

Supporting Information for

Delivery of an Immunogenic Cell Death-Inducing Copper Complex to Cancer Stem Cells Using Polymeric Nanoparticles

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Table of Content

Fig. S1	UV-Vis spectrum of 1 (25 μM) in DMSO over the course of 24 h at 37 $^{\circ}\text{C}$.
Fig. S2	UV-Vis spectrum of 1 (25 μM) in DMF over the course of 24 h at 37 $^{\circ}\text{C}$.
Fig. S3	UV-Vis spectrum of 1 (25 μM) in PBS:DMSO (200:1) with 10% FBS over the course of 24 h at 37 $^{\circ}\text{C}$.
Fig. S4	UV-Vis spectrum of 1 (25 μM) in PBS:DMSO (200:1) in the presence of ascorbic acid (250 μM) over the course of 24 h at 37 $^{\circ}\text{C}$.
Fig. S5	UV-Vis spectrum of 1 (25 μM) in PBS:DMSO (200:1) in the presence of glutathione (250 μM) over the course of 24 h at 37 $^{\circ}\text{C}$.
Fig. S6	UV-Vis spectrum of 1 (50 μM) in the presence of ascorbic acid (500 μM) and bathocuproine disulfonate, BCS (100 μM) in PBS:DMSO (200:1) over the course of 24 h at 37 $^{\circ}\text{C}$.
Fig. S7	UV-Vis spectrum of 1 (50 μM) in the presence of glutathione (500 μM) and bathocuproine disulfonate, BCS (100 μM) in PBS:DMSO (200:1) over the course of 24 h at 37 $^{\circ}\text{C}$.
Fig. S8	ESI mass spectra (positive mode) of 1 (500 μM) in H_2O :DMSO (10:1) (A), and in the presence of ascorbic acid (5 mM) (B) or in the presence of glutathione (5 mM) (C) after incubation for 24 h at 37 $^{\circ}\text{C}$.
Fig. S9	Dynamic light scattering size distribution of 1 NP ¹⁰ suspended in water. Size refers to diameter of nanoparticles in nm.
Fig. S10	Dynamic light scattering size distribution of empty PEG-PLGA nanoparticles suspended in water. Size refers to diameter of nanoparticles in nm.

- Fig. S11** Variation in **1 NP¹⁰** diameter upon incubation in water, PBS with 10% FBS, and mammary epithelial growth medium (MEGM) over the course of 72 h at 37 °C.
- Fig. S12** Copper content in HMLER and HMLER-shEcad cells treated with **1 NP¹⁰** (110 nM for 24 h) or **1** (110 nM for 24 h) at 37 °C.
- Fig. S13** Copper content in HMLER and HMLER-shEcad cells treated with **1 NP¹⁰** (110 nM for 4 h) at 4 °C or 37 °C.
- Fig. S14** Copper content in HMLER-shEcad cells treated with **1 NP¹⁰** only (16 nM for 24 h), and upon pre-incubation with ammonium chloride (50 mM for 2 h) or chloroquine (100 μM for 2 h) at 37 °C. Error bars represent standard deviations and Student *t test*, * = $p < 0.05$.
- Fig. S15** The amount of copper released from **1 NP¹⁰** upon incubation in PBS (pH 7.4) or sodium acetate buffer (pH 5.2) over the course of 72 h at 37 °C.
- Fig. S16** Representative dose-response curves for the treatment of HMLER and HMLER-shEcad cells with **1 NP¹⁰**.
- Fig. S17** Representative dose-response curves for the treatment of HMLER and HMLER-shEcad cells with empty PEG-PLGA nanoparticles.
- Fig. S18** Representative bright-field images ($\times 10$) of HMLER-shEcad mammospheres in the absence and presence of salinomycin at its respective IC₂₀ values for 5 days.
- Fig. S19** Representative dose-response curve for the treatment of HMLER-shEcad mammospheres with **1 NP¹⁰**.
- Fig. S20** Representative dose-response curve for the treatment of HMLER-shEcad mammospheres with empty PEG-PLGA nanoparticles.
- Fig. S21** Representative dose-response curves for the treatment of HMLER-shEcad cells with **1 NP¹⁰** after 72 h incubation in the presence of salubrinal (10 μM).
- Fig. S22** Immunoblotting analysis of proteins related to the unfolded protein response (UPR). Protein expression in HMLER-shEcad cells following treatment with **1 NP¹⁰** (40–191 nM) for (A) 4 h or (B) 24 h.
- Fig. S23** Immunoblotting analysis of proteins related to apoptosis. Protein expression in HMLER-shEcad cells following treatment with **1 NP¹⁰** (37–146 nM for 72 h).
- Fig. S24** Representative dose-response curves for the treatment of HMLER-shEcad cells with **1 NP¹⁰** after 72 h incubation in the presence of z-VAD-FMK (5 μM).
- Fig. S25** Representative histograms displaying the green fluorescence emitted by anti-CRT Alexa Fluor 488 nm antibody-stained HMLER-shEcad cells untreated (red), and treated with **1** (0.2 μM for 24 h) (blue).
- Fig. S26** Representative histograms displaying the green fluorescence emitted by anti-CRT Alexa Fluor 488 nm antibody-stained HMLER-shEcad cells untreated (red), and treated with cisplatin (150 μM for 24 h) and thapsigargin (7 μM for 24 h) (blue).
- Fig. S27** Immunoblotting analysis of high mobility group box 1 (HMGB-1). Protein expression in HMLER-shEcad cells following treatment with **1 NP¹⁰** (95–764 nM for 24 h).

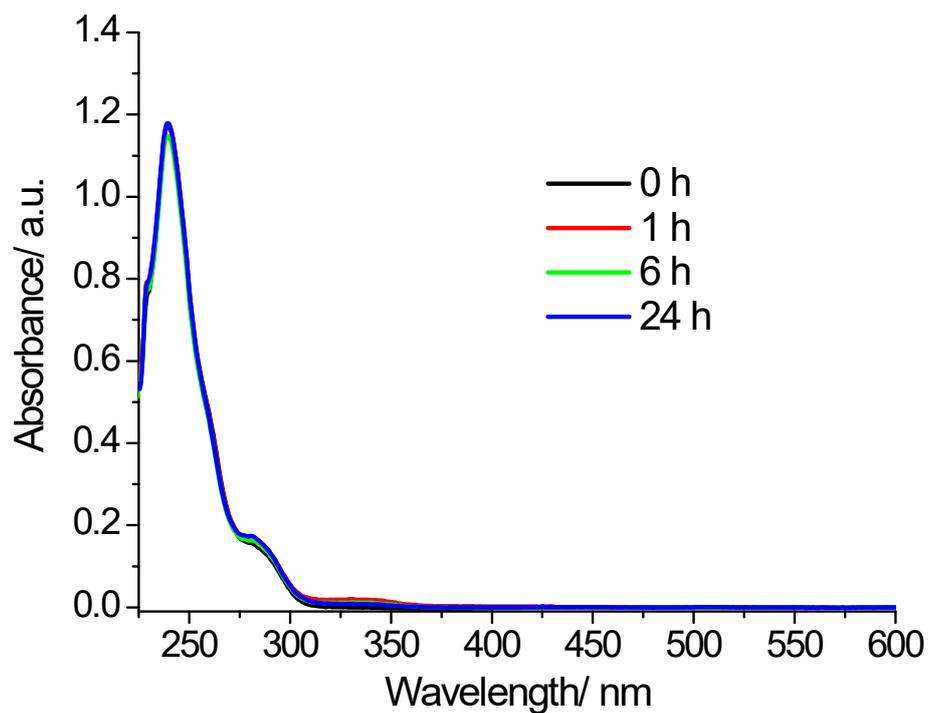


Fig. S1 UV-Vis spectrum of **1** (25 μM) in DMSO over the course of 24 h at 37 °C.

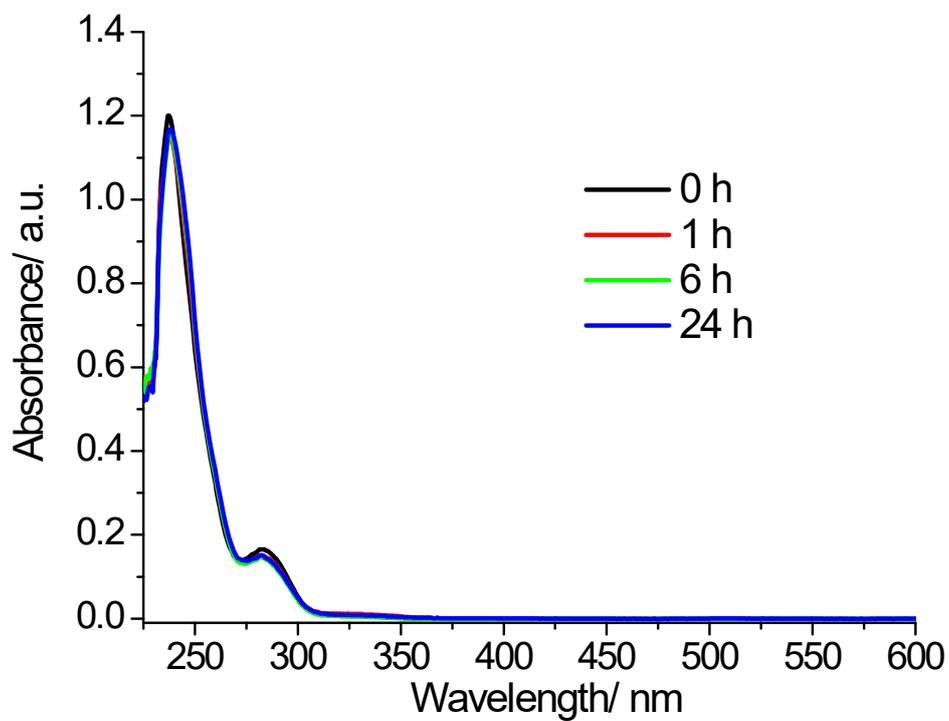


Fig. S2 UV-Vis spectrum of **1** (25 μM) in DMF over the course of 24 h at 37 °C.

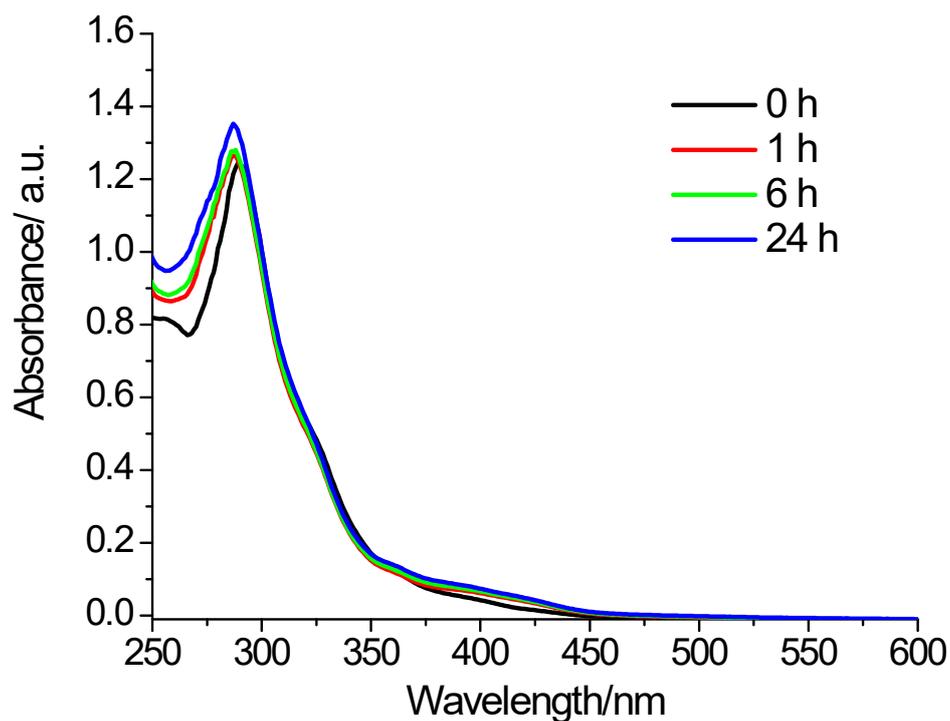


Fig. S3 UV-Vis spectrum of **1** (25 μM) in PBS:DMSO (200:1) with 10% FBS over the course of 24 h at 37 °C.

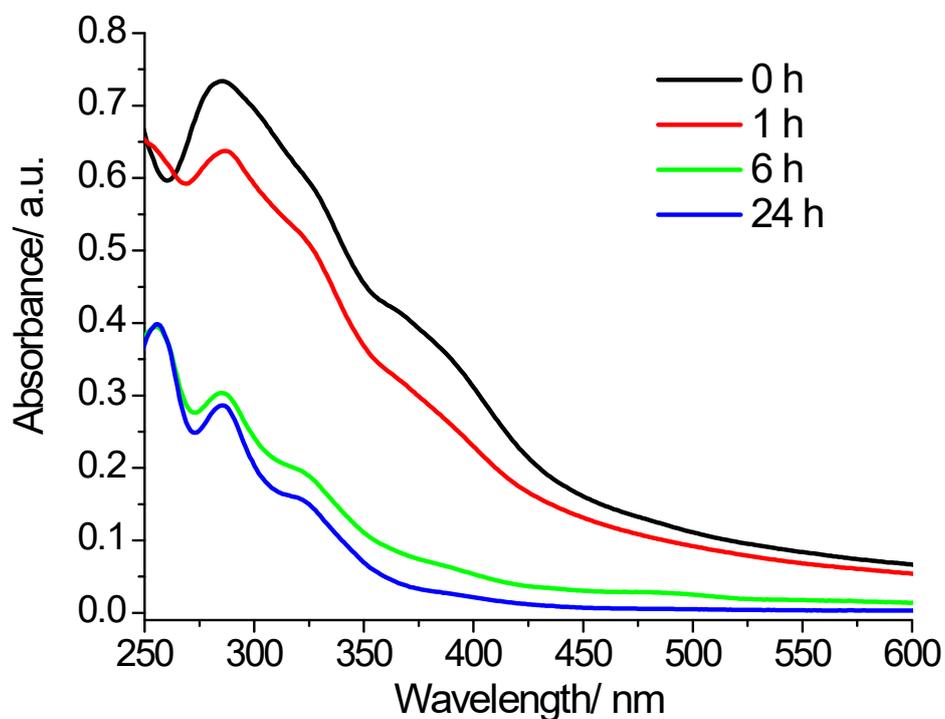


Fig. S4 UV-Vis spectrum of **1** (25 μM) in PBS:DMSO (200:1) in the presence of ascorbic acid (250 μM) over the course of 24 h at 37 °C.

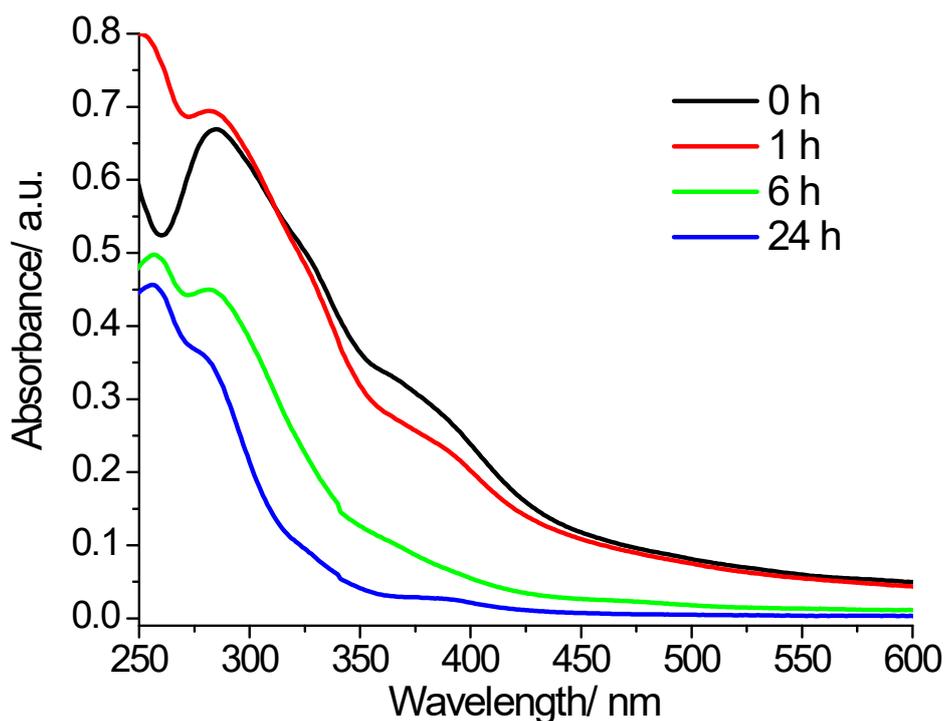


Fig. S5 UV-Vis spectrum of **1** (25 μM) in PBS:DMSO (200:1) in the presence of glutathione (250 μM) over the course of 24 h at 37 $^{\circ}\text{C}$.

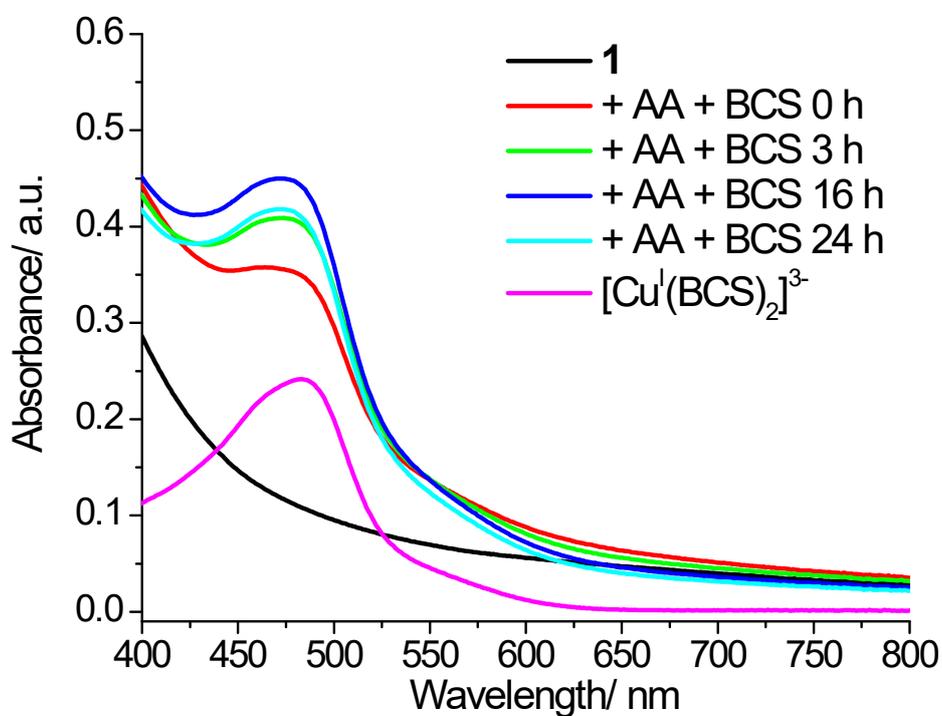


Fig. S6 UV-Vis spectrum of **1** (50 μM) in the presence of ascorbic acid (500 μM) and bathocuproine disulfonate, BCS (100 μM) in PBS:DMSO (200:1) over the course of 24 h at 37 $^{\circ}\text{C}$.

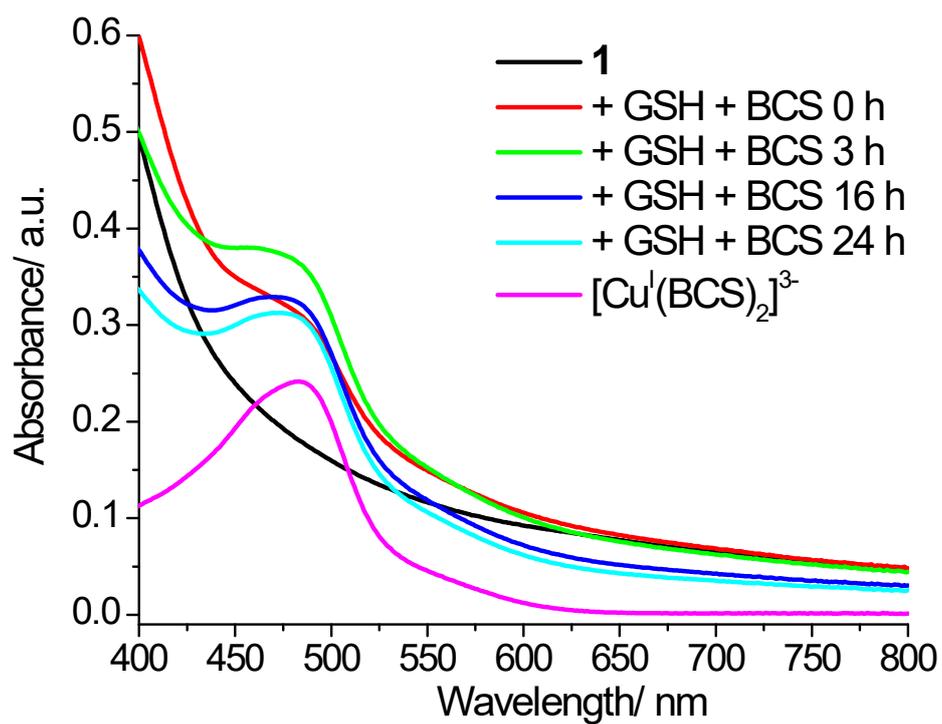


Fig. S7 UV-Vis spectrum of **1** (50 μM) in the presence of glutathione (500 μM) and bathocuproine disulfonate, BCS (100 μM) in PBS:DMSO (200:1) over the course of 24 h at 37 $^{\circ}\text{C}$.

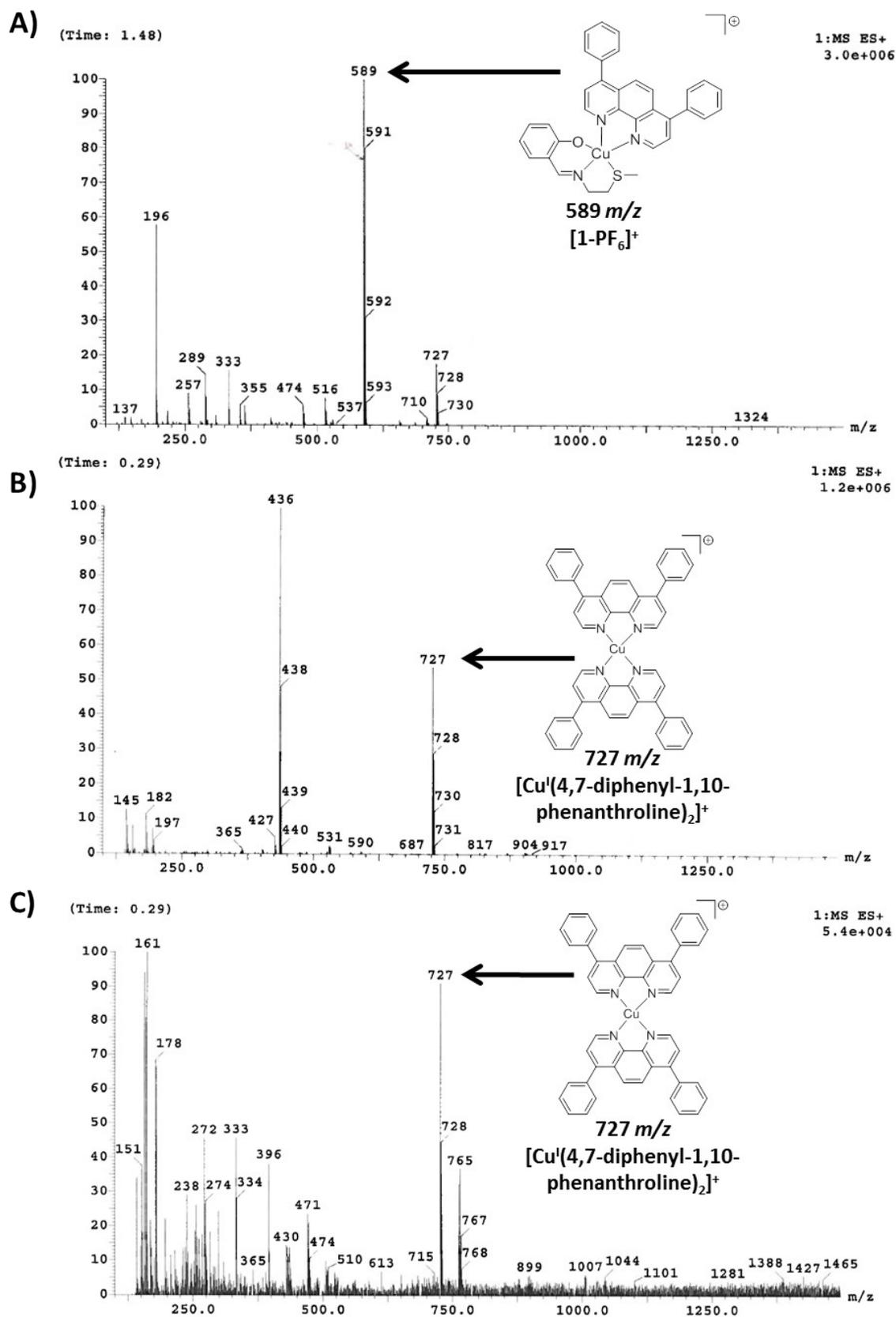


Fig. S8 ESI mass spectra (positive mode) of **1** (500 μ M) in $\text{H}_2\text{O}:\text{DMSO}$ (10:1) (A), and in the presence of ascorbic acid (5 mM) (B) or in the presence of glutathione (5 mM) (C) after incubation for 24 h at 37 $^\circ\text{C}$.

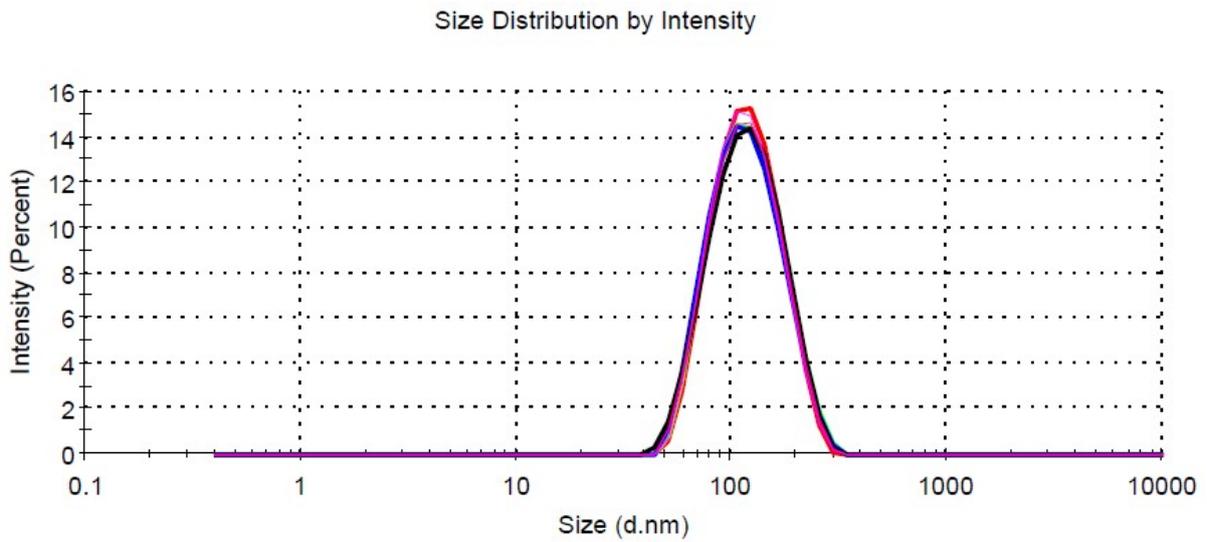


Fig. S9 Dynamic light scattering size distribution of 1 NP¹⁰ suspended in water. Size refers to diameter of nanoparticles in nm.

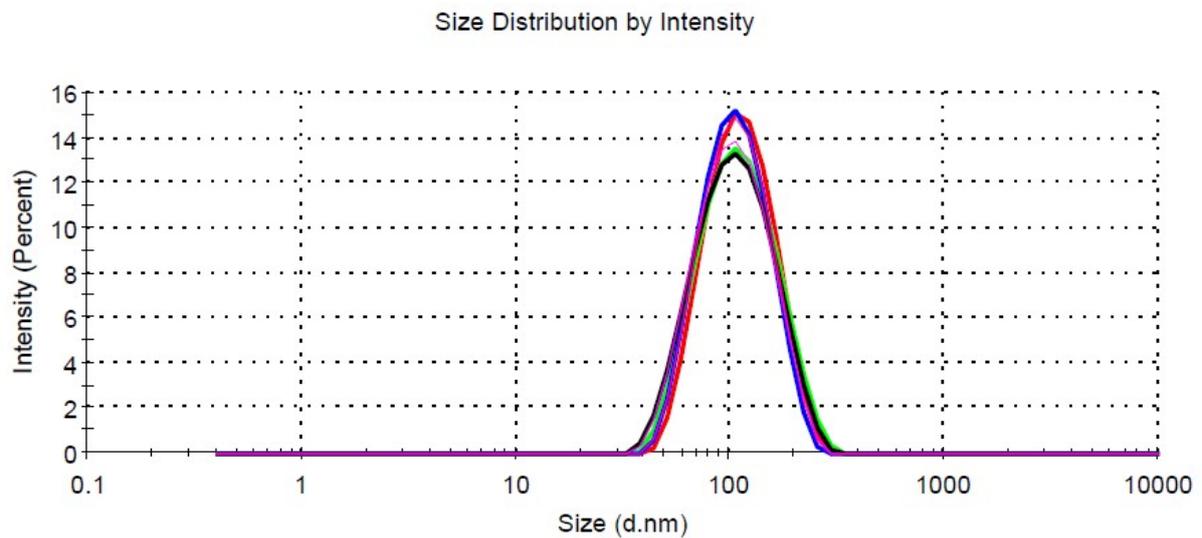


Fig. S10 Dynamic light scattering size distribution of empty PEG-PLGA nanoparticles suspended in water. Size refers to diameter of nanoparticles in nm.

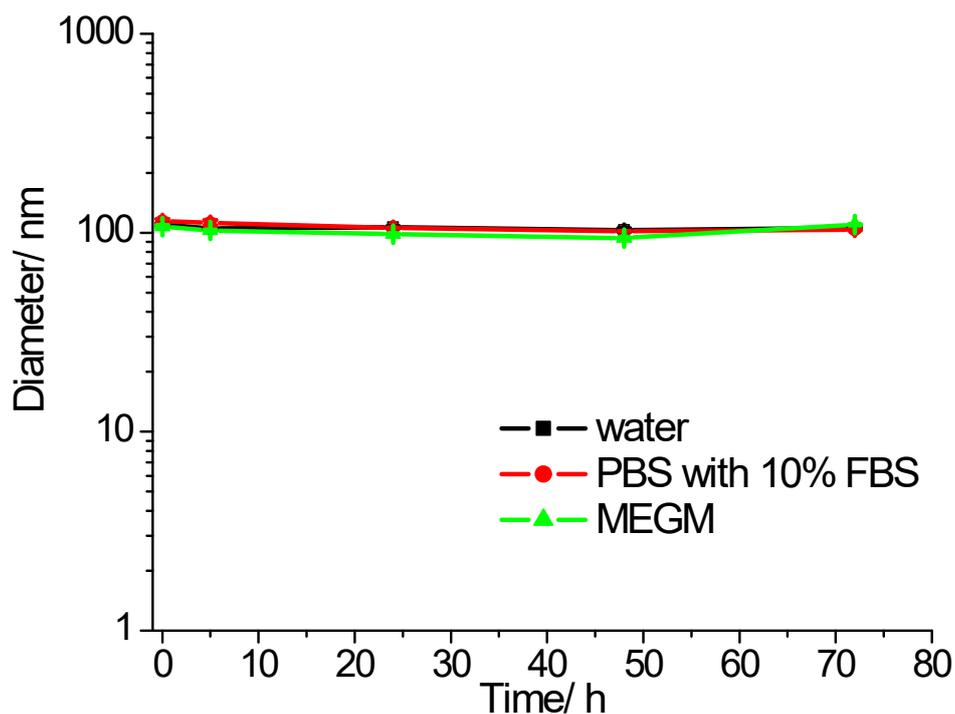


Fig. S11 Variation in **1 NP¹⁰** diameter upon incubation in water, PBS with 10% FBS, and mammary epithelial growth medium (MEGM) over the course of 72 h at 37 °C.

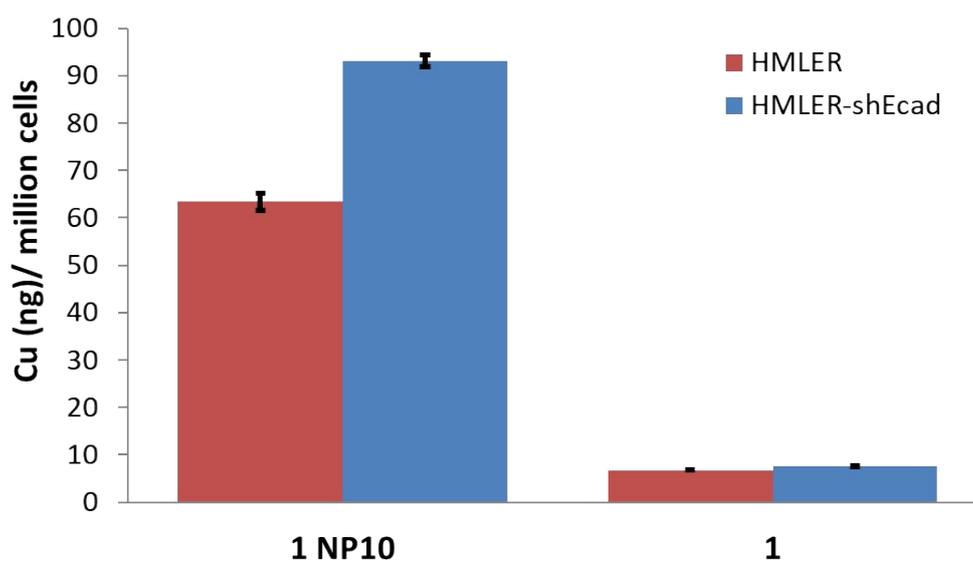


Fig. S12 Copper content in HMLER and HMLER-shEcad cells treated with **1 NP¹⁰** (110 nM for 24 h) or **1** (110 nM for 24 h) at 37 °C.

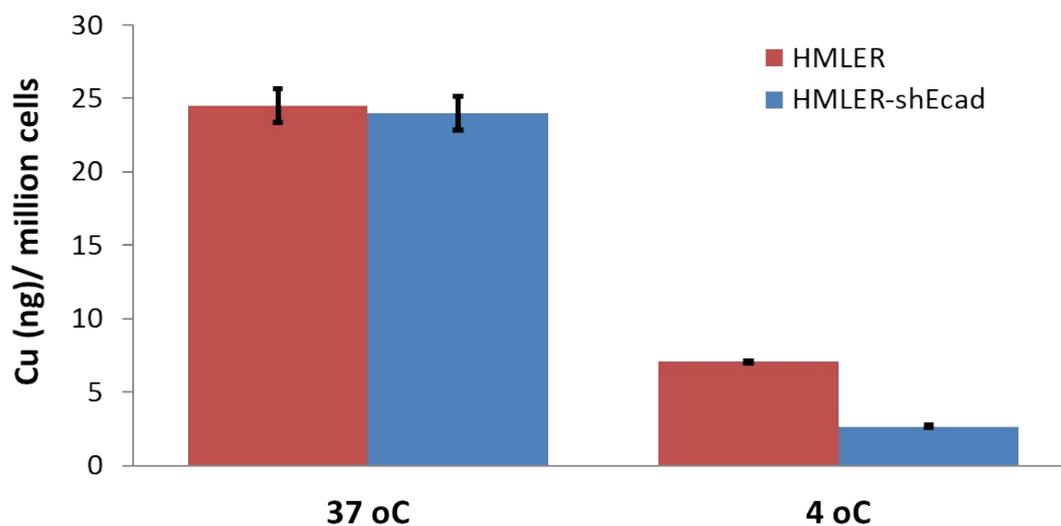


Fig. S13 Copper content in HMLER and HMLER-shEcad cells treated with **1 NP¹⁰** (110 nM for 4 h) at 4 °C or 37 °C.

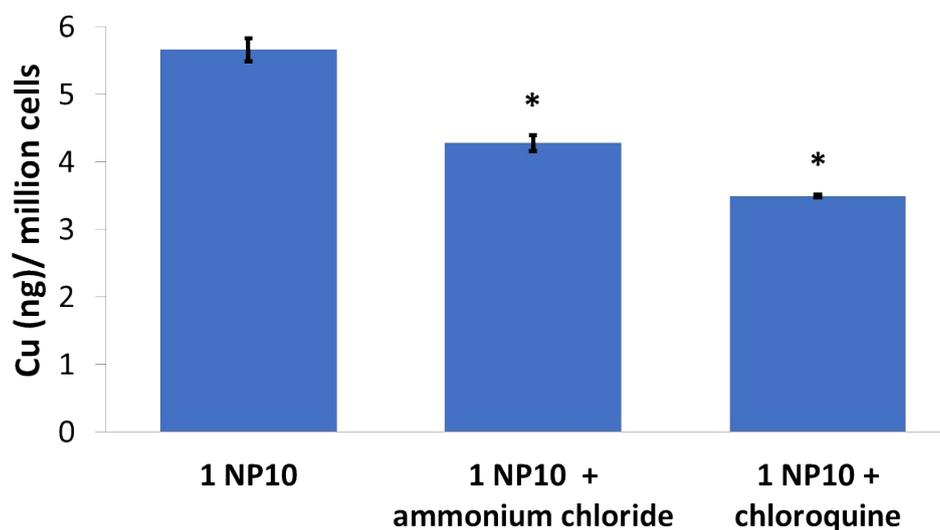


Fig. S14 Copper content in HMLER-shEcad cells treated with **1 NP¹⁰** only (16 nM for 24 h), and upon pre-incubation with ammonium chloride (50 mM for 2 h) or chloroquine (100 μ M for 2 h) at 37 °C. Error bars represent standard deviations and Student *t test*, * = $p < 0.05$.

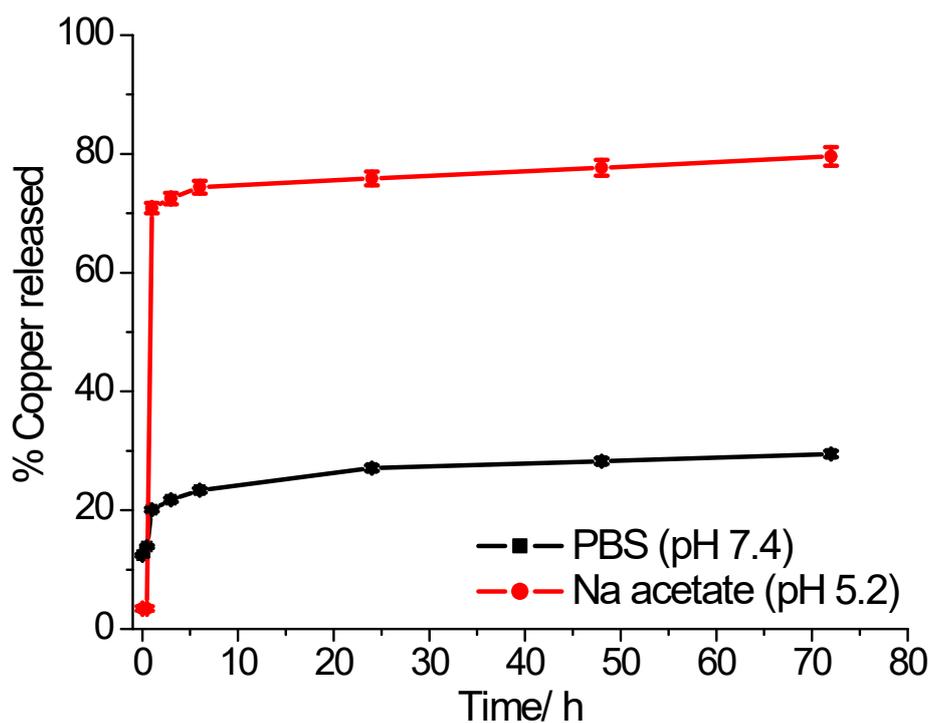


Fig. S15 The amount of copper released from **1 NP¹⁰** upon incubation in PBS (pH 7.4) or sodium acetate buffer (pH 5.2) over the course of 72 h at 37 °C.

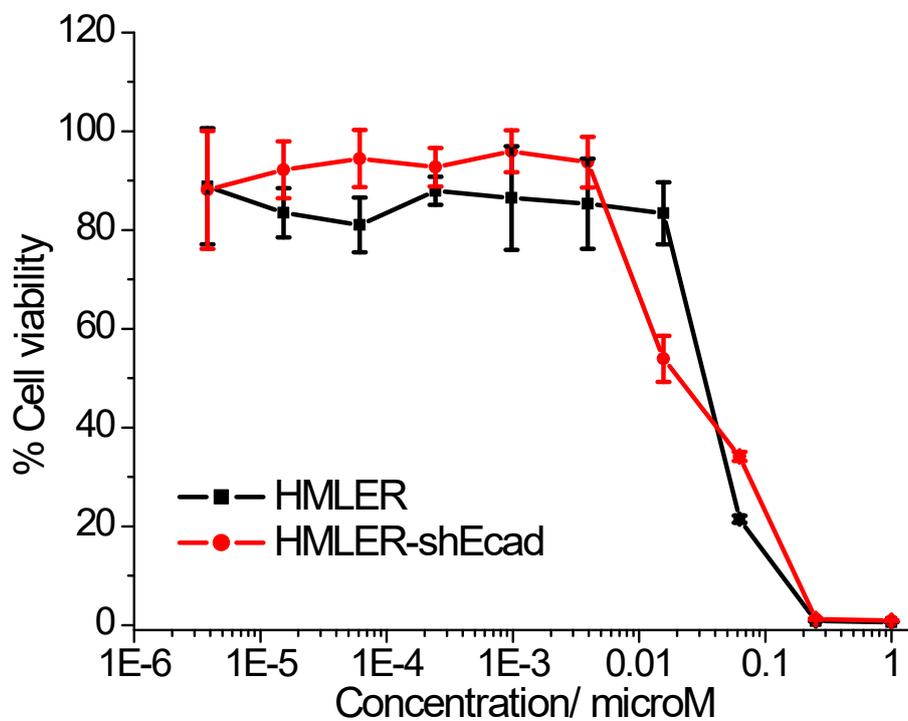


Fig. S16 Representative dose-response curves for the treatment of HMLER and HMLER-shEcad cells with **1 NP¹⁰**.

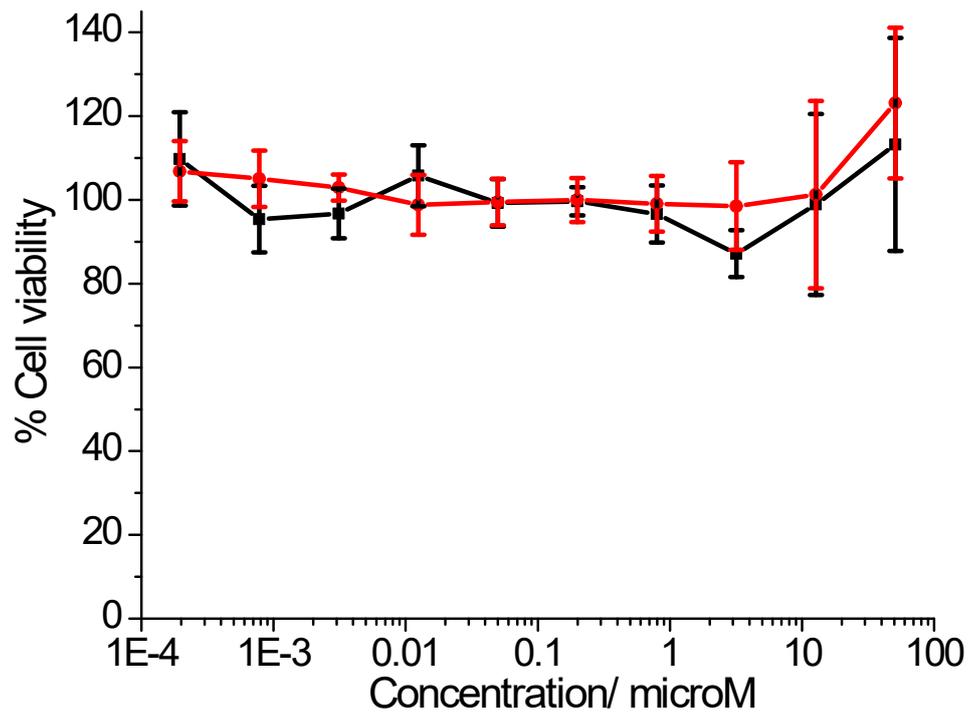


Fig. S17 Representative dose-response curves for the treatment of HMLER and HMLER-shEcad cells with empty PEG-PLGA nanoparticles.

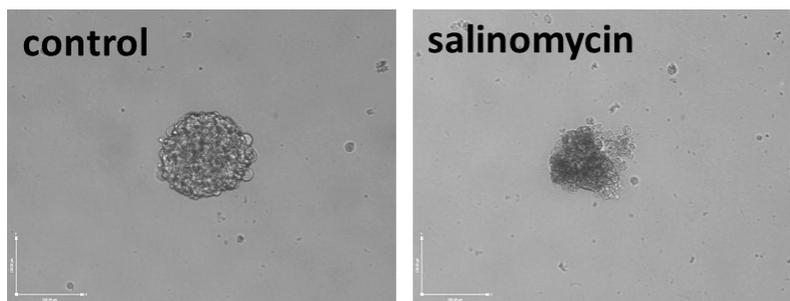


Fig. S18 Representative bright-field images ($\times 10$) of HMLER-shEcad mammospheres in the absence and presence of salinomycin at its respective IC_{20} values for 5 days.

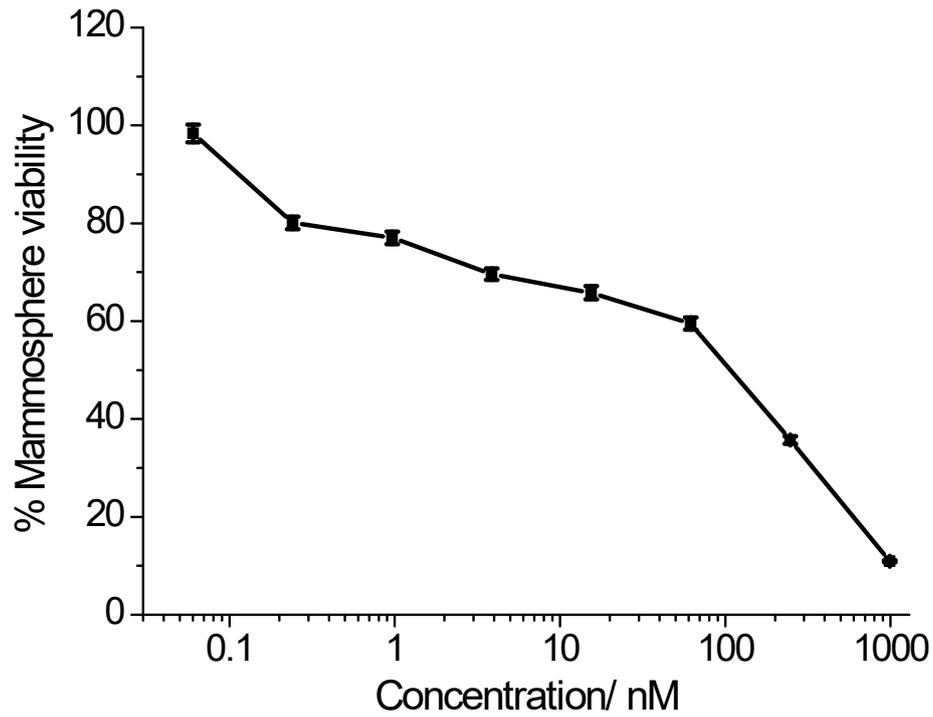


Fig. S19 Representative dose-response curve for the treatment of HMLER-shEcad mammospheres with 1 NP¹⁰.

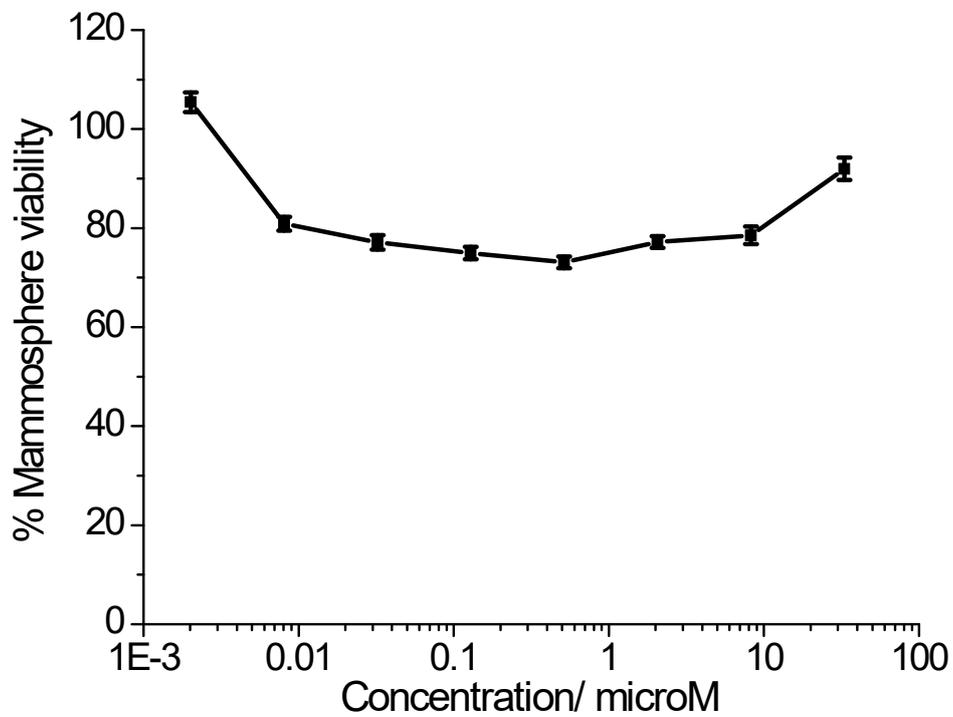


Fig. S20 Representative dose-response curve for the treatment of HMLER-shEcad mammospheres with empty PEG-PLGA nanoparticles.

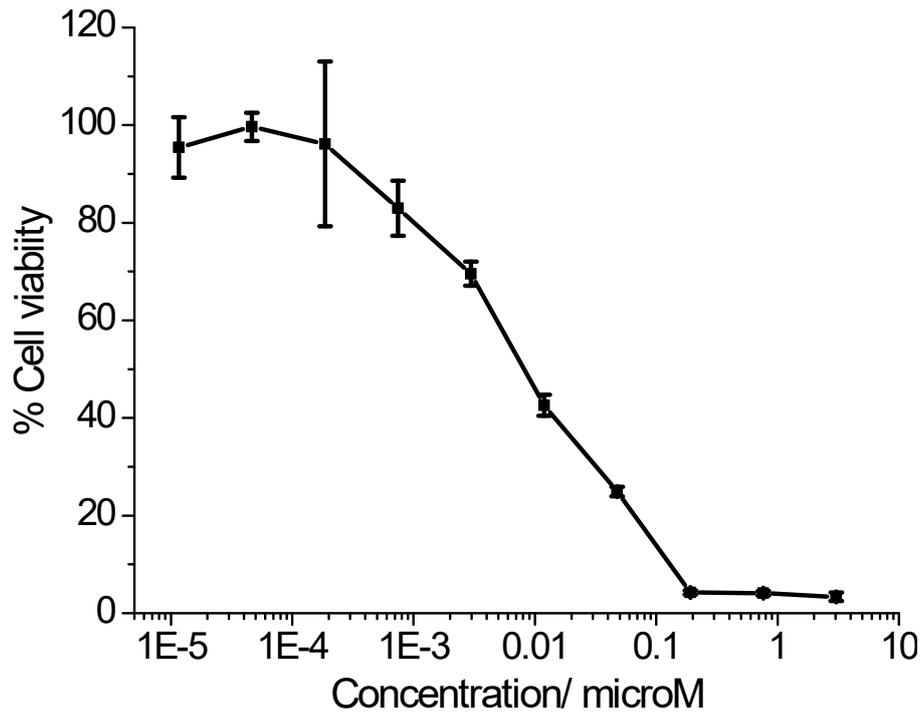


Fig. S21 Representative dose-response curves for the treatment of HMLER-shEcad cells with **1 NP¹⁰** after 72 h incubation in the presence of salubrinal (10 μ M).

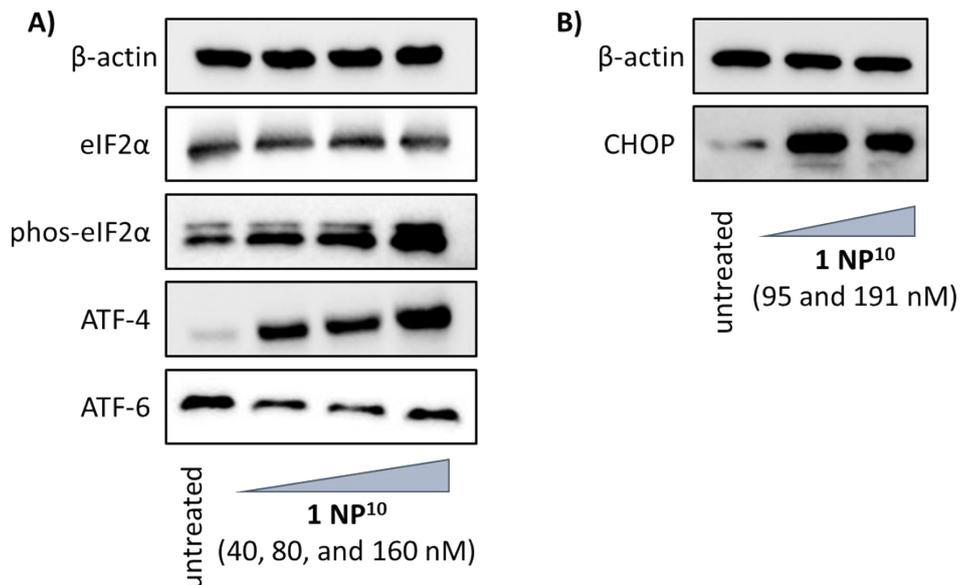


Fig. S22 Immunoblotting analysis of proteins related to the unfolded protein response (UPR). Protein expression in HMLER-shEcad cells following treatment with **1 NP¹⁰** (40–191 nM) for (A) 4 h or (B) 24 h.

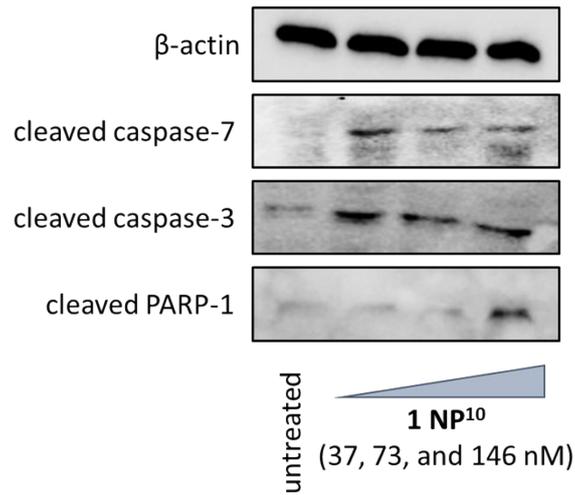


Fig. S23 Immunoblotting analysis of proteins related to apoptosis. Protein expression in HMLER-shEcad cells following treatment with **1 NP¹⁰** (37–146 nM for 72 h).

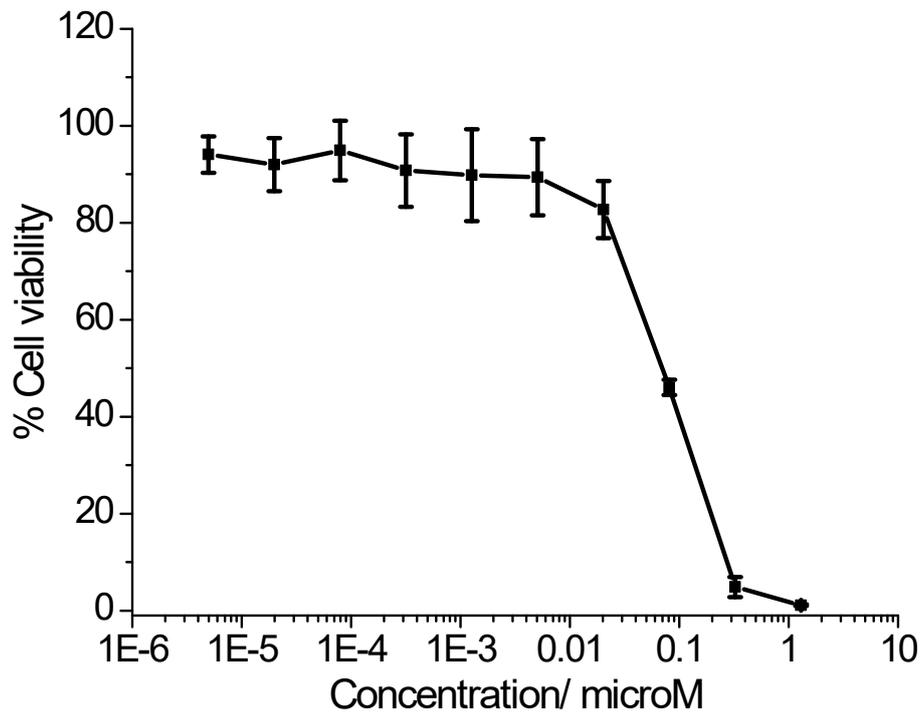


Fig. S24 Representative dose-response curves for the treatment of HMLER-shEcad cells with **1 NP¹⁰** after 72 h incubation in the presence of z-VAD-FMK (5 μ M).

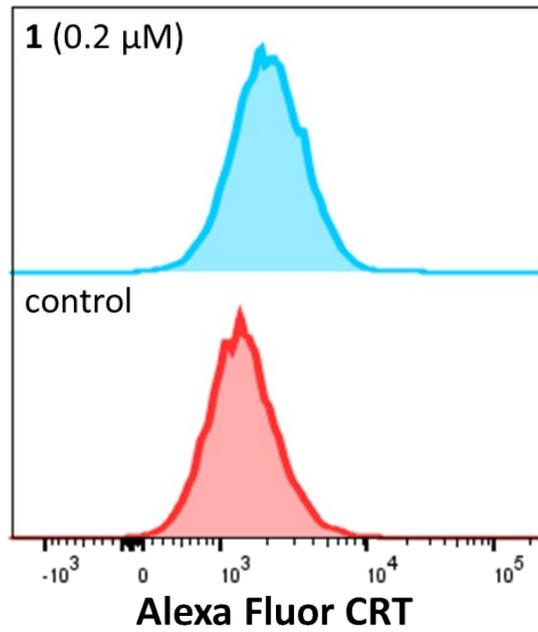


Fig. S25 Representative histograms displaying the green fluorescence emitted by anti-CRT Alexa Fluor 488 nm antibody-stained HMLER-shEcad cells untreated (red), and treated with **1** (0.2 μM for 24 h) (blue).

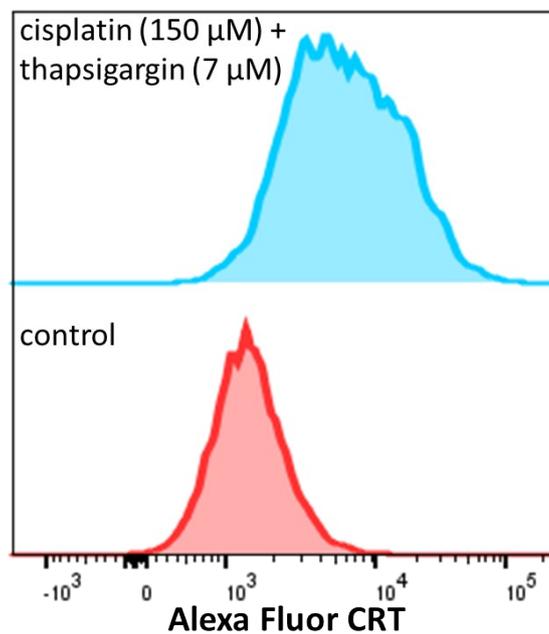


Fig. S26 Representative histograms displaying the green fluorescence emitted by anti-CRT Alexa Fluor 488 nm antibody-stained HMLER-shEcad cells untreated (red), and treated with cisplatin (150 μM for 24 h) and thapsigargin (7 μM for 24 h) (blue).

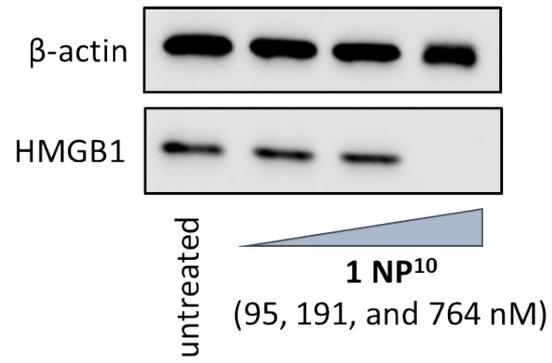


Fig. S27 Immunoblotting analysis of high mobility group box 1 (HMGB-1). Protein expression in HMLER-shEcad cells following treatment with **1 NP¹⁰** (95–764 nM for 24 h).