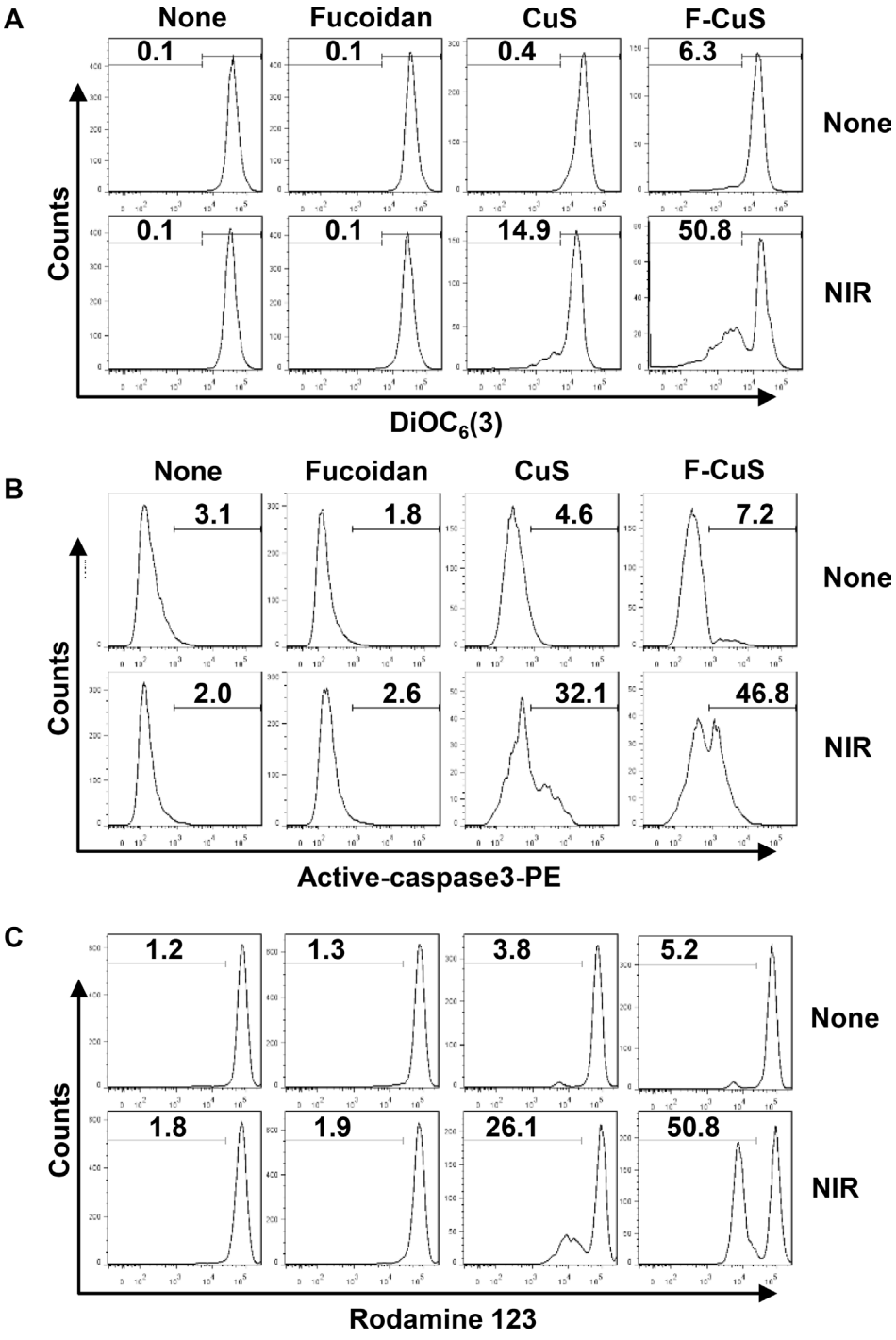

**Supplementary Figure 6: Photothermal heating curves of CuS (red) and F-CuS (blue) with different concentration dissolved in PBS irradiated using 808 nm laser with power density of 2 W/cm² for 5 min.**



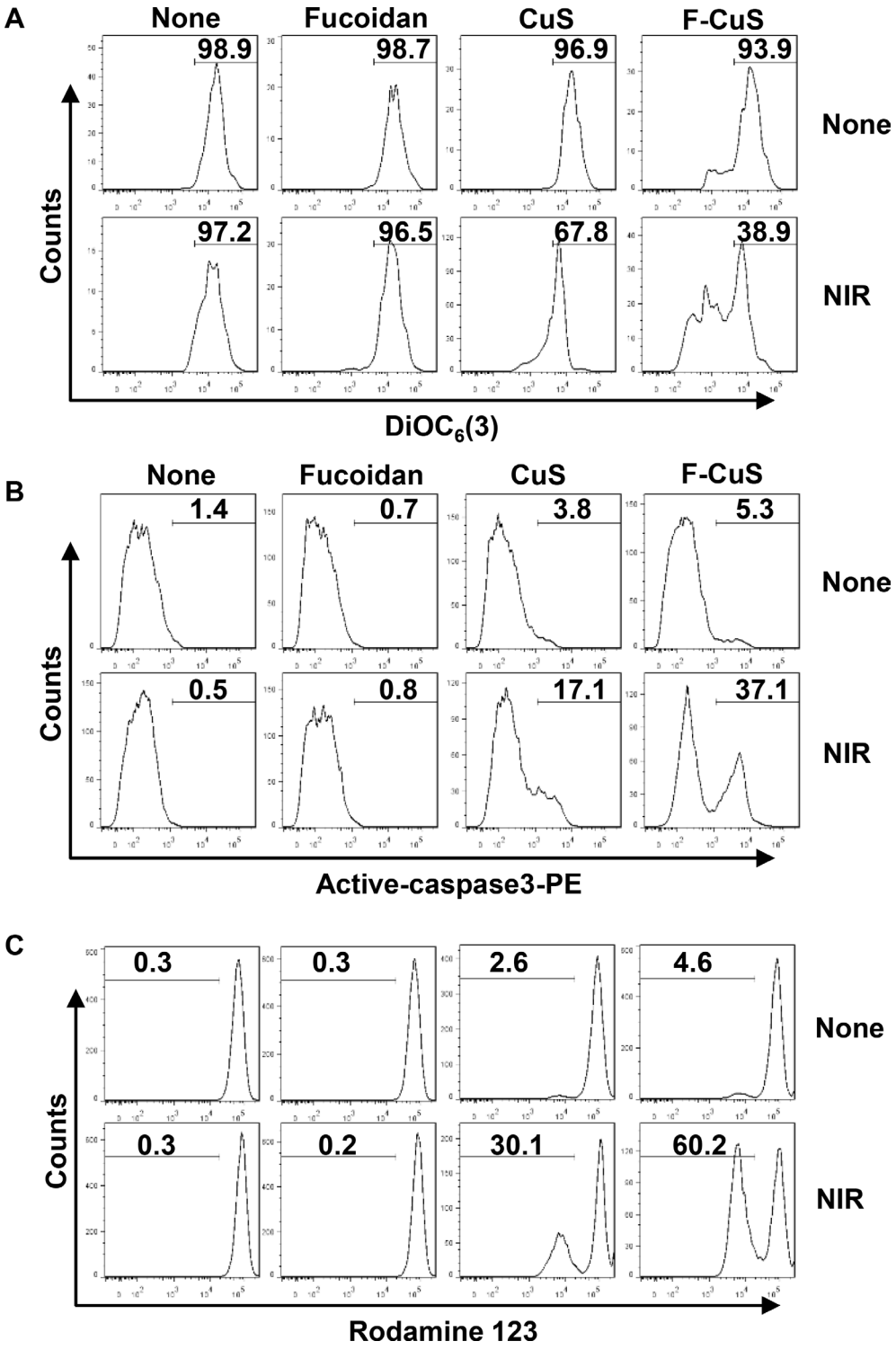

**Supplementary Figure 7: RAW cells were treated with an indicated concentration of F-CuS for 24 h.** Cell viability was analyzed by MTT assay.



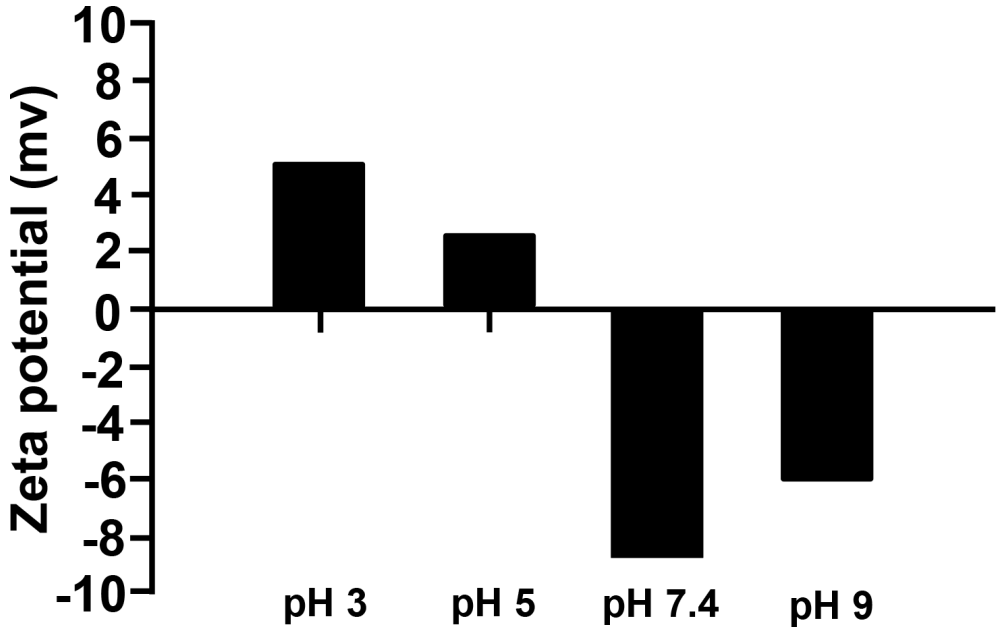

**Supplementary Figure 8: Apoptotic effect of F-CuS with laser irradiation against A549 cells.** A549 cells ($2 \times 10^5$) were incubated with fucoidan, CuS and F-CuS-FITC and irradiated with 808 nm laser for 5 min. (**A**) Apoptosis of A549 cells were analyzed by annexin-V and 7AAD staining on a flow cytometry after 24 h of laser irradiation (left panel). (**B**) Mean percentage of early apoptotic cells (Annexin-V+7AAD- Cells) and late apoptotic/necrotic cells (Annexin-V+7AAD+ Cells) were shown (right panel). $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ early apoptotic cells, $^{*}p < 0.05$, $^{**}p < 0.01$ late apoptotic/necrotic cells. Data are representative of or the average of analyses of 6 independent samples (2 samples per experiment, 3 independent experiments).



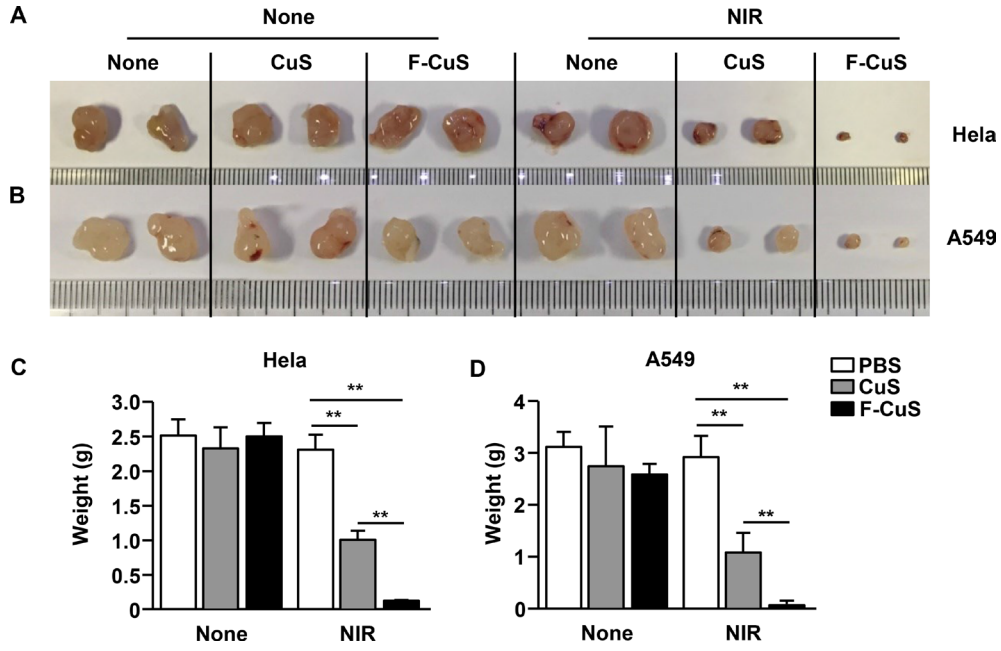

**Supplementary Figure 9: Mitochondria permeability and activation of caspase 3 by F-CuS.** HeLa cells ($2 \times 10^5$) were incubated with fucoidan, CuS and F-CuS-FITC and irradiated with 808 nm laser for 5 min. (**A**) Mitochondria permeability was measured by $DiOC^6(3)$ reduction on a flow cytometry. (**B**) Activation of caspase 3 was analyzed by flow cytometry. (**C**) The mictochondrial membrane potential were measured by rodamine 123 staining. Data are representative of analyses of 6 independent samples (2 samples per experiment, 3 independent experiments).



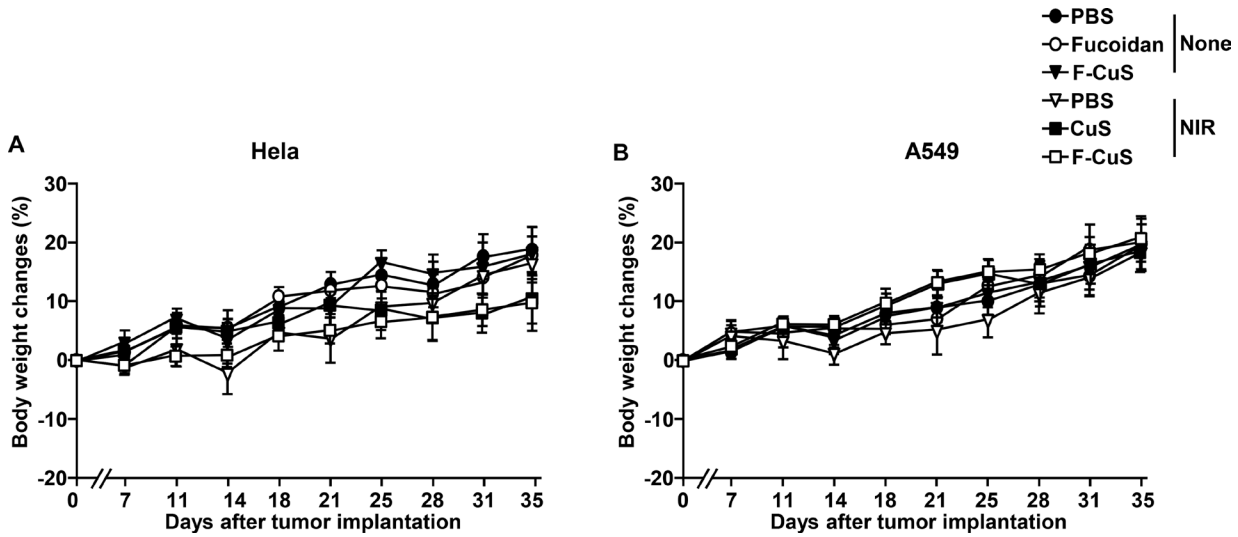

**Supplementary Figure 10: Mitochondria permeability and activation of caspase 3 by F-CuS.** A549 cells ($2 \times 10^5$) were incubated with fucoidan, CuS and F-CuS-FITC and irradiated with 808 nm laser for 5 min. (**A**) Mitochondria permeability was measured by $DiOC^6(3)$ reduction on a flow cytometry. (**B**) Activation of caspase 3 was analyzed by flow cytometry. (**C**) The mictochondrial membrane potential were measured by rodamine 123 staining. Data are representative of analyses of 6 independent samples (2 samples per experiment, 3 independent experiments).



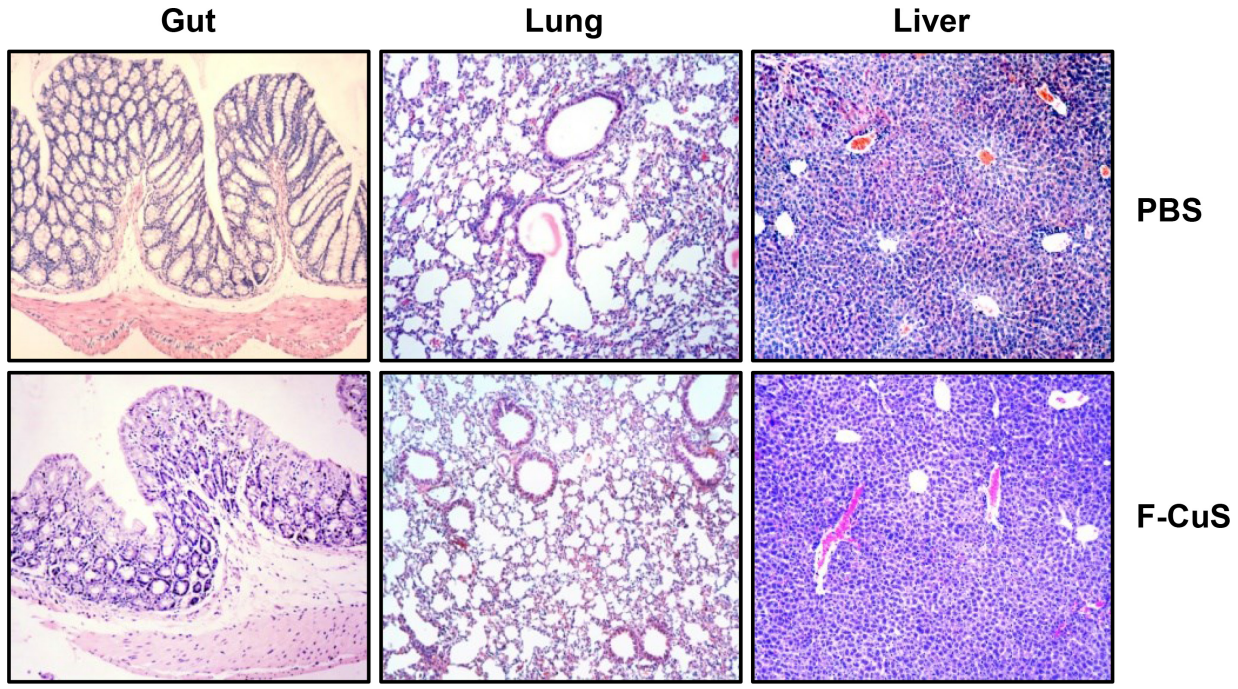

**Supplementary Figure 11: Release feature of fucoidan from NPs.** Zeta potential value of F-CuS changed from -8.23 (at pH 7.4) to 5.05 (at pH 3), 2.64 (at pH 5), and -5.92 (at pH 9) after 2 h exposure.



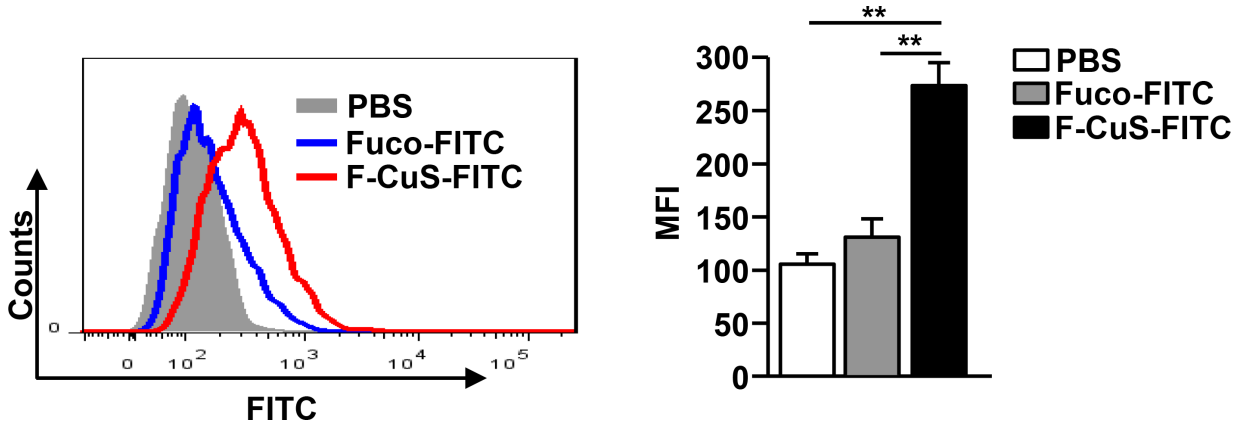

**Supplementary Figure 12: Therapeutic effect of F-CuS with laser irradiation against HeLa and A549 tumor.** Nude mice were injected s.c. with $5 \times 10^6$ HeLa cells and $5 \times 10^6$ A549 cells, respectively. Once tumors were measured ~5.0 mm after 14 days, the mice were treated i.t. with 40 μg/kg fucoidan, 10 mg/kg CuS and 2.5 mg/kg F-CuS. Two hours after treatment, the mice were irradiated by 808 nm laser at 2 W/cm$^2$ for 5 min. (**A**) HeLa and (**B**) A549 tumor mass were shown on day 35 of tumor cell injection. (**C**) Hela and (**D**) A549 tumor weight were shown on day 35. Data are representative of analyses of 6 independent samples (2 mice per experiment, total 3 independent experiments).



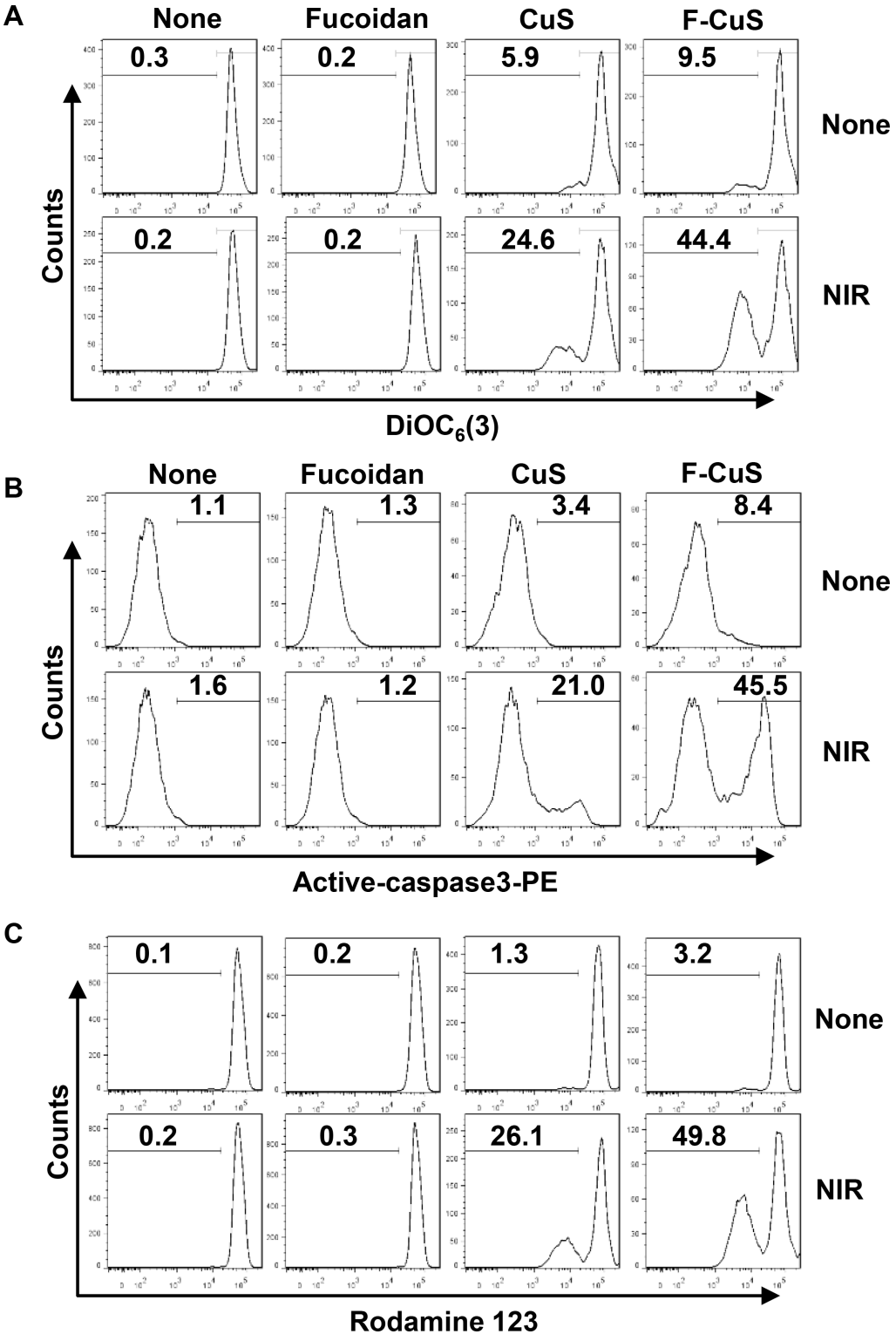

**Supplementary Figure 13: Changes of body weight during treatment of HeLa and A549 tumor by F-CuS.** Nude mice were injected s.c. with $5 \times 10^6$ HeLa cells and $5 \times 10^6$ A549 cells, respectively. Once tumors were measured ~5.0 mm after 14 days, the mice were treated i.t. with 40 μg/kg fucoidan, 10 mg/kg CuS and 2.5 mg/kg F-CuS. Two hours after treatment, the mice were irradiated by 808 nm laser at 2 W/cm$^2$ for 5 min. The body weight of (**A**) HeLa and (**B**) A549 tumor implanted mice were measured during treatment of the tumor. Data are a average of analyses of 6 independent samples (2 mice per experiment, total 3 independent experiments).



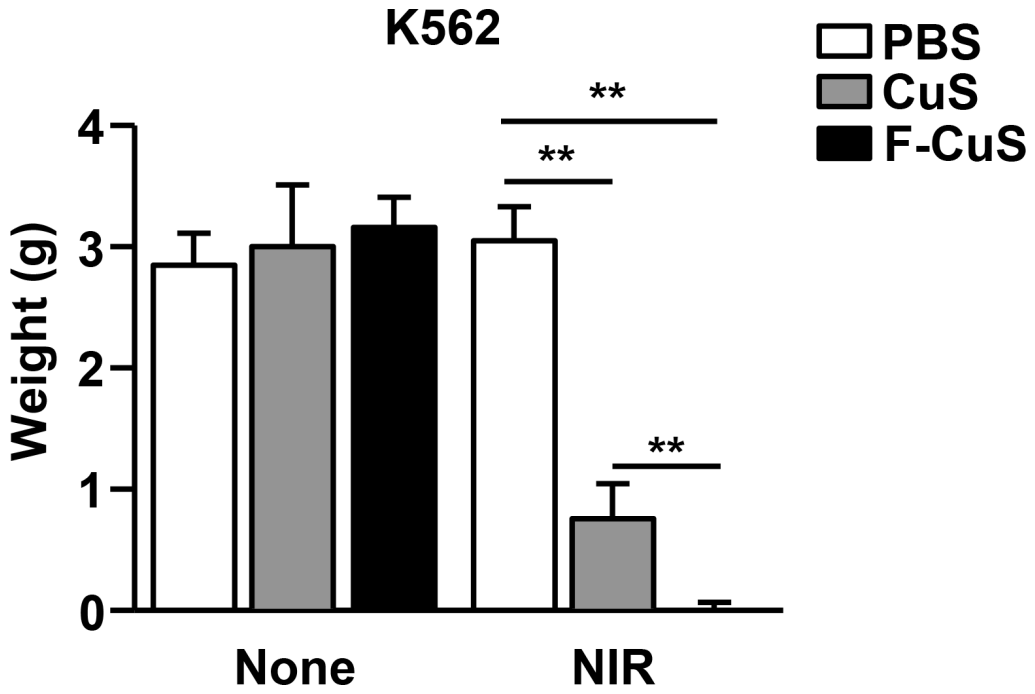

**Supplementary Figure 14: Histological analysis of peripheral tissue damage.** HeLa tumor-bearing nude mice were treated i.t. with F-CuS and irradiated by 808 nm laser at 2 W/cm$^2$ for 5 min. Twenty-one days after F-CuS treatment and laser irradiation, Gut, lung and liver were harvested and stained with haematoxylin and eosin (H&E). Data are representative of analyses of 6 independent samples (2 mice per experiment, total 3 independent experiments).



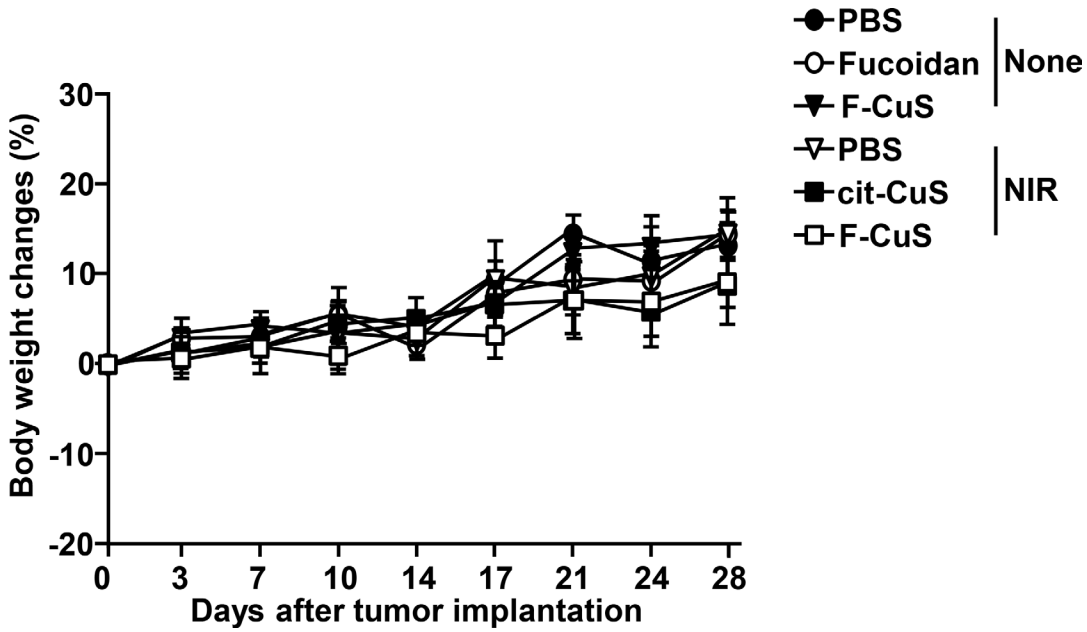

**Supplementary Figure 15: Intracellular uptake of fucoidan in K562 cells by F-CuS.** K562 cells ($2 \times 10^5$) were incubated with fucoidan-FITC (Fuco-FITC) and F-CuS-FITC for 2 h. The intracellular uptake of fucoidan was analyzed on a flow cytometry (left panel). Mean fluorescence intensity (MFI) of FITC positive cells was shown (right panel), $^{**}p < 0.01$. Data are representative of or the average of analyses of 6 independent samples (2 samples per experiment, 3 independent experiments).




**Supplementary Figure 16: Mitochondria permeability and activation of caspase 3 by F-CuS.** K562 cells ($2 \times 10^5$) were incubated with fucoidan, CuS and F-CuS-FITC and irradiated with 808 nm laser for 5 min. (**A**) Mitochondria permeability was measured by $DiOC^6(3)$ reduction on a flow cytometry. (**B**) Activation of caspase 3 was analyzed by flow cytometry. (**C**) The mictochondrial membrane potential were measured by rodamine 123 staining. Data are representative of analyses of 6 independent samples (2 samples per experiment, 3 independent experiments).




**Supplementary Figure 17: Nude mice were injected s.c.** with $5 \times 10^6$ K562 cells and treated F-CuS with or without laser irradiation as described in Figure 5C. Weight of K562 tumor mass was shown on day 35. Data are representative of analyses of 6 independent samples (2 mice per experiment, total 3 independent experiments).




**Supplementary Figure 18: Changes of body weight during treatment of K562 tumor by F-CuS.** Nude mice were injected s.c. with $5 \times 10^6$ K562 cells. Fourteen days after tumor injection, the mice were treated i.t. with 40 μg/kg fucoidan, 10 mg/kg CuS and 2.5 mg/kg F-CuS. Two hours after treatment, the mice were irradiated by 808 nm laser at 2.5 W/cm$^2$ for 5 min. The body weight of the mice were monitored during treatment of the tumor. Data are a average of analyses of 6 independent samples (2 mice per experiment, total 3 independent experiments).