Electronic Supplementary Material (ESI) for Nanoscale.

Supporting Information

Light triggered selective ROS dependent autophagy by bioactive nanoliposomes for efficient cancer theranostics.

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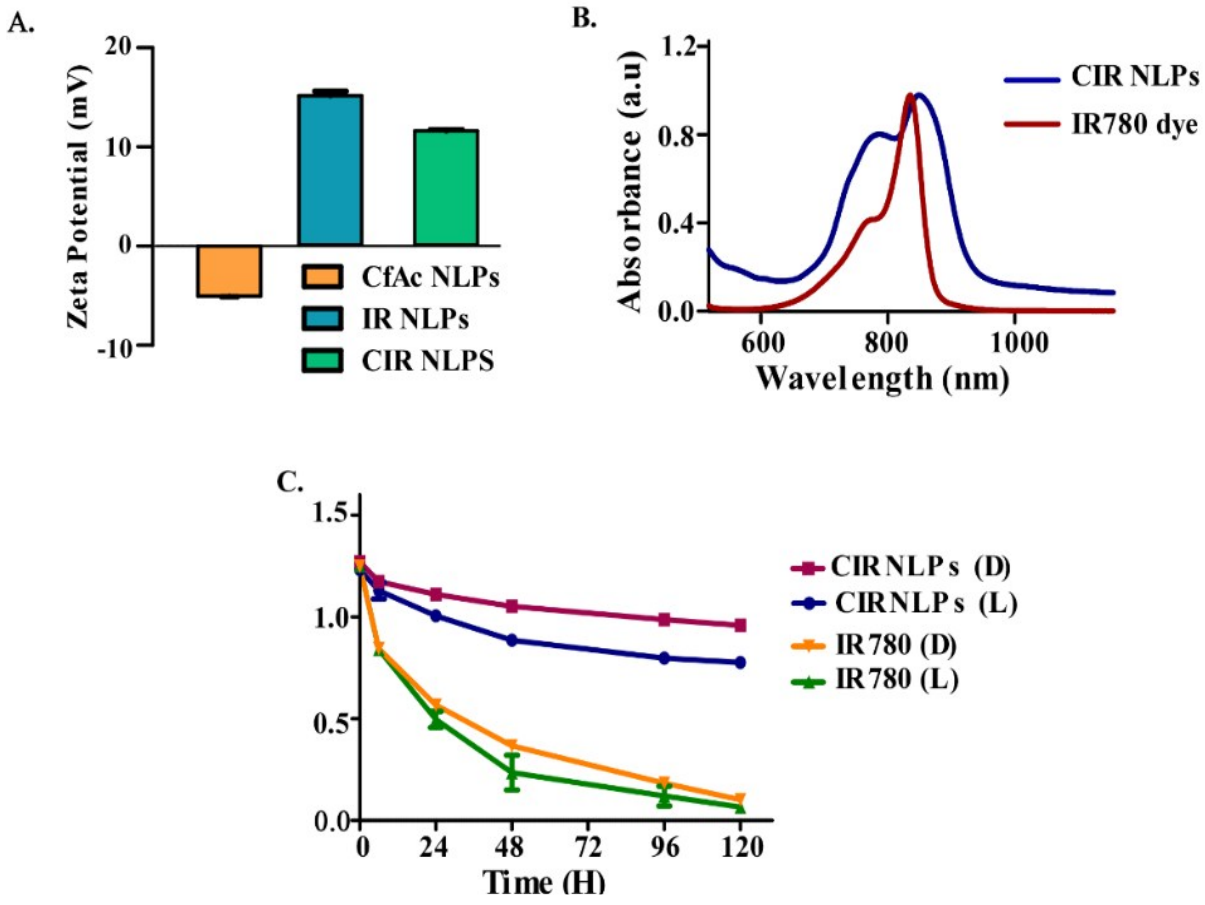
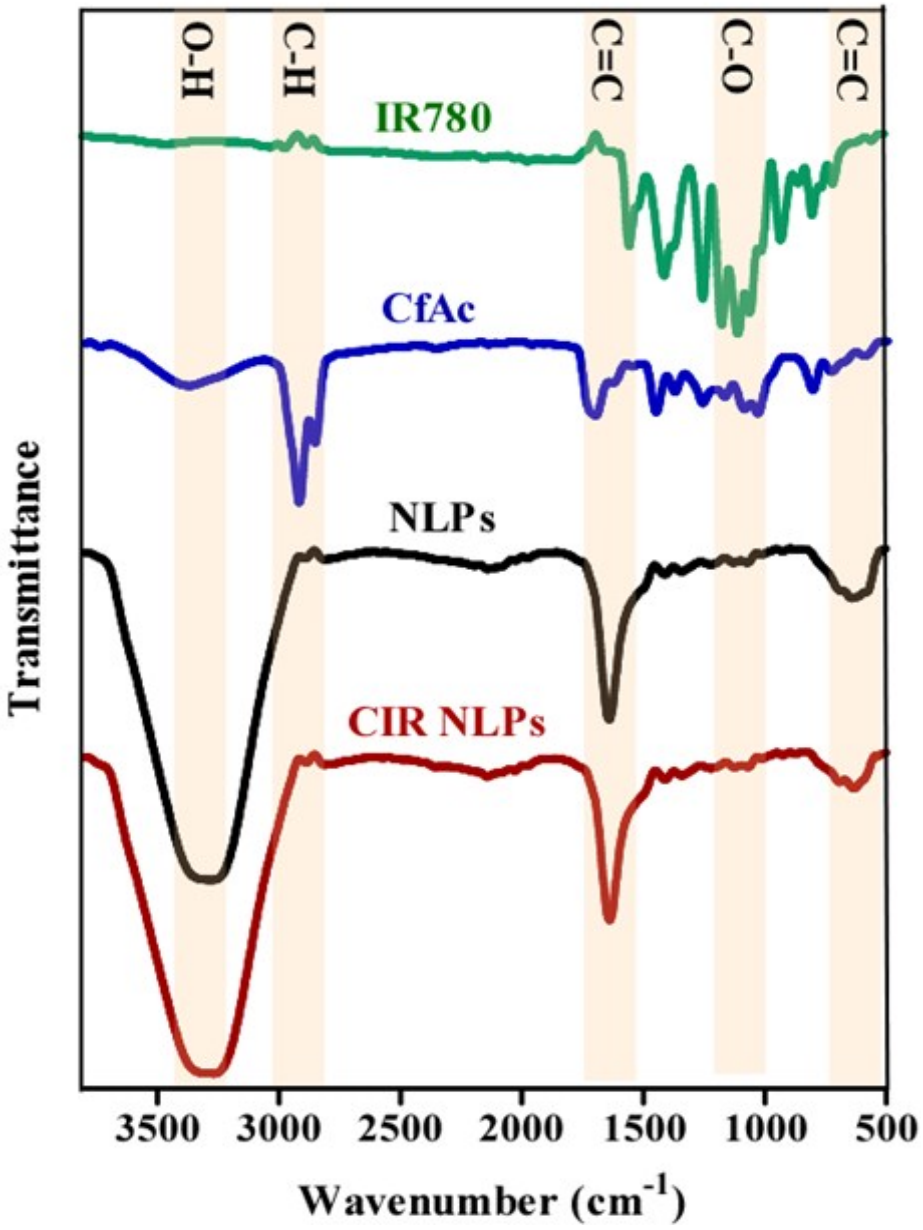


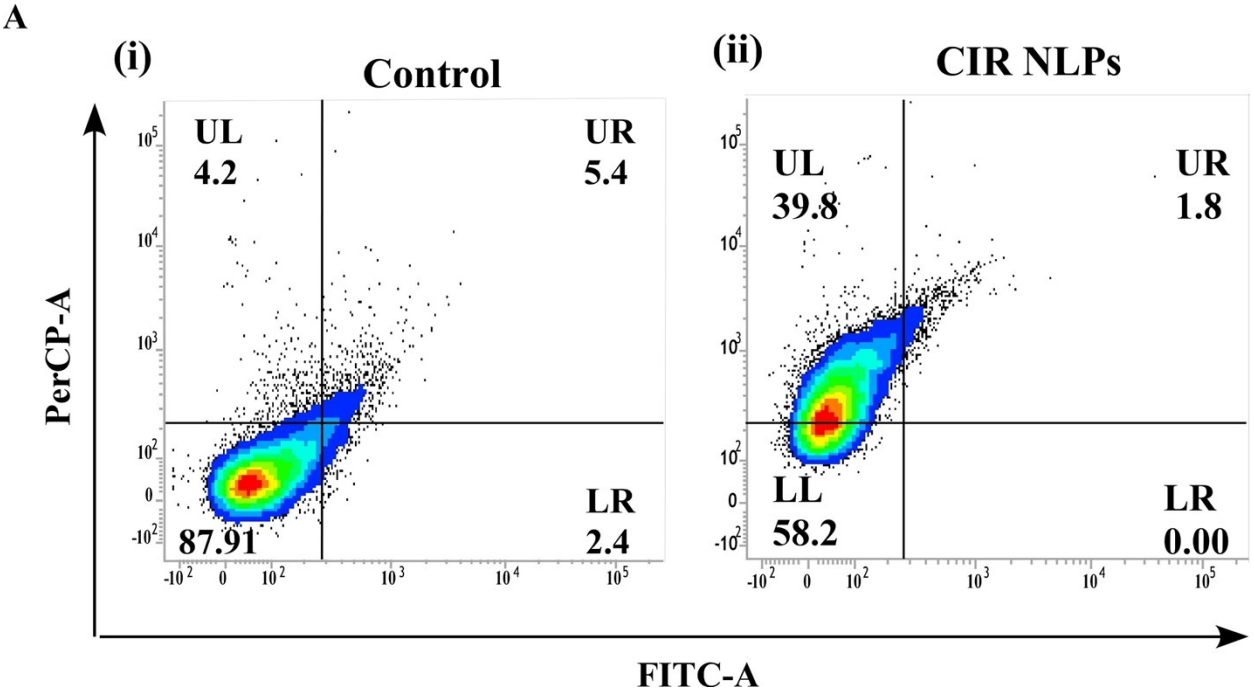
SI Figure 1: A) Zeta Potential of CfAc, IR & CIR NLPs. B) UV-Spectra of CIR NLPs and IR780 dye C) Stability of CIR NLPs over free IR780 stored under light (L) and dark (D) conditions.



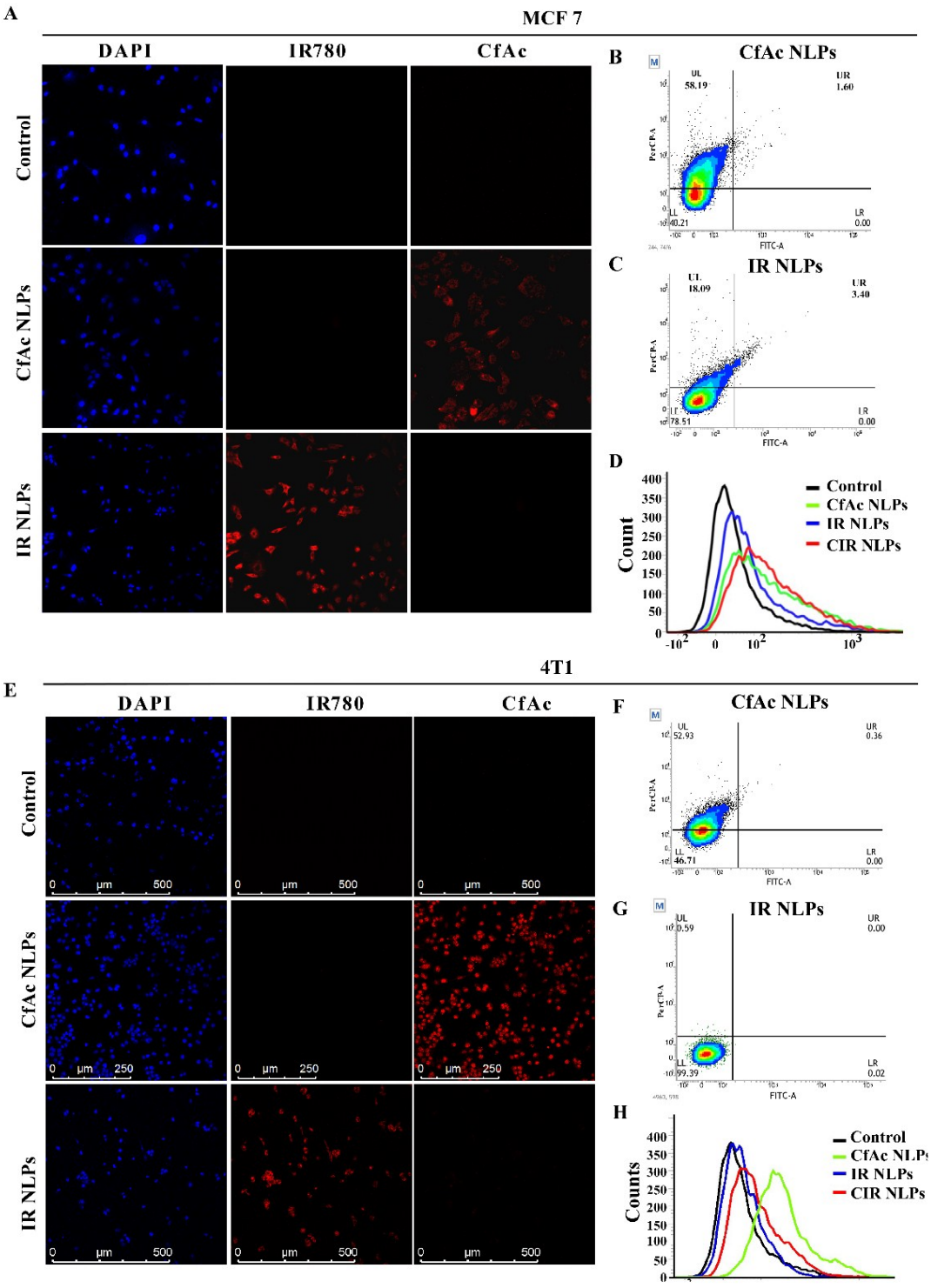
SI Figure 2: FTIR analysis of the IR780 dye, CfAc, blank nanoliposomes (NLPs) and CIR NLPs.

Cellular internalization of CIR NLPs.

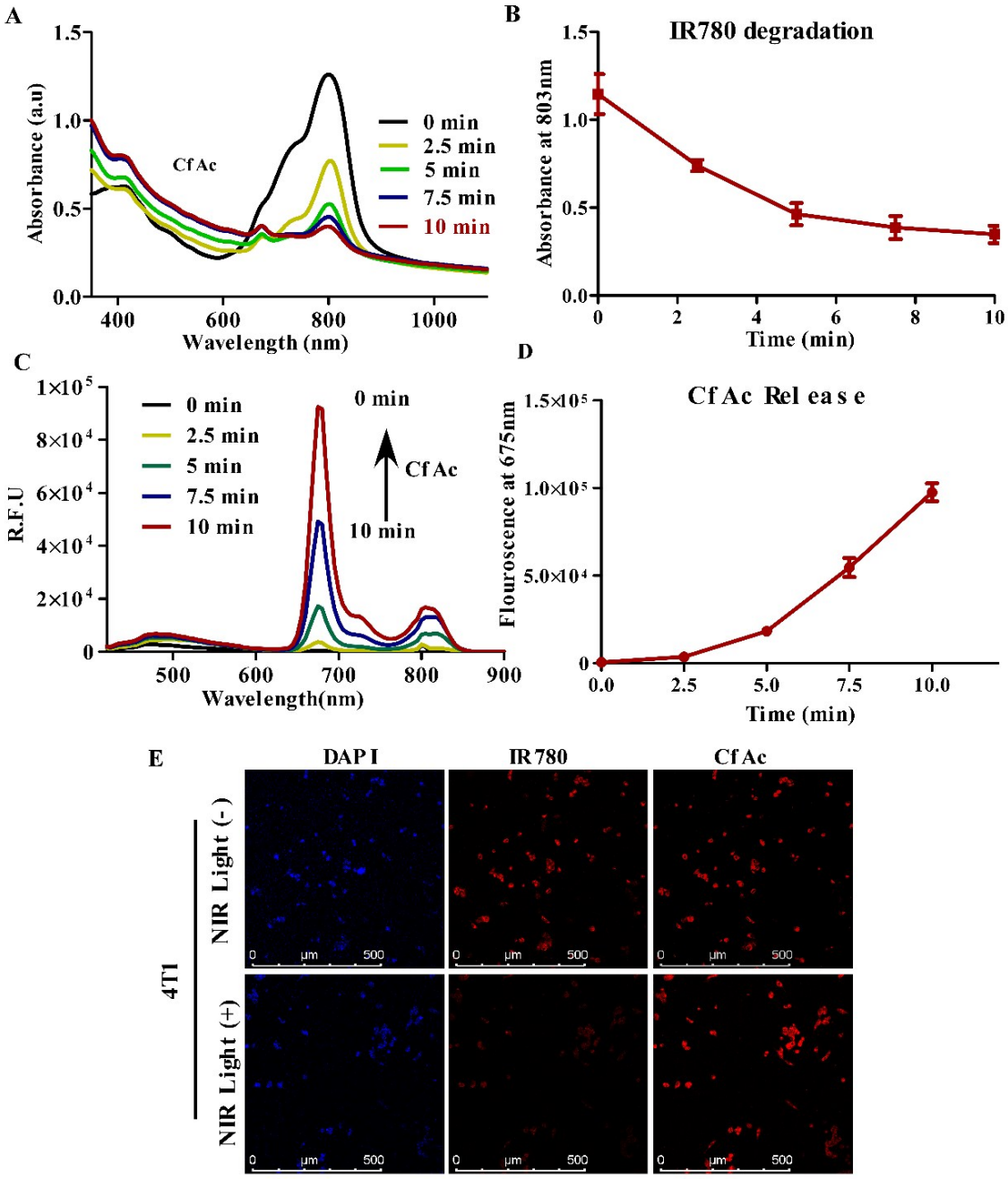
The NLPs were studied to understand their cellular uptake in MCF-7 and 4T1 cell lines using confocal microscopy and flow cytometry [26].The inherent red fluorescence of CfAc (Ex/Em @ 400/680) and IR780 (Ex/Em @ 630/780) was used to detect the cellular uptake of NLPs [19] [27]. Briefly, the cells were seeded in 6-well plates at an estimated density of 2×10^5 cells/well. Next day, fresh media containing, aqueous CfAc (100 µg/ml), CfAc, IR and CIR NLPs (the final concentration of CfAc @ 7.5 µg/ml and IR780 @ 3.5 µg/ml) were incubated with cells for 5 hours. For microscopic analysis, cells were rinsed thrice with ice-cold 1x PBS and then fixed with 4% formaldehyde for 20 min. The plates were washed thrice with PBS and stained with DAPI (1 µg/ml). Finally, the cells were observed in DAPI (Nucleus), UV (CfAc) and IR780 (IR780) channels of a confocal microscope (Leica, Germany). Similar groups without DAPI staining were subjected to flow cytometry analysis (FACS Fortessa, BD Biosciences) at an emission wavelength of 660 nm (Per-Cp channel).



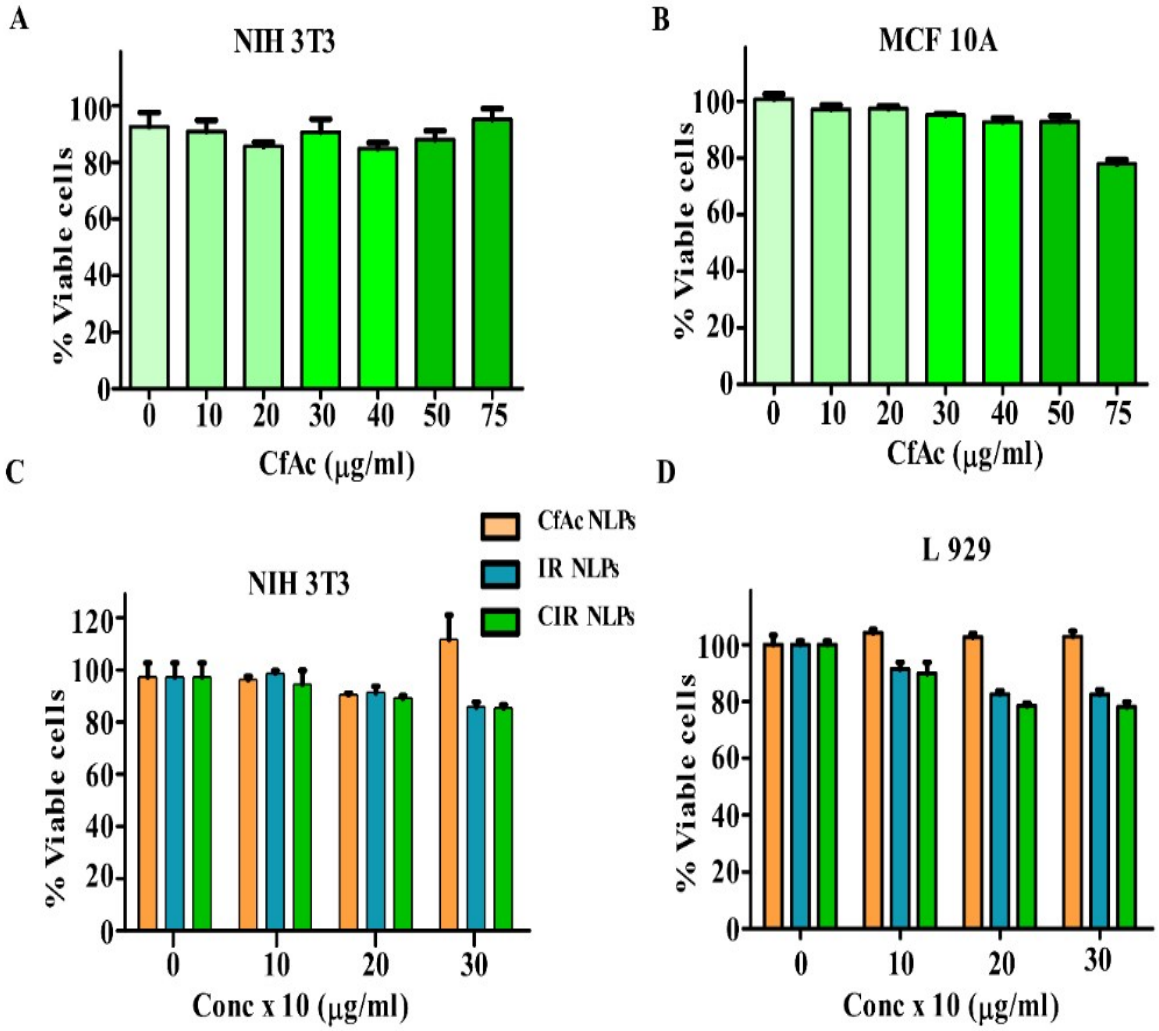
SI Figure 3: Cellular uptake of CIR NLPs in MCF 7 and 4T1 cell lines. Flow cytometry analysis in MCF 7 cell lines (i) Control group (ii) CIR NLPs treated group. (Quadrant abbreviation: UL=Upper Left, LL= Lower Left, UR=Upper Right, LR=Lower Right).



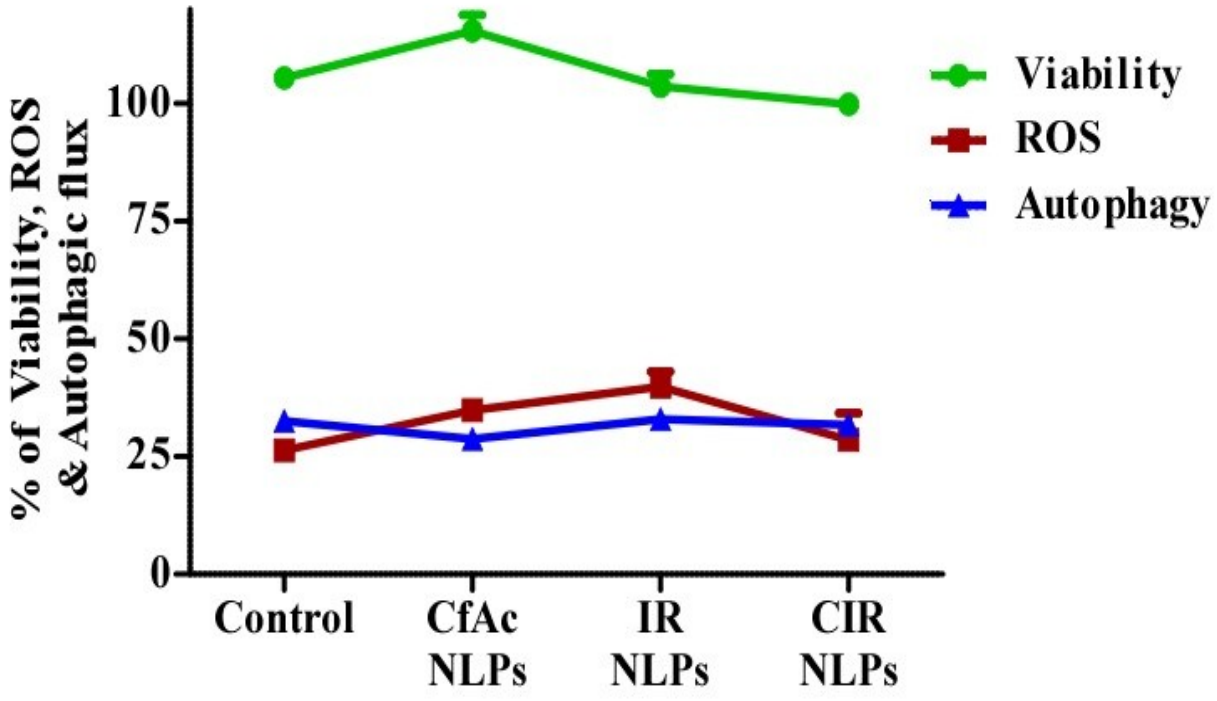
SI Figure 4: A) Confocal microscopy images of CfAc & IR NLPs in MCF 7 cell line. B, C) Flow cytometric analysis of MCF 7 cell line incubated with CfAc & IR NLPs. D) Overlay of FACS analysis in MCF7 cell line. E) Confocal microscopy imaging of CfAc & IR NLPs in 4T1 cell line. F, G) Flow cytometric analysis of CfAc & IR NLPs in 4T1, MCF7 cell lines. H) Overlay of FACS analysis in 4T1 cell line.



SI Figure 5: A) Absorbance spectra of CIR NLPs showing the degradation of IR780 and release of CfAc. B) Degradation profile of IR780 in CIR NLPs after NIR light irradiation. C) Fluorescence spectra of CIR NLPs showing the rise in CfAc peak with PTT. D) The time-dependent release profile of CfAc from CIR NLPs when subjected to NIR light irradiation. E) Confocal microscopy images of CIR NLPs when exposed to NIR light.



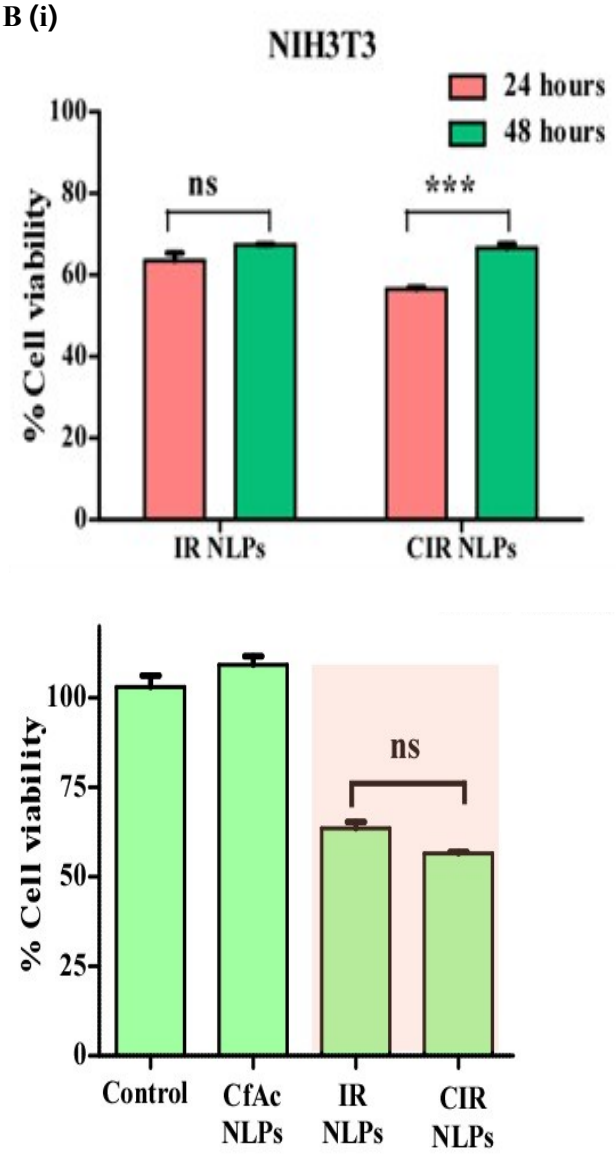
SI Figure 6: Biocompatibility studies of CfAc in A) NIH3T3, B) MCF 10A. Biocompatibility studies of NLPs (CfAc, IR & CIR) in C) NIH3T3 and D) L929 Cell lines.



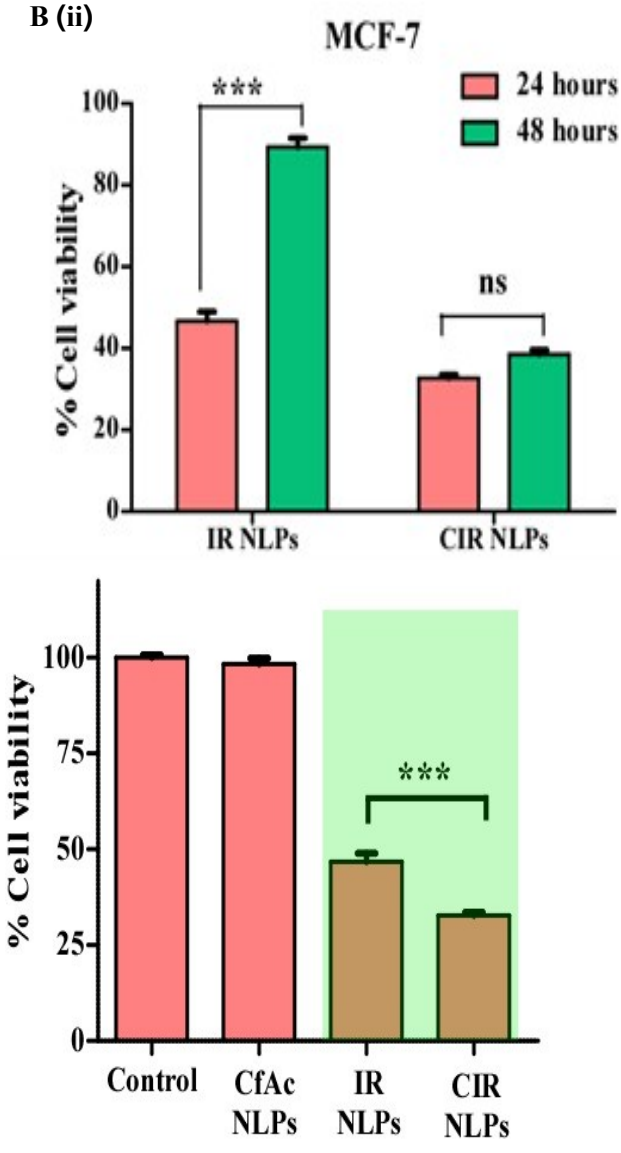
SI Figure 7: Effect of NLPs on ROS, autophagic flux and viability in NIH3T3 cell line.

A (i) **NIH 3T3 (24 h)** **A (ii)** **MCF 7 (24 h)**

B (i)

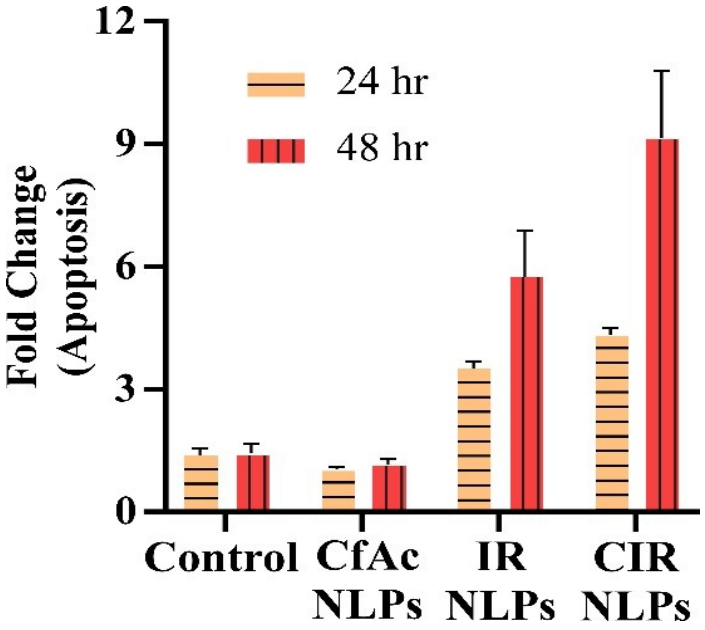

NIH3T3
24 hours
48 hours
% Cell viability
ns
***
IR NLPs
CIR NLPs
% Cell viability
ns
Control
CfAc NLPs
IR NLPs
CIR NLPs

B (ii)


MCF-7
24 hours
48 hours
% Cell viability
***
ns
IR NLPs
CIR NLPs
% Cell viability
***
Control
CfAc NLPs
IR NLPs
CIR NLPs

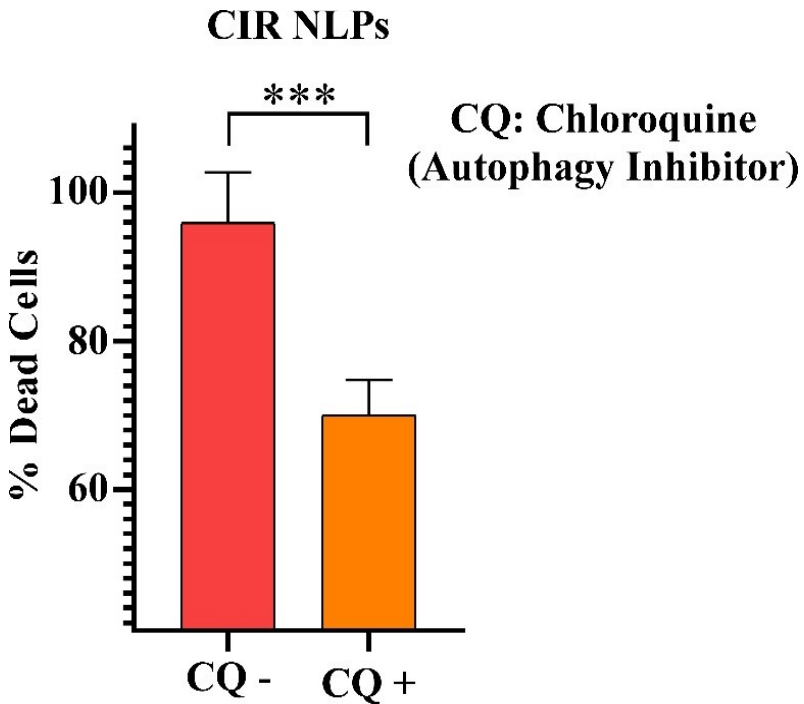
SI Figure 8 A) Cell viability (24 h) in (i) normal (NIH3T3) and (ii) cancerous (MCF-7) cells after incubation & treatment with NLPs/NIR light. **B)** Comparison of cell viability in (i) NIH3T3 and (ii) MCF-7 cells.

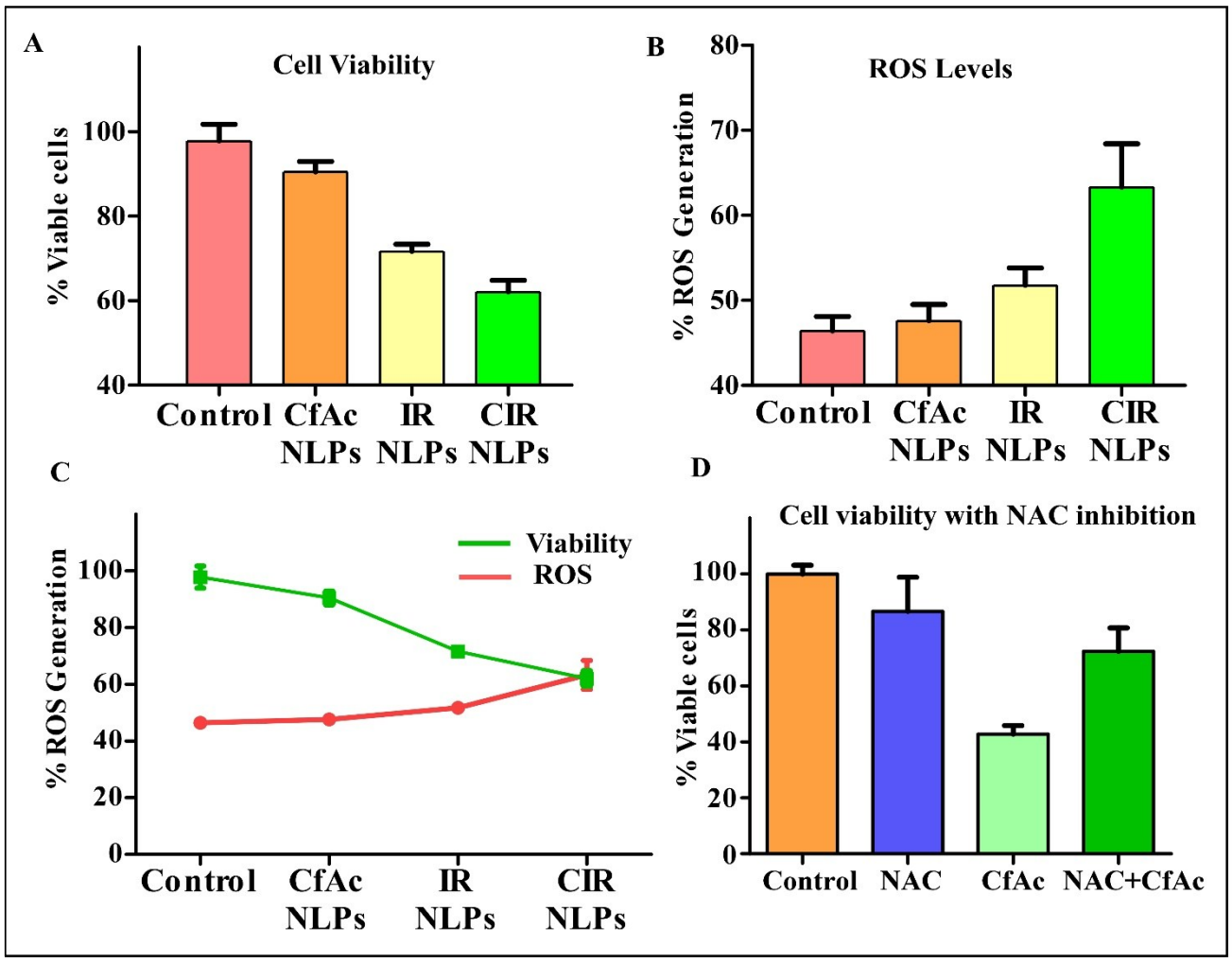
A



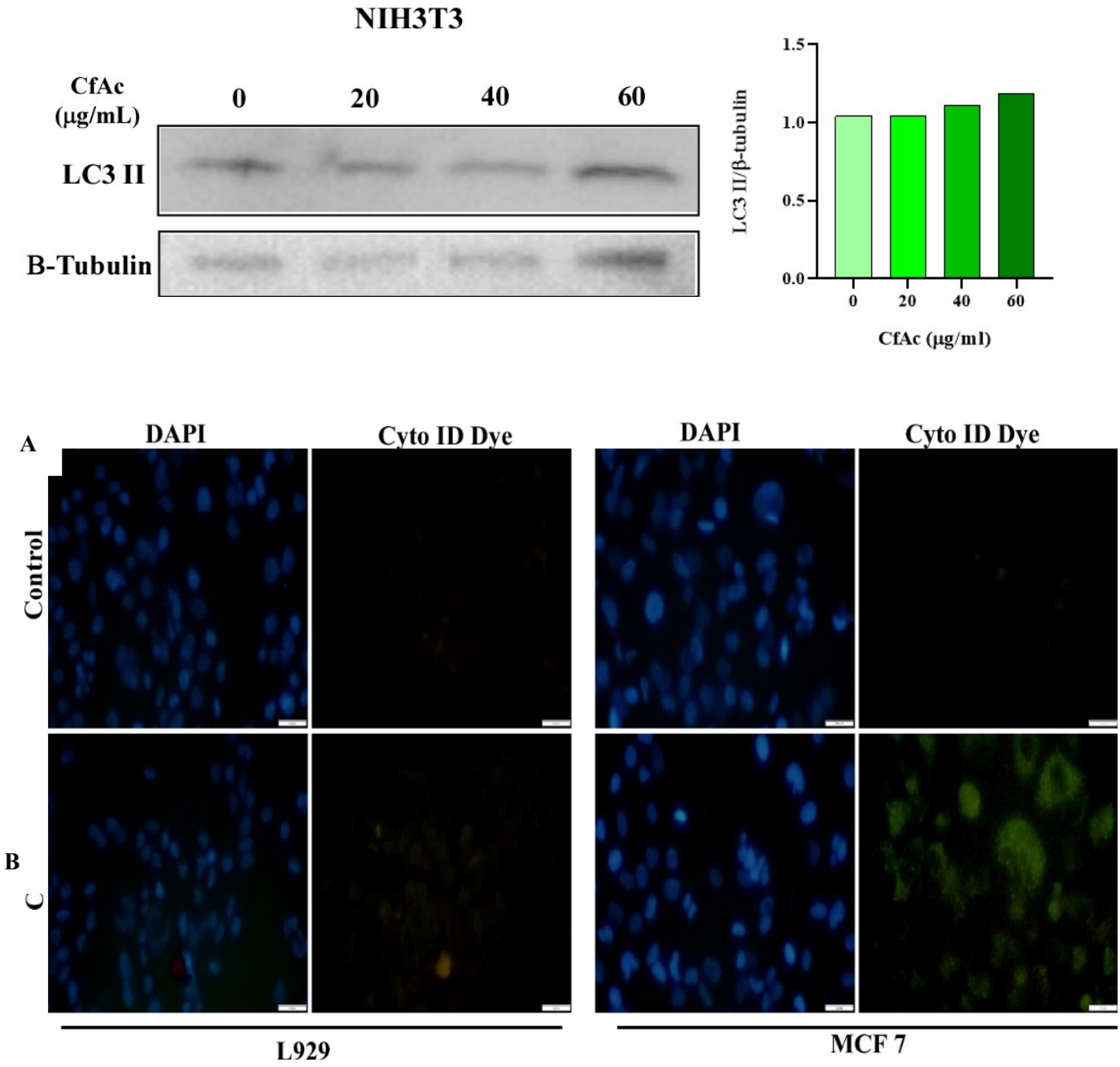
SI Figure 9: A) Apoptosis assay in MCF-7 cell lines after incubation with NLPs and exposure to NIR light (808nm, 650 mW). B) Fluorescence intensity of dead cells (MCF-7) incubated with NLPs/NIR light.

B

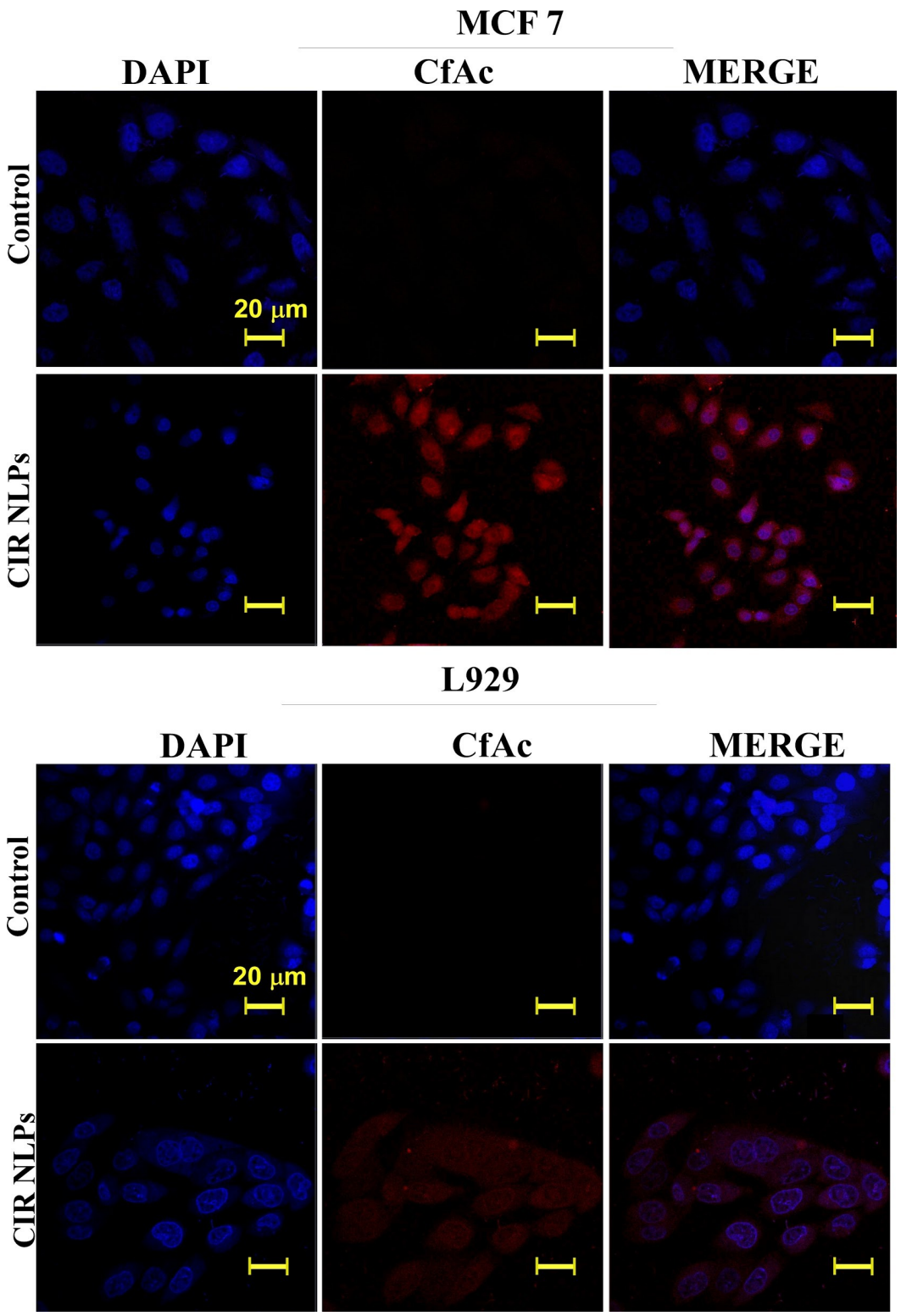




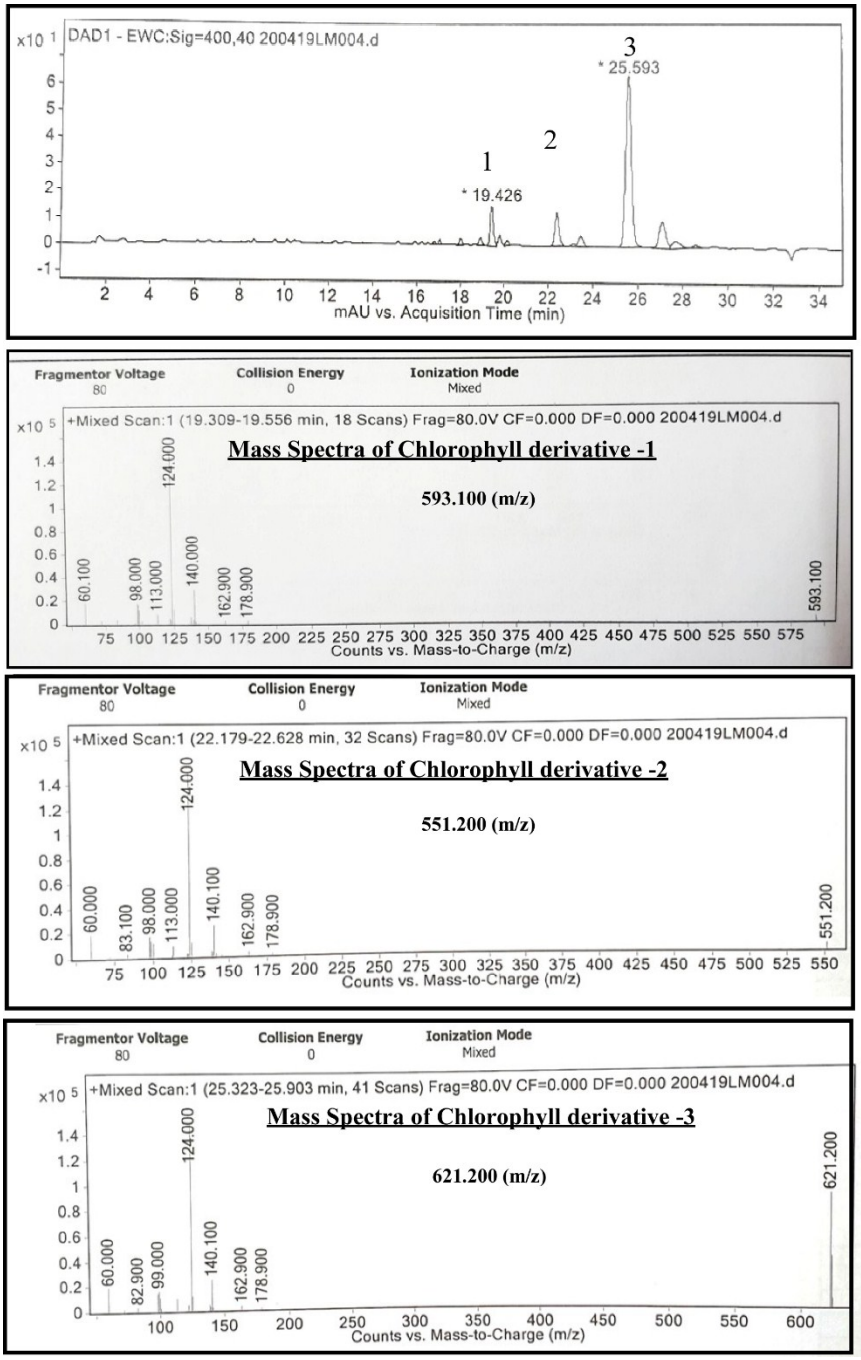
SI Figure 10: A) Cell viability by alamar blue assay B) ROS assay (DCF-DA) measured in 4T1 cell line after exposure to NLPs/NIR light. C) Interplay of ROS and cell viability in 4T1 cell line. D) Bar graph indicating the viability upon ROS inhibition by NAC in 4T1 cell line.



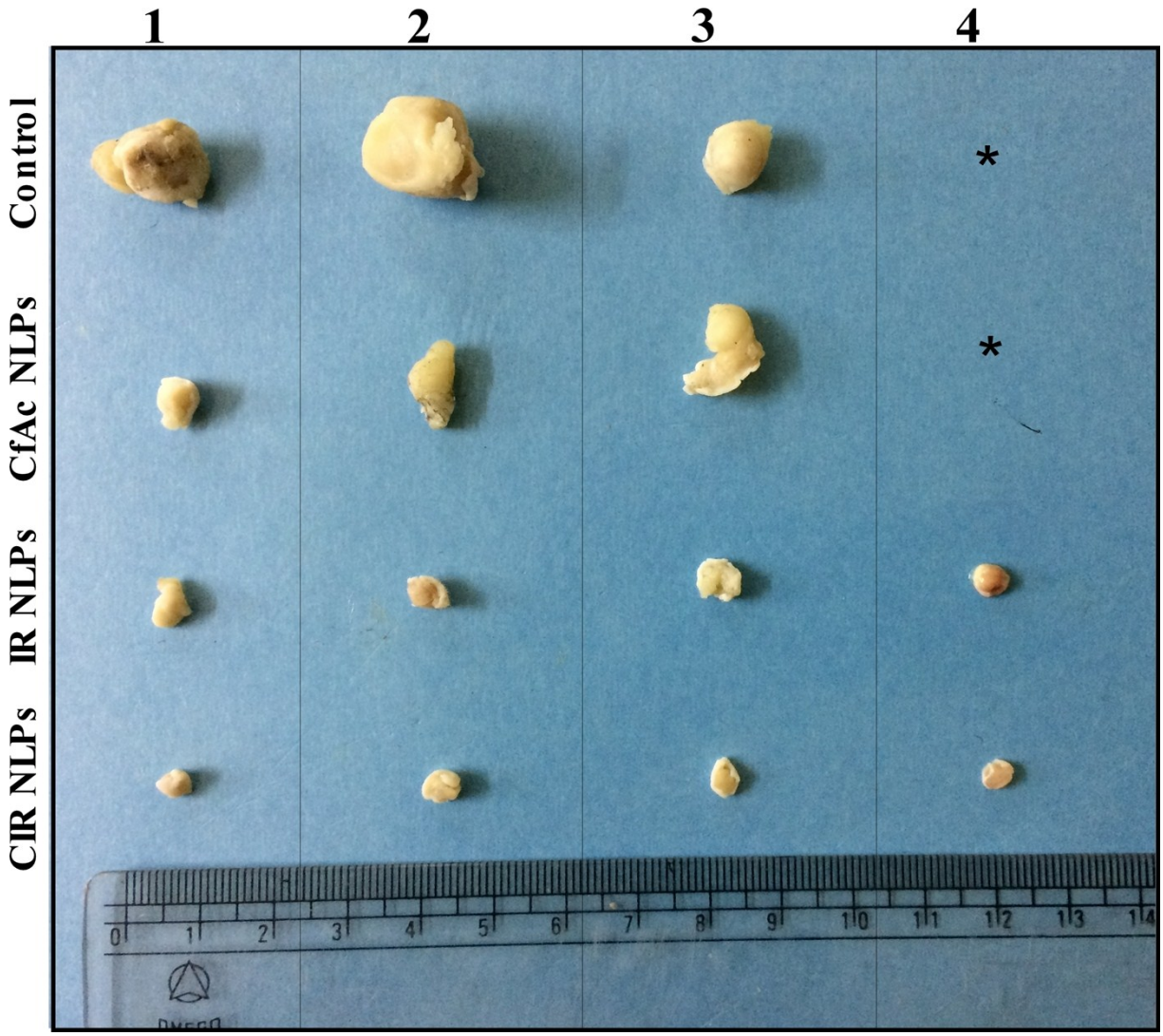
SI Figure 11A: Immunoblot images and quantified bar graphs indicating the expression of LC3 protein in NIH3T3 cell line**. B)** Effect of CfAc in modulating autophagy effect (LC3) in normal (L929) and cancerous (MCF 7) cells. The green fluorescence indicates the amount of autophagic flux in the cells.



SI Figure 11C: Cellular uptake of CIR NLPs by MCF 7 and L929 cell lines. The red fluorescence indicates the amount of CfAc in the cells.

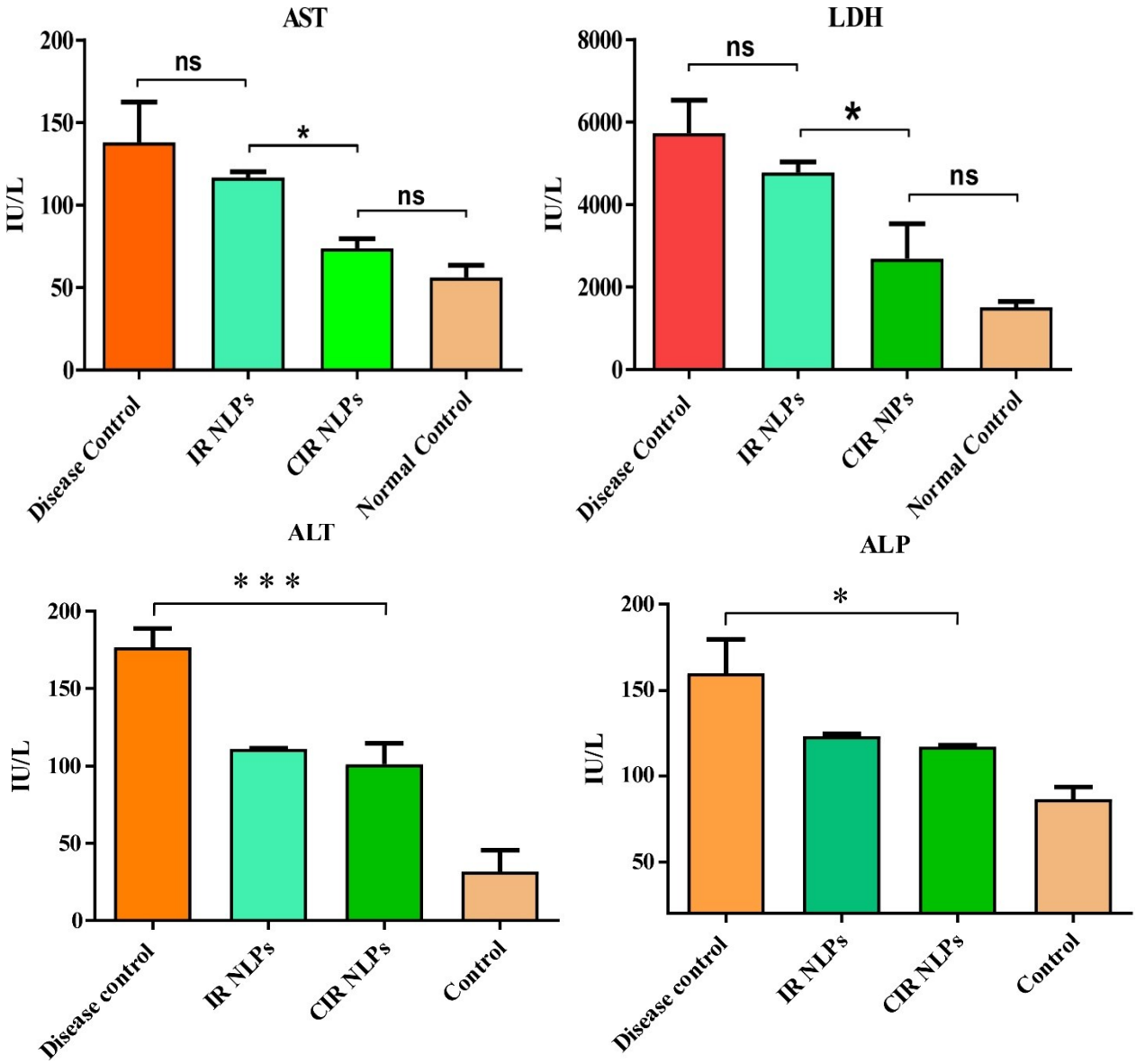


SI Figure 12: i**)** Liquid chromatography spectra studies of CfAc (1, 2, 3 denotations indicate different compounds in the CfAc). ii, iii & iv**)** Mass spectra of the Chlorophyll derivative compounds indicating their individual mass**.**



SI Figure 13A: Mice tumor images after treatment with NLPs (CfAc, IR & CIR).

(∗The tumor of the mouse had grown beyond the recovery phase and hence was excluded from the study)



SI Figure 13B. Effect of NLPs on various serum Parameters A) AST, B) LDH, C) ALT and D) ALP.

S.No.	Parameter	D.Control (IU/L)	IR NLPs (IU/L)	CIR NLPs (IU/L)	Control (IU/L)	Normal Range (IU/L) ⁎SI Ref :(1-3)
1	Lactate Dehydrogenase (LDH)	5730.18± 461.35	4774.22± 148.50	2680.50± 600.11	1500.08± 99.97	1000-2000
2	Aspartate Aminotransferase (AST)	137.97 ± 14.07	116 13 ± 2.06	73.65 ± 0.28	54.13±3.52	50-150
3	Alanine Aminotransferase (ALT)	176 ± 6.96	110 ± 0.29	101.16 ±9.44	31.53 ± 9.88	25-60
4	Alkaline Phosphatase (ALP)	159.83 ± 9.8	123.05 ± 0.78	116.95 ± 0.67	86.31 ± 5.17	30-130

SI Table 1: Tabular column representing levels of various serum parameters in the mice at the end of study.

⁎Supporting Information References:

1. Sher Y, Hung M. Blood AST, ALT and UREA/BUN Level Analysis. Bio-protocol. 2013;3(19):e931.
2. Mohd Tamsir N, Mohd Esa N, Shafie NH, Hussein MZ, Hamzah H, Abdullah MA. The Acute Effects of Oral Administration of Phytic Acid-Chitosan-Magnetic Iron Oxide Nanoparticles in Mice. International journal of molecular sciences. 2019;20(17):4114.
3. Liu W-X, Jia F-L, He Y-Y, Zhang B-X. Protective effects of 5-methoxypsoralen against acetaminophen-induced hepatotoxicity in mice. World Journal of Gastroenterology: WJG. 2012;18(18):2197.