Electronic Supplementary Material (ESI) for RSC Advances.

SUPPLEMENTARY INFORMATION

Chemo sensitization of IκBα overexpressing U87MG cells towards anti-cancer agents

Subhamoy Banerjee,[a] Amaresh Kumar Sahoo,[b] Arun Chattopadhyay[b, c] and Siddhartha Sankar Ghosh *[a, b]

a- Department of Biotechnology, IIT Guwahati, Assam-781039, Guwahati, India
b- Centre for Nanotechnology, IIT Guwahati, Assam-781039, Guwahati, India
c- Department of Chemistry, IIT Guwahati, Assam-781039, Guwahati, India

Materials and Methods Section

List of primers-

1. β-Actin- Fwd: 5' CTGTCTGGCGGCACCACCAT 3'
   Rev: 5' GCAACTAAGTCATAGTCCGC 3'

2. Cyclin D1- Fwd: 5' CGCCCCACCCCTCCAG 3'
   Rev: 5' CGCCCAGACCCTCAGACT 3'

3. Cyclin D2- Fwd: 5' AAAGTTGGCTCCAAAGGGTCCTT 3'
   Rev: 5' GAAACTGGCTGAACCTGTAAAAAT 3'

4. p27- Fwd: 5' CTGCAACCGACGATTCTTCTACT 3'
   Rev: 5' GGGCGTCTGCTCCACCAGA 3'

5. p21- Fwd: 5' GGACAGCAGAGGAAGACCATGT 3'
   Rev: 5' TGGAGTGGTAGAAATCTGTCATGC 3'

6. IκBα- Fwd: 5' TGCACTTGGCCATCATCCAT 3'
   Rev: 5' CGTGTGGCCATTGTAGTTGG 3'

Results and Discussions-

1. Size distribution of BSA loaded curcumin NPs

The particle size distribution of BSA loaded curcumin NPs was calculated by ImageJ™ software. The total number of particles calculated from separate TEM images taken on the same day. It was found that mean size of the BSA loaded curcumin NPs were 225 nm with standard deviation of 40 nm.

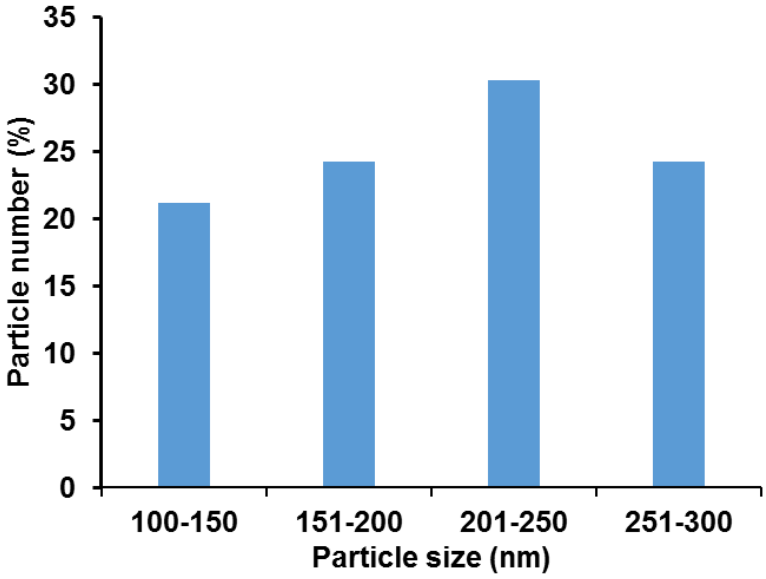

Fig. S1 Particle size distribution of BSA loaded curcumin NPs obtained by transmission electron microscopy. The mean± S.D. of particles were found to be 225±40 nm.

2. Stability of curcumin nano-conjugate upon addition of PLL and BSA

Curcumin NPs was stabilized by addition of PLL and BSA. The z average diameter of synthesized curcumin NPs was found to be 131.7 nm (Fig. S2 A) and zeta potential was -20±6.4 mV. Upon addition of PLL, the nanoparticles were found agglomerated with time (Fig. S2 B) with Z average diameter as 369 nm and surface charge 27 ±4.6 mV. Both cases are not suitable for cellular uptake [48, 49]. With addition of BSA, the size and zeta potential was found to be optimum for cellular uptake (Section 3.4 and Fig. 3C).

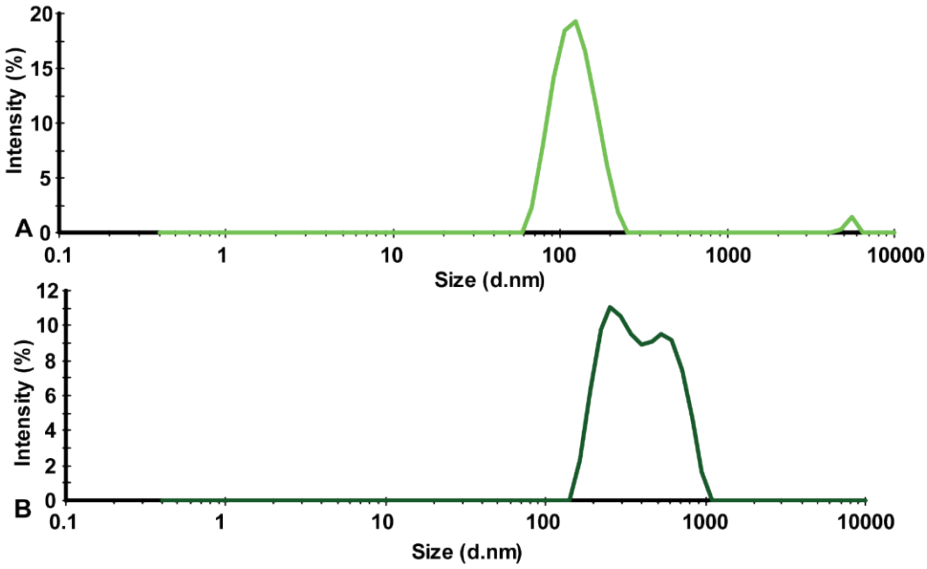

Fig. S2 Hydrodynamic diameter of (A) curcumin nanoparticle and (B) PLL added on curcumin NPs.

3. Interaction of BSA loaded curcumin nano-conjugate with cells

BSA loaded curcumin nano-conjugate added to the U87MG and U87-IκBα cell line and the interaction with the cells were checked by epi-fluorescence microscopy with bis-benzimide Hoescht 33342 tri-hydrochloride dye (Sigma, USA), which readily enters into the cell and bind with DNA. It's excitation and emission maximum is 346 nm and 460 nm respectively. Thus, the nuclear staining was done to ensure the BSA loaded curcumin nano-conjugate interaction with the cells as nucleus was emitting blue fluorescence for both untreated and treated cells but the treated cells were glowing with characteristic green fluorescence around the nucleus.

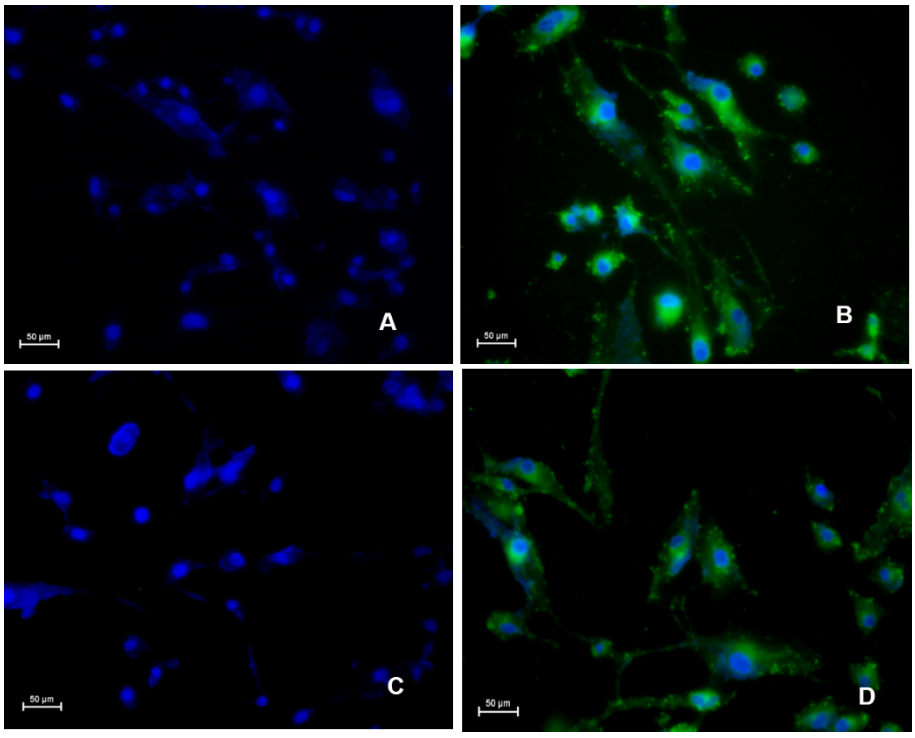

Fig. S3 Fluorescent microscopy image of curcumin nano-conjugate treated and untreated U87MG and U87-IκBα cells stained with Hoescht dye untreated (A) U87MG cells, (C) U87-IκBα cells and treated (B) U87MG cells and (D) U87-IκBα cells.

4. Cell viability assay upon treatment with 5-Fluorouracil for 72 h

The effect of 5-FU on U87MG and U87-IκBα cells was checked upon treatment with 72 h (Fig. S4). It was found that the cell viability was dropped slightly as compared to the 48 h treatment (Fig. 2A). Although, U87-IκBα cells had been found to be more sensitized as compared to its parental cell line which unambiguously proved that the expression of IκBα played crucial role in the sensitivity of U87-IκBα cells towards 5-FU.

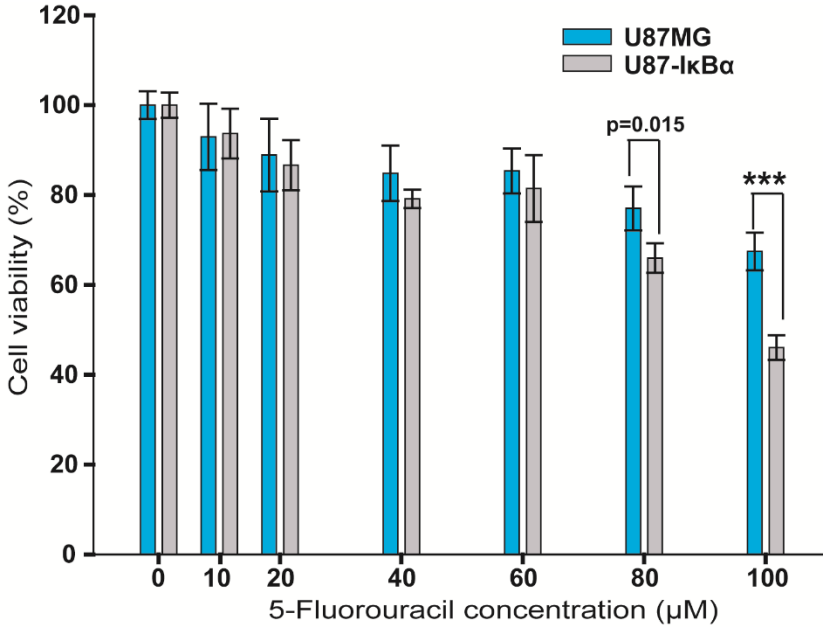

Fig. S4 Anti-cell proliferative effect of 5-FU on U87MG and U87-IκBα cells after 72 h. All data are represented as Mean ± S.D. and statistical analysis was done by two way ANOVA in Sigma Plot software. Statistical significance between treated samples with significant p value (<0.05) are mentioned and p<0.001 are denoted by ***.

5. Cell viability assay by curcumin NPs

The effect of only curcumin NPs were checked on both U87MG and U87-IκBα cells by MTT based cell viability assay. It was found that both the cells were not affected by curcumin NPs may be because of high surface charge upon curcumin NPs that hindered cellular uptake. Still, U87-IκBα cells were found to be more sensitized than U87MG cells Fig. S5).

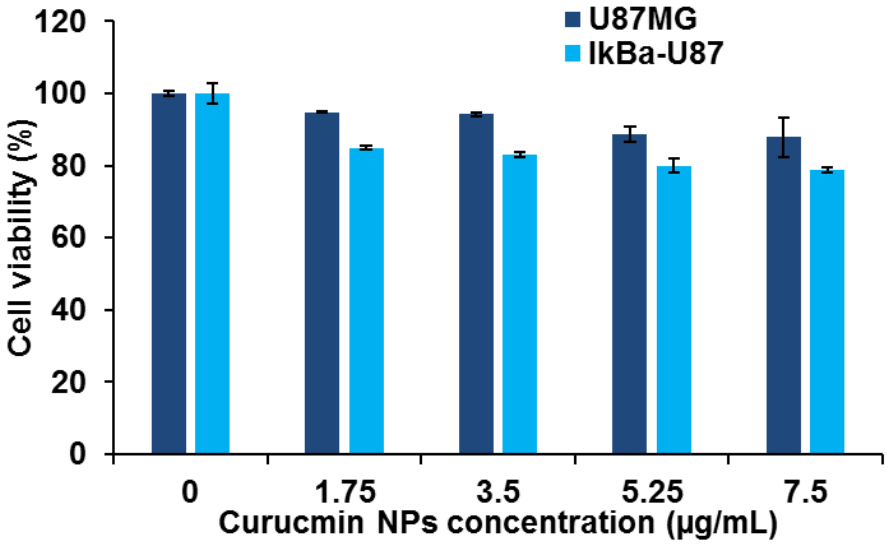

Fig. S5 Cell viability of U87MG and U87-IκBα cells treated by curcumin NPs. All data are represented as Mean ± S.D of three individual experiments.

6. Induction of ROS by only curcumin nanoparticle

Only curcumin NPs was found to differentially induce ROS in both U87MG and U87-IκBα cells (Fig. S6). Although, for U87MG cells, the ROS generation was not so significant (Fig. S6 A) that will induce apoptosis. Similarly, although for U87-IκBα the generation of ROS was little higher than U87MG (Fig. S6 B).

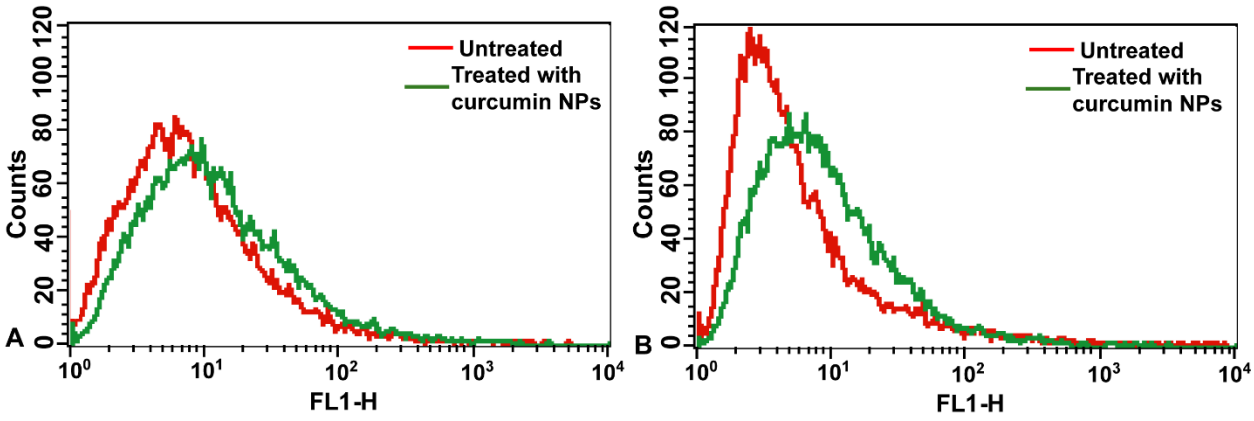

Fig. S6 Induction of ROS in (A) U87MG cells and (B) U87-IκBα cells treated by curcumin NPs.