

Supplementary Information

Camptothecin loaded Casein nanosystem for tuning the therapeutic efficacy against highly metastatic triple-negative breast cancer cells

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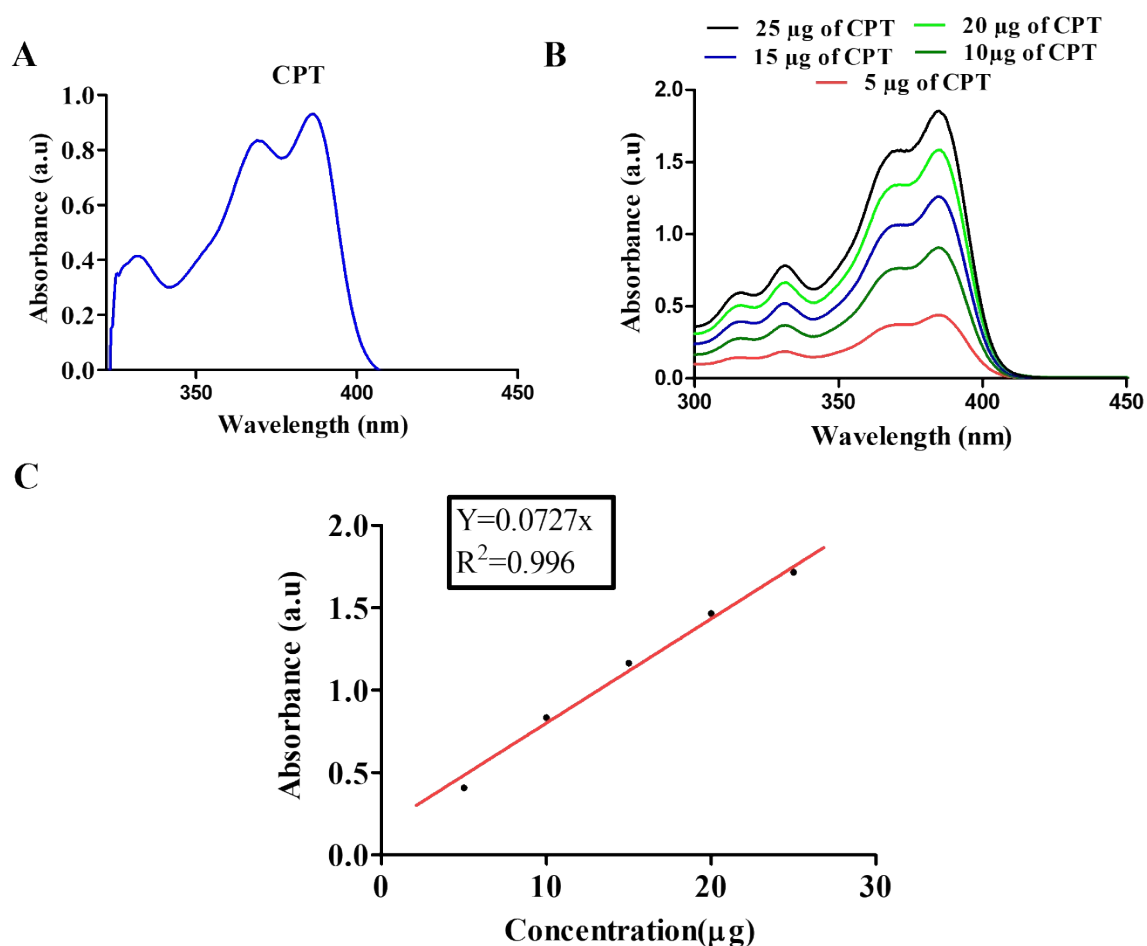


Fig. S1 (A) UV-visible and fluorescence spectra of CPT. (B & C) Concentrations dependent UV absorbance of CPT.

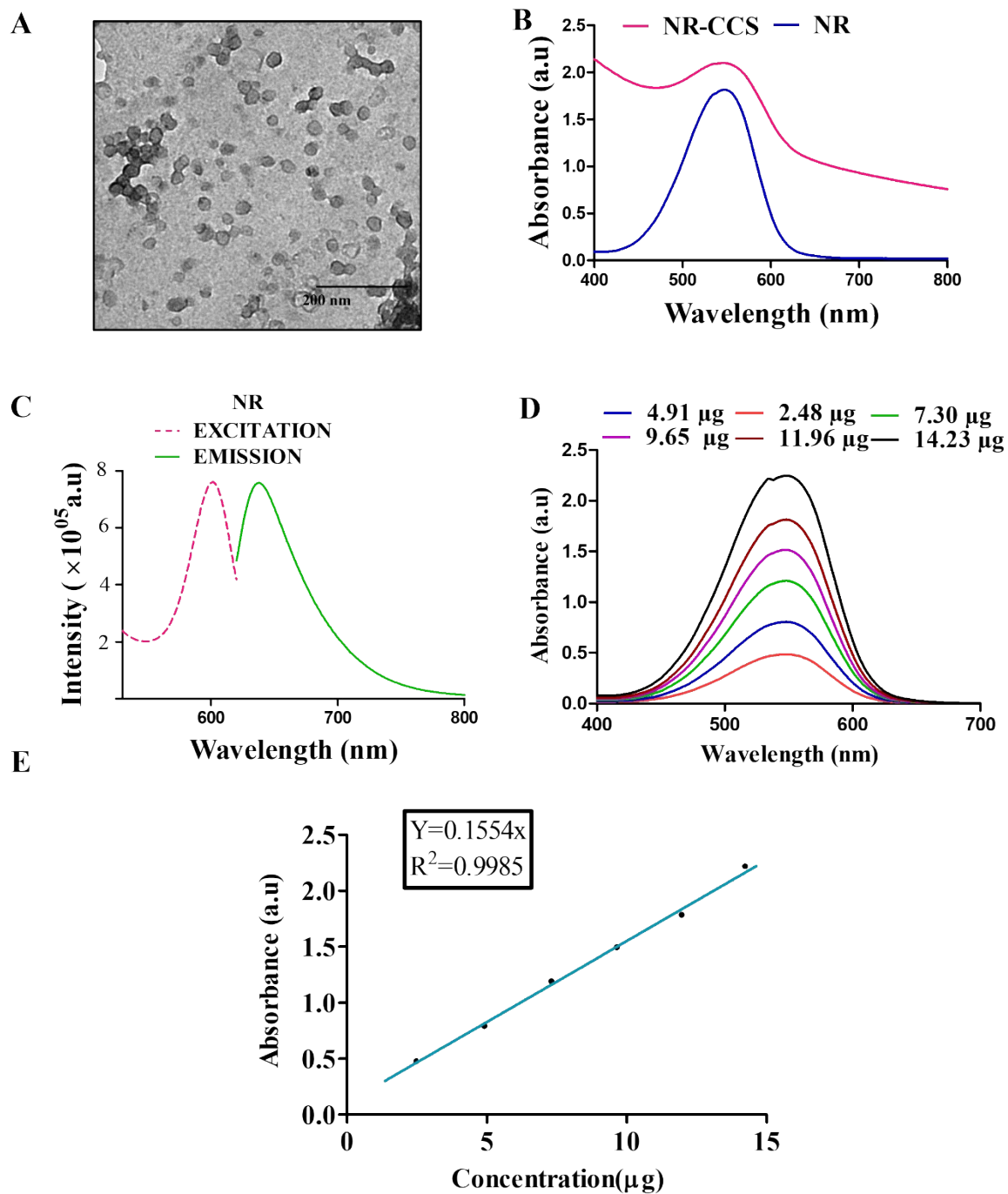


Fig. S2 (A) TEM image of CS NPs. (B) UV-visible spectra of NR and NR-CCS NPs. (C) Fluorescence spectra of NR. (D & E) Concentrations dependent UV absorbance of NR.

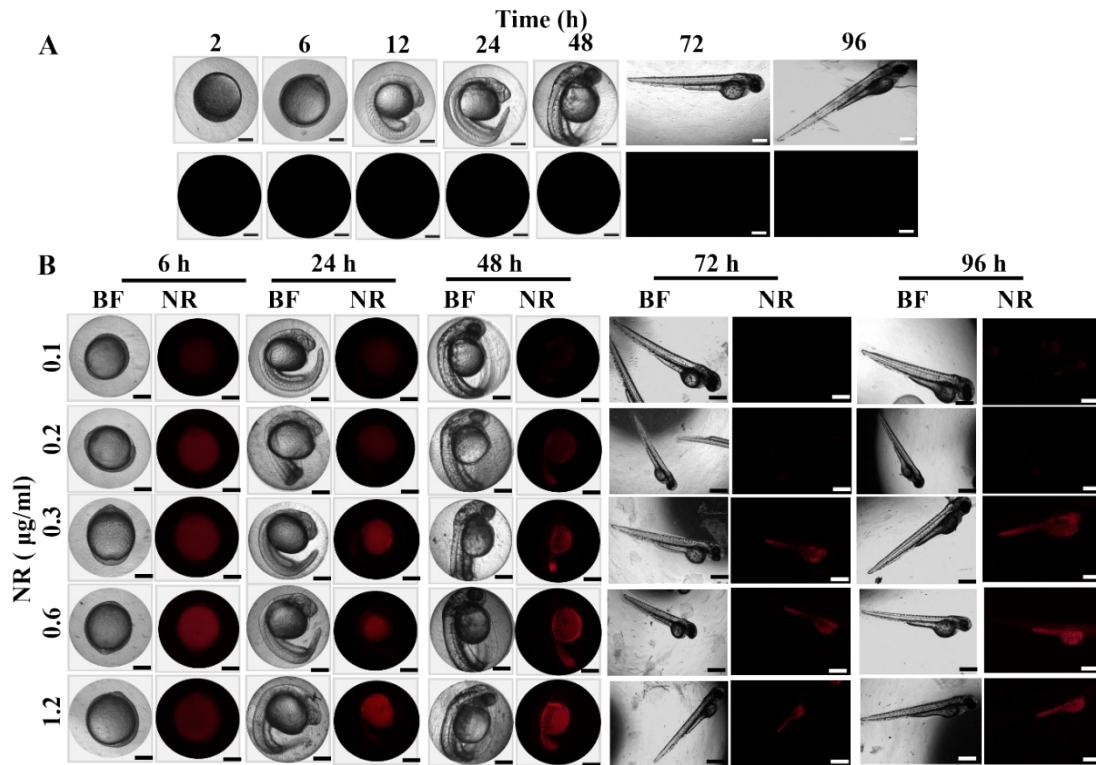


Fig. S3 (A) Images of untreated control of zebra fish embryo for biocompatibility study. (B) Treated with free NR, positive control group for biocompatibility study. Scale bar:100 µm.

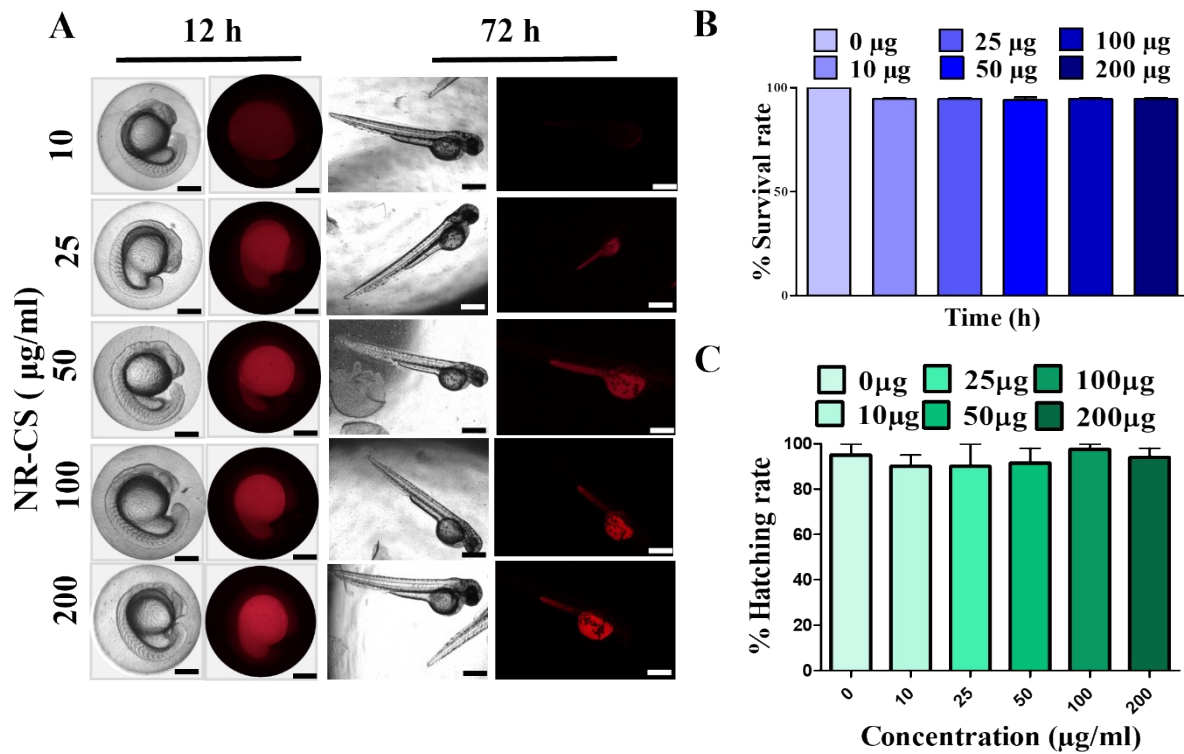


Fig. S4 (A) Analysis of NR-CS NPs biocompatibility in zebra fish embryo for 12 and 72 h, treated with various concentration of NR-CS NPs. NR was used to see the presence of CS NPs inside the embryo. Red fluorescent corresponds to the localization of NR-CS NPs. Scale bar:100 µm. Graphical representation of (B) survival rate and (C) hatching rate of embryo on different time points upon treatment with NR-CS NPs.

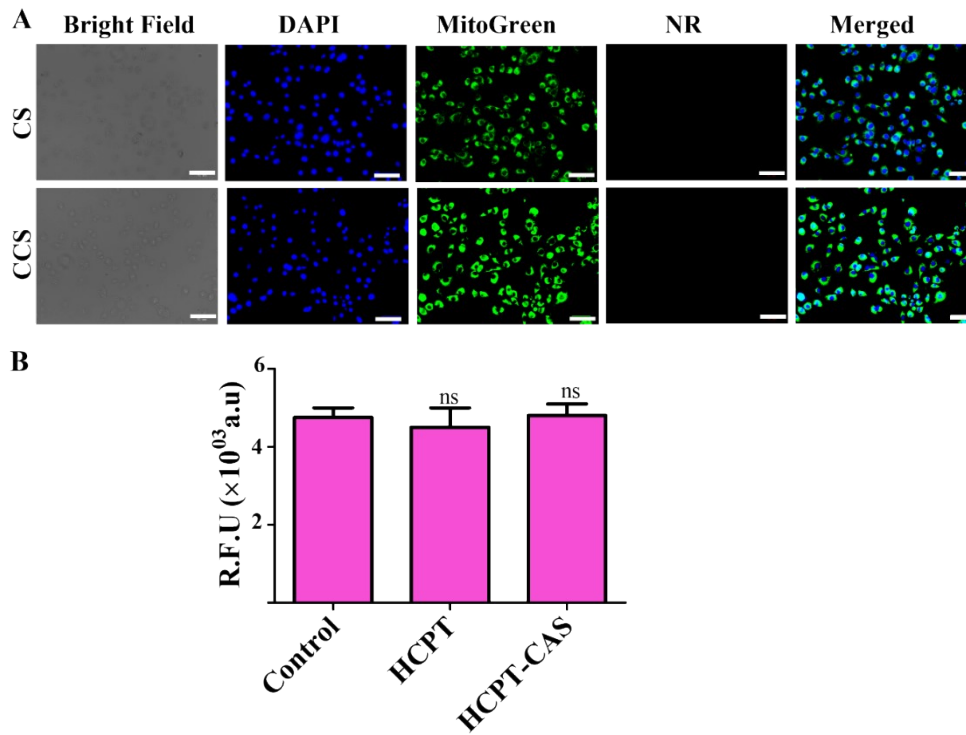


Fig. S5 (A) Cellular uptake study of CS and CCS NPs in 4T1 cells. Scale bar: 50 μ m. (B) Graphical representation of cellular uptake of CS and CCS NPs.

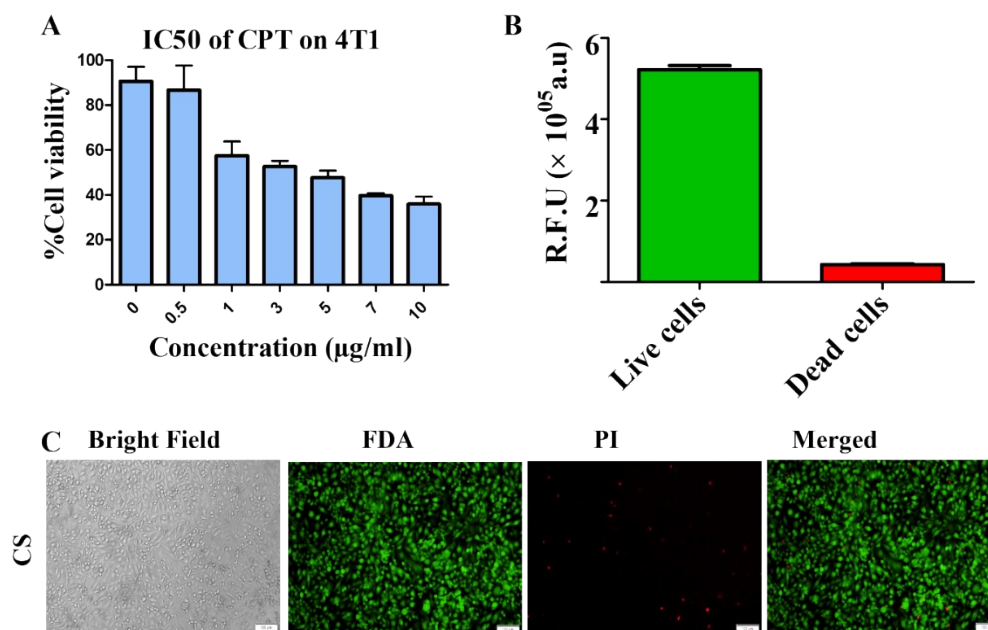


Fig. S6 (A) IC 50 value of CPT in 4T1 cells after 24 h treatment. (B) Graphical representation of live and dead cells treated with CS NPs. (C) Live-dead imaging of 4T1 cells treated with CS NPs for 24 h. Green and red fluorescence corresponds to live and dead cells respectively. Scale bar: 100 μ m.

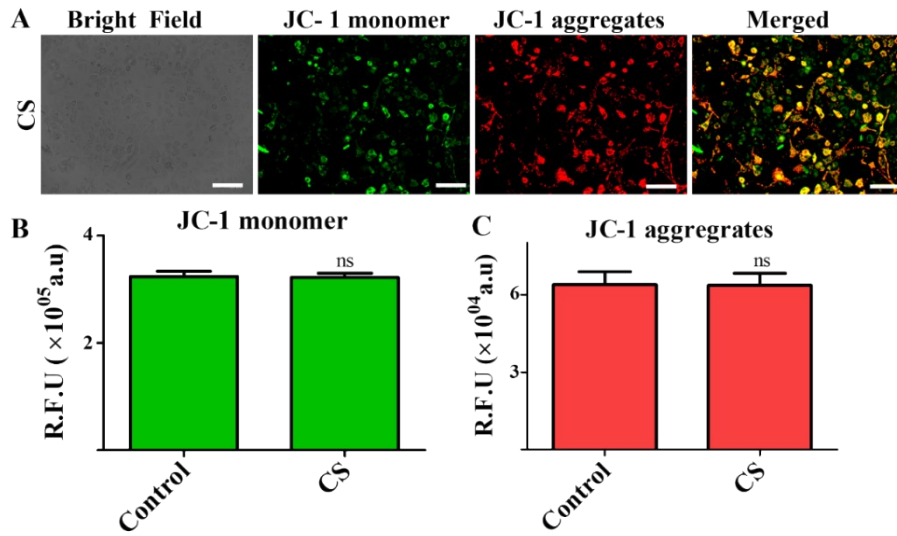


Fig. S7 (A) Mitochondrial membrane potential analysis of 4T1 cells after 10 h treatment with CS NPs, using JC-1 stain. Green fluorescence exhibited JC-1 monomer in cytoplasm and red fluorescence exhibited JC-1 aggregates in core mitochondria, merged image to show the co-localization of monomer and aggregates. Scale bar: 100 μ m. (B, C) Graphical representation of JC-1 monomer and JC-1 aggregates respectively.

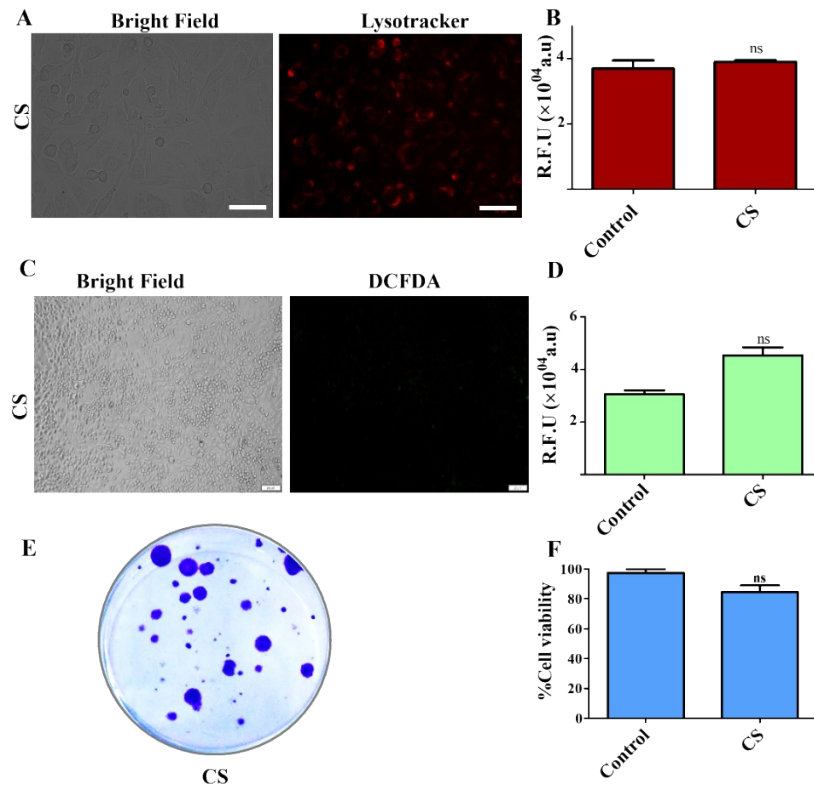


Fig. S8 (A) Analysis of lysosomal localization of CS NPs after 10 h incubation in 4T1 cells using deep red lysotracker. Scale bar: 50 μ m. (B) Graphical representation of lysosomal localization of CS NPs. (C) DCFDA analysis in 4T1 cells on treatment with CS NPs for 24 h to see the reactive oxygen species (ROS) generation. Scale bar: 100 μ m. (D) Graphical representation for DCFDA analysis. (E) Clonogenic assay in 4T1 cells to see the regrowth on post treatment. (F) Graphical representation of cell viability % in clonogenic assay.

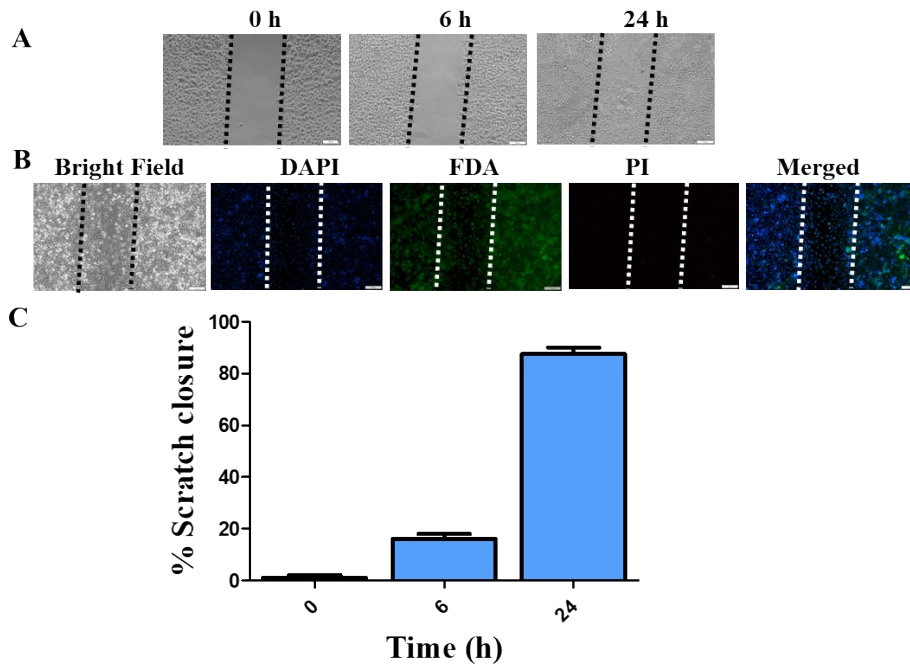


Fig. S9 (A) Scratch assay in 4T1 cells on treatment with CS NPs for 0, 6 and 24 h. (B) Live-dead imaging after 24 hr of migrated cells treated with CS NPs; blue fluorescence (DAPI) to see the nucleus of cells, green fluorescence (FDA) corresponds to live cells, red fluorescence (PI) corresponds to dead cells and merged image to see the co-localization. All scale bar: 100 μ m. (C) Graphical representation of % scratch closure on 0, 6 and 24 h.

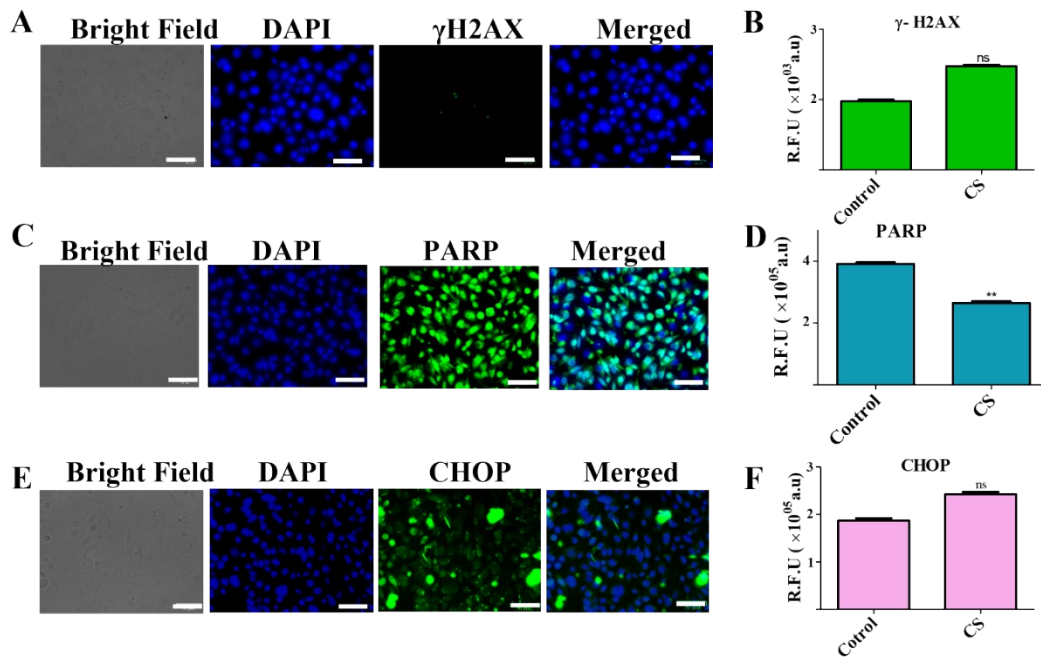


Fig. S10 (A, C & E) Immunofluorescence imaging of 4T1 cells using Alexa Fluor[®] 488 to re-ensure the expression of γ H2Ax, PARP and CHOP respectively on treatment with CS NPs. Green fluorescence corresponds to the expression of γ H2Ax, PARP and CHOP respectively. Scale bar: 50 μ m. (B, D & F) Graphical representations of fluorescence intensity corresponds to expression of γ H2Ax, PARP and CHOP respectively.

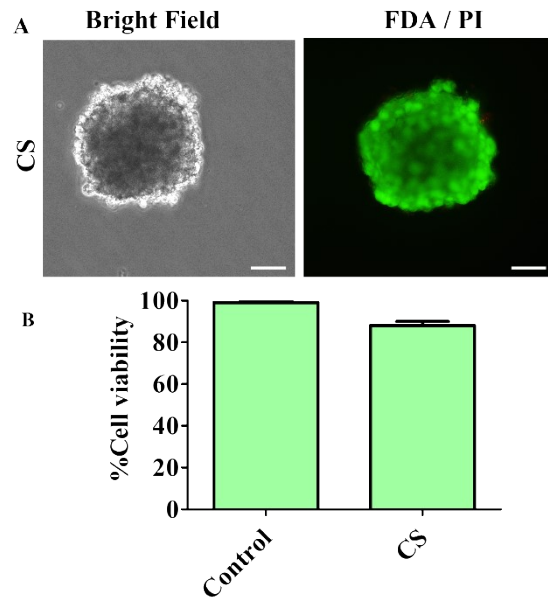


Fig. S11 (A) *In-vitro* analysis of deep tumor tissue penetration of CS NPs in 3-D spheroids of 4T1 cells after 24 h treatment with CS NPs. Green fluorescence corresponds to live cells, red fluorescence corresponds to dead cells and merged image represents the co-localization of live and dead cells. Scale bar:100 μ m. (B) Graphical representation of cell viability in 3-d spheroids.