

Supporting Information

Generation of GPC-3-Targeted Radicals for Precise Therapy of Hepatocellular Carcinoma

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#Wanting Hao and Meng Zhang contributed equally to this work.

Table

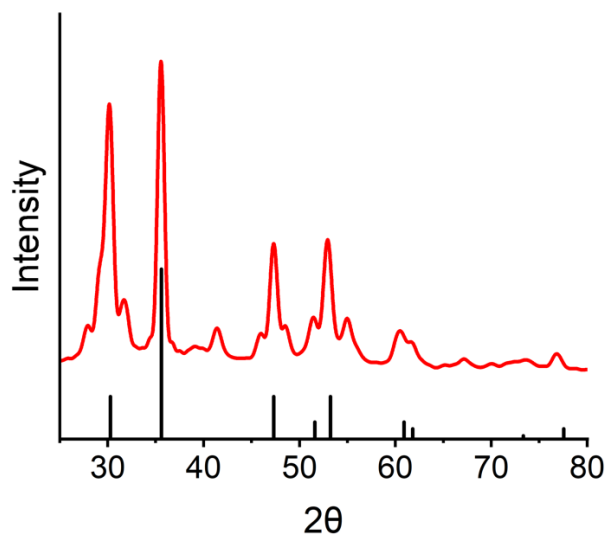
Supplementary Table S1. The loading ratio and encapsulation efficiency of dCPO and TA.

Samples	1	2	3	Average
Encapsulated dCPO / μ mol	12.56	13.42	11.96	/
Total dCPO for production / μ mol	15	15	15	/
Encapsulation efficiency (dCPO) %	83.7%	89.5%	79.7%	84.3%
Encapsulated TA / μ mol	1.85	1.76	2.04	/
Total TA for production / μ mol	2.53	2.53	2.53	/
Encapsulation efficiency (TA) %	73.3%	69.4%	80.7%	74.5%
Total lipids for production / μ mol	33	33	33	/
Loading ratio (dCPO) (% , w/w)	3.66	3.92	3.45	3.68
Loading ratio (TA) (% , w/w)	12.73	12.16	13.89	12.93

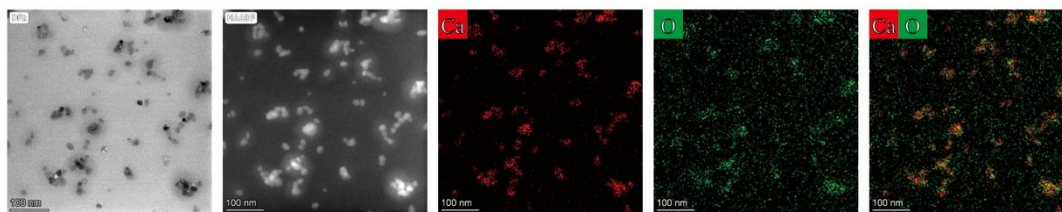
Supplementary Table S2. Semi-quantitative histopathology scoring of major organs by three blinded pathologists following treatment.

Organ	e-LNP			TA LNP			dCPO LNP			TA/dCPO LNP		
Heart	0	0	0	1	0	0	1	1	1	0	0	0
Liver	1	0	0	0	0	0	1	2	1	0	0	0
Spleen	0	0	0	1	1	0	1	0	0	0	1	0
Lung	0	1	0	0	0	1	0	0	0	0	0	0
Kidney	1	0	0	0	0	0	0	1	0	0	0	0

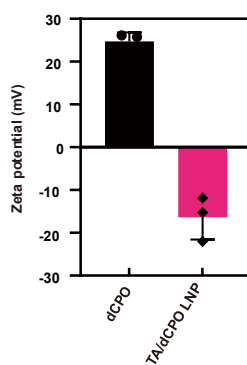
Supplementary Figures



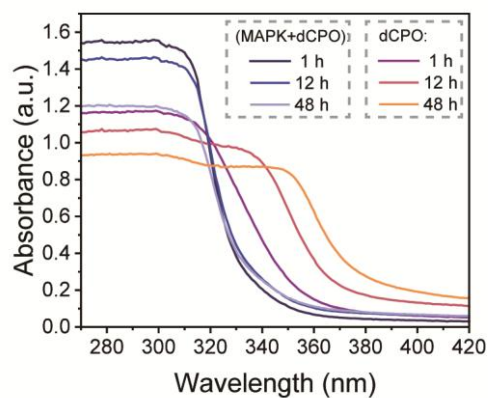
Supplementary Figure 1. XRD spectrum of CPO NPs.



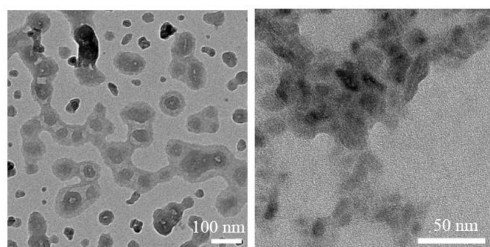
Supplementary Figure 2. EDS elemental mapping images of CPO NPs.



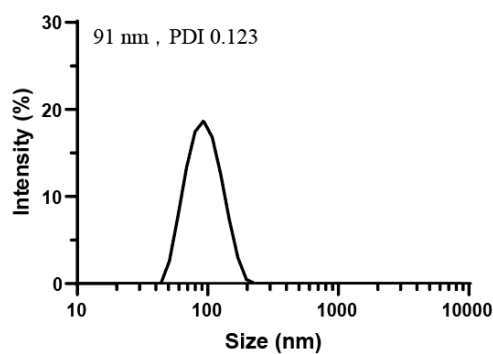
Supplementary Figure 3. Zeta-potential analysis of dCPO NPs and TA/dCPO LNP. Data are presented as mean $\pm$ SD (n=3 independent experiments).



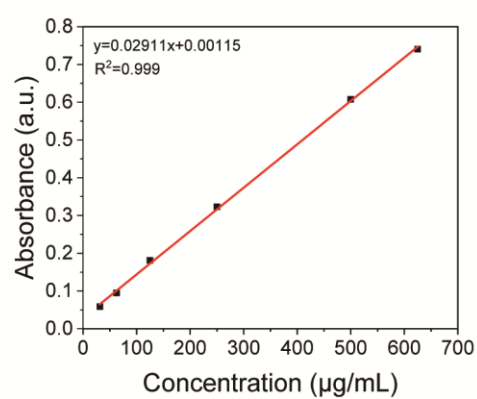
Supplementary Figure 4. TA degradation measurement over time when TA was mixed with dCPO or MAPK+dCPO.



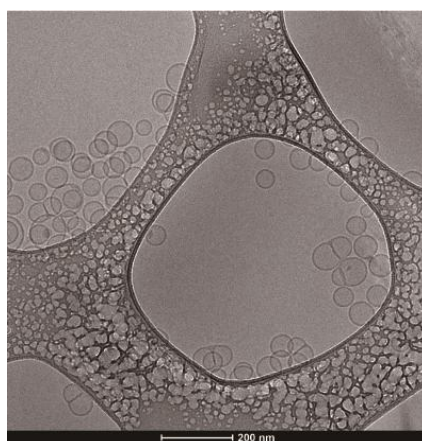
Supplementary Figure 5. TEM images of the mixture of dCPO+TA (left) and MAPK+dCPO+TA (right).



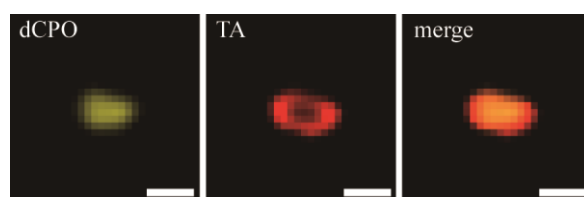
Supplementary Figure 6. Size distribution of the TA LNPs by DLS.



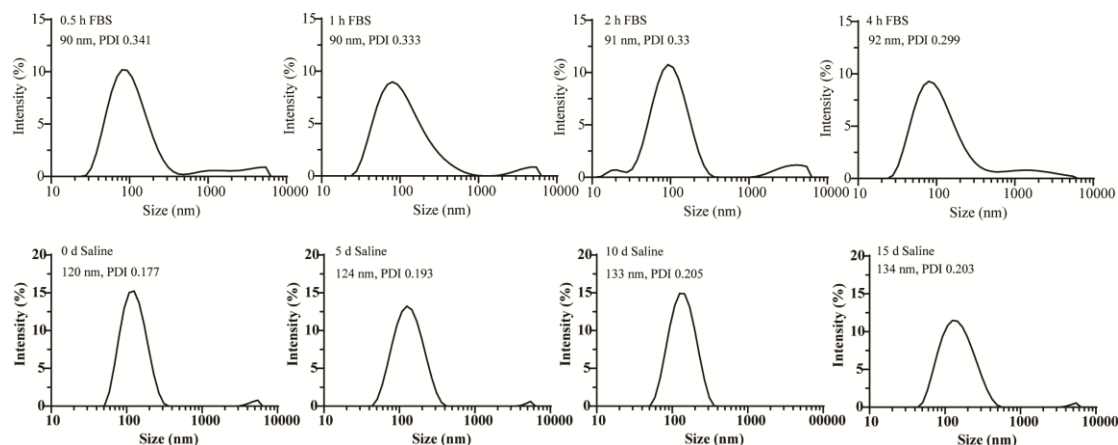
Supplementary Figure 7. Calibration curve of the UV-vis absorbance intensity versus standard TA concentrations.



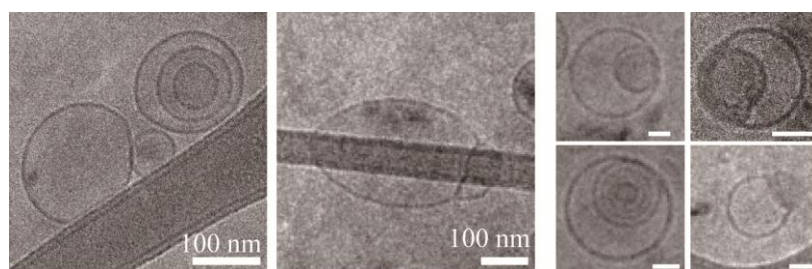
Supplementary Figure 8. Cryo-TEM image of TA LNP.



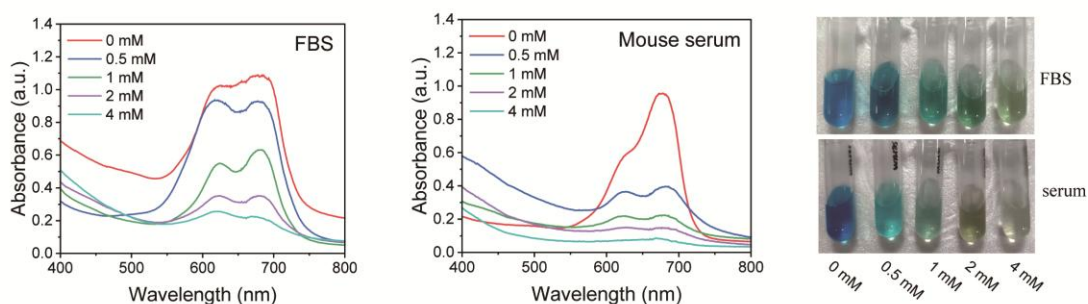
Supplementary Figure 9. High-resolution confocal fluorescence images of the double labeled TA/dCPO LNP (yellow - sulforhodamine B-labeled dCPO; red - Cy5-labeled TA). Scale bar: 500 nm.



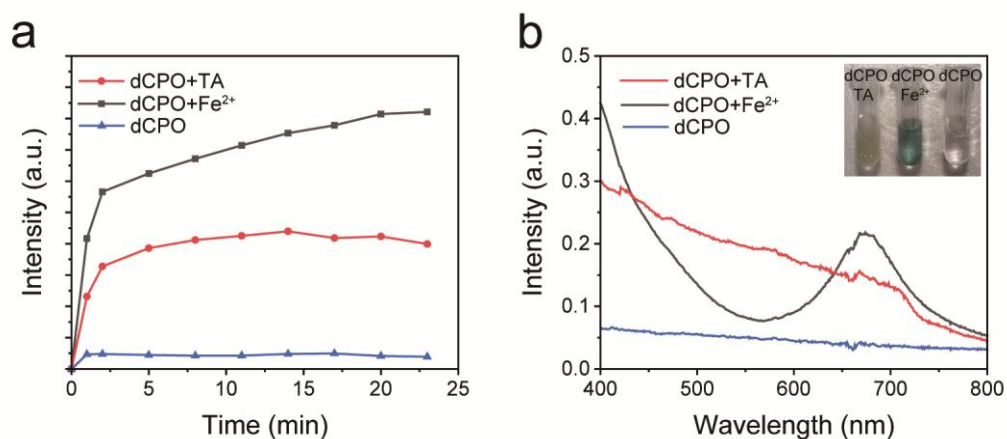
Supplementary Figure 10. Size distribution of the TA/dCPO LNP in physiological saline over a 15-day period by DLS (down), and in FBS-containing physiological fluids within 4 h (up), a duration representative of typical systemic circulation time for nanoparticles prior to tissue accumulation.



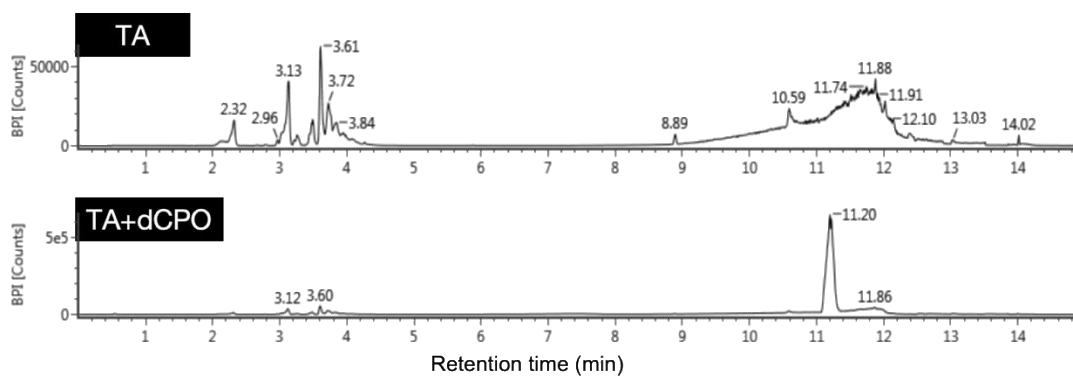
Supplementary Figure 11. Cryo-TEM images of dCPO/TA LNPs treated in PBS medium, showing oval or eccentric morphologies with structural defects. Scale bar: 50 nm (right).



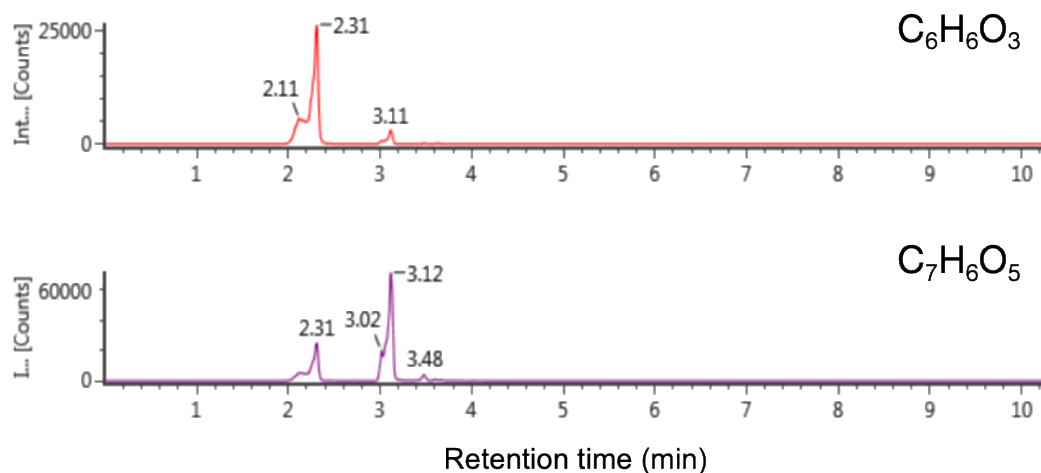
Supplementary Figure 12. UV-vis spectroscopy curves of MB solution in serum-containing environments, following reaction with dCPO NPs at different concentrations in the presence of TA.



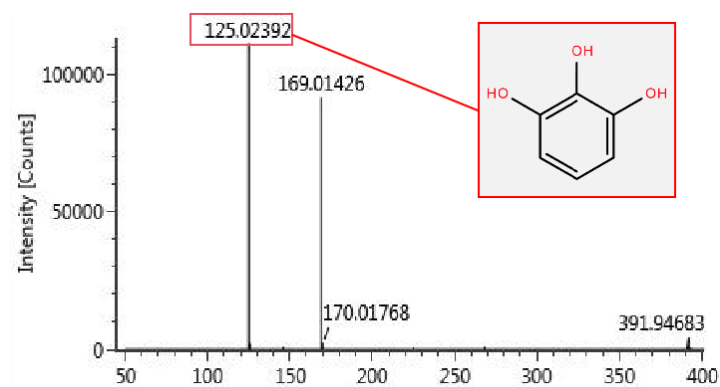
Supplementary Figure 13. a) The oxidation kinetics of TMB induced by hydroxyl radicals (generated from dCPO and Fe²⁺), phenoxyl radicals (GA•) (produced by the dCPO and TA) or dCPO alone. b) UV-vis spectra of TMB oxidation induced by dCPO+TA, dCPO+Fe²⁺ or dCPO alone at 23 min.



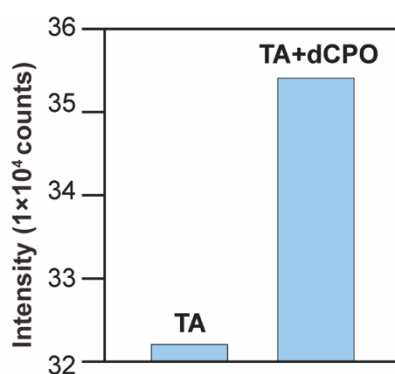
Supplementary Figure 14. Total Ion Chromatogram of the TA and TA+dCPO groups.



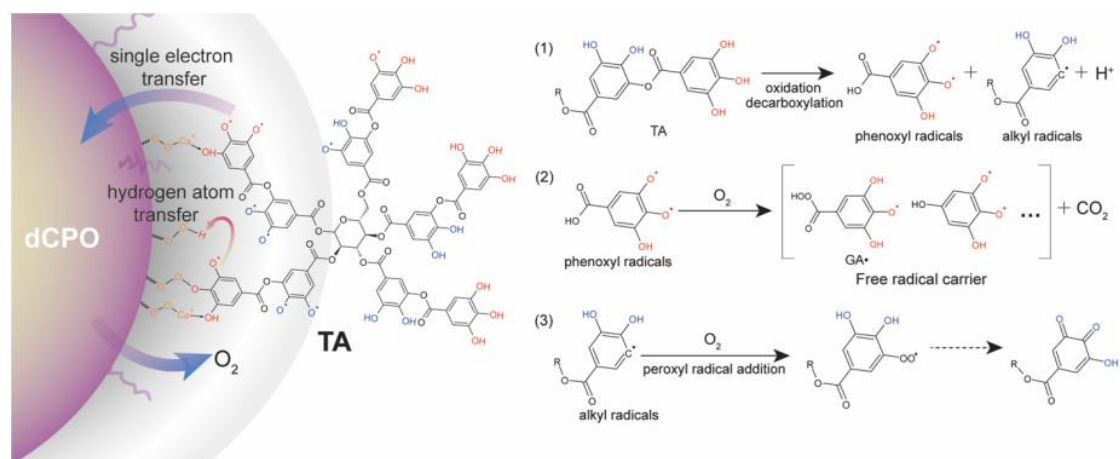
Supplementary Figure 15. HPLC analysis of the TA/dCPO groups detected peaks corresponding to $C_6H_6O_3$ (retention time = 2.31 min) and $C_7H_6O_5$ (retention time = 3.12 min) in the chromatograms.



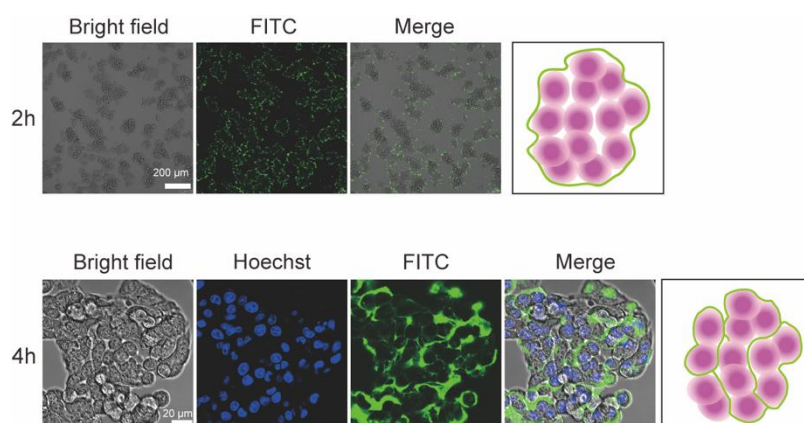
Supplementary Figure 16. HPLC-MS chromatograms of the TA/dCPO groups showed peaks at retention times of 2.31 min.



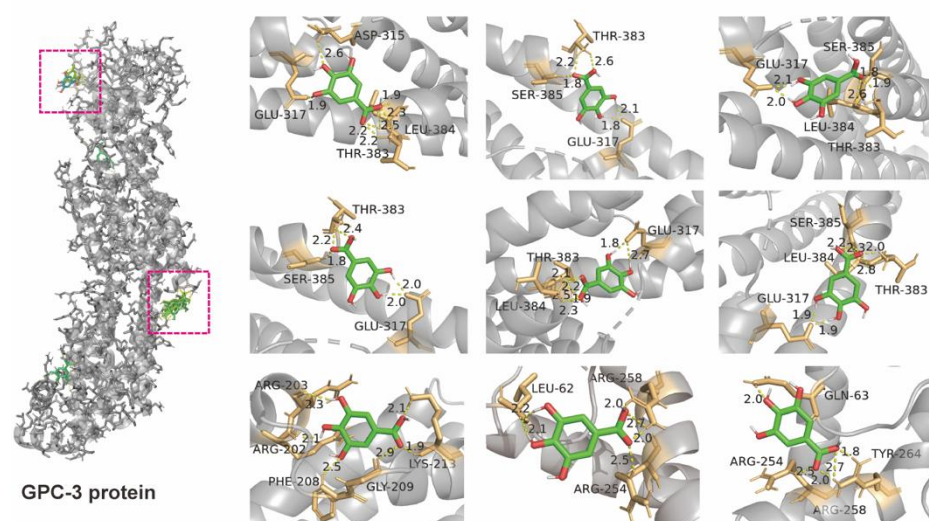
Supplementary Figure 17. Integration of the peak at retention times of 3.12 min.



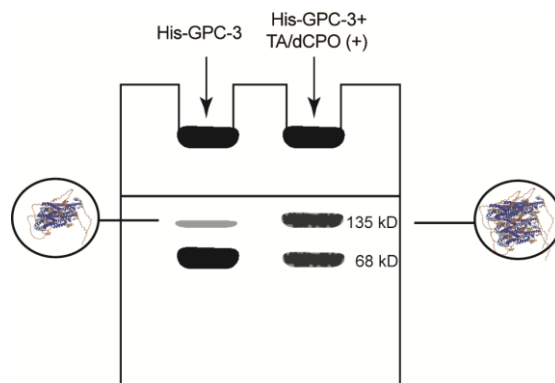
Supplementary Figure 18. Chemical reaction mechanism of the TA/dCPO system.



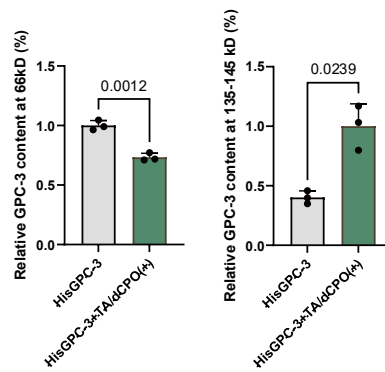
Supplementary Figure 19. The interaction of TA/dCPO LNPs with cellular membranes.



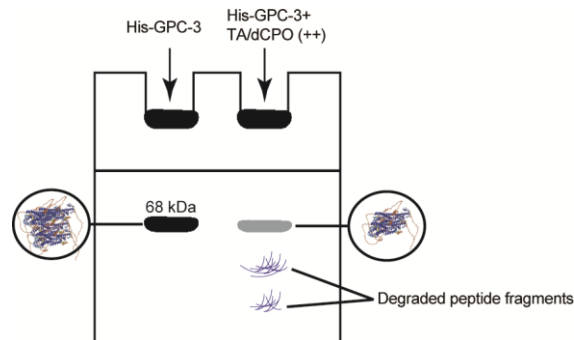
Supplementary Figure 20. Molecular docking simulation of GPC-3 protein and GA.



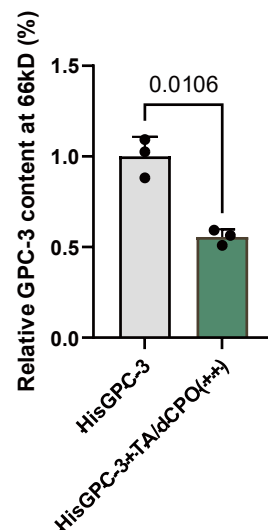
Supplementary Figure 21. Schematic demonstration of the electrophoresis for His-GPC-3 protein treated with low dose of TA/dCPO complex.



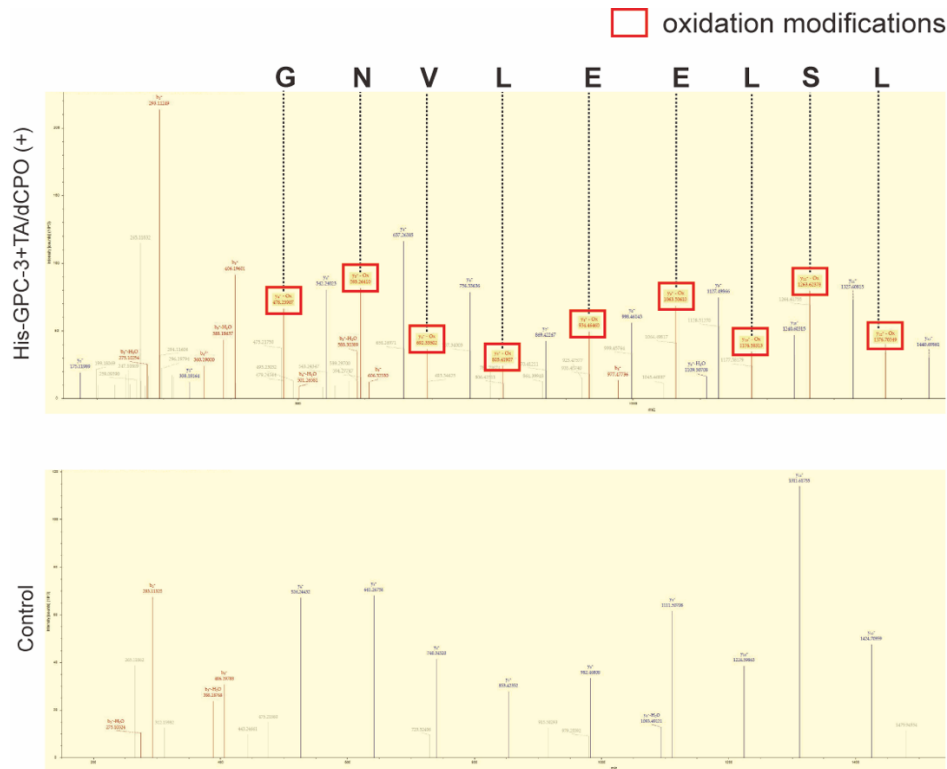
Supplementary Figure 22. Quantification of Coomassie blue staining of purified recombinant proteins His GPC-3 with or without treatment with a low-dose TA/dCPO complex ($n = 3$ independent experiments). Data are presented as mean $\pm$ SD. Statistical significance was determined using Welch's t test.



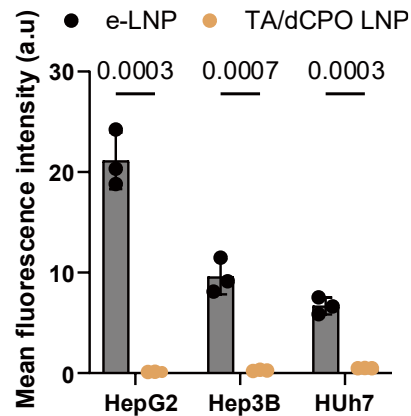
Supplementary Figure 23. Schematic demonstration of the electrophoresis for His-GPC-3 protein treated with high dose of TA/dCPO complex.



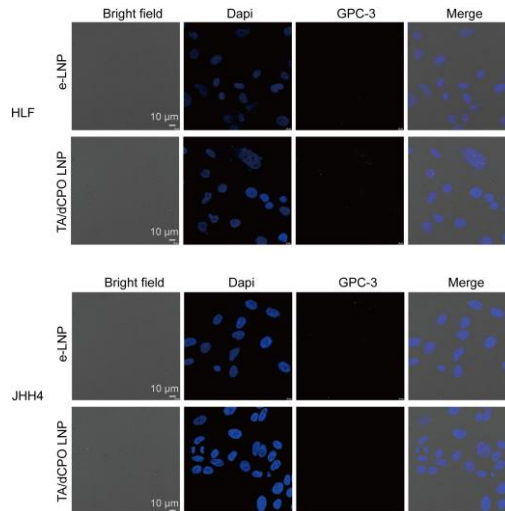
Supplementary Figure 24. Quantification of Coomassie blue staining of purified recombinant proteins His GPC-3 with or without treatment with a high-dose TA/dCPO complex ($n = 3$ independent experiments). Data are presented as mean $\pm$ SD. Statistical significance was determined using Welch's t test.



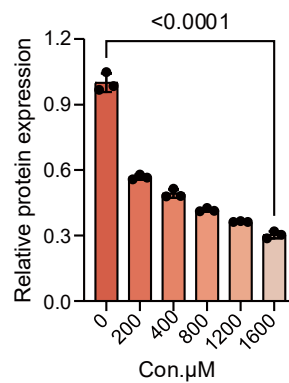
Supplementary Figure 25. The second-order mass spectrum of His-GPC-3 protein when treated with low dose of TA/dCPO complex. The oxidated amino acids were marked at the top.



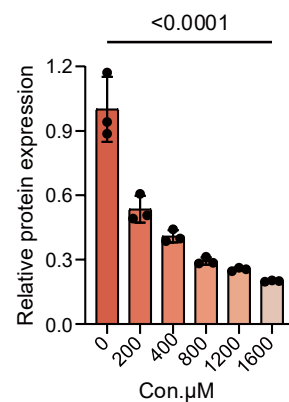
Supplementary Figure 26. Quantification analysis of mean fluorescent intensities of Confocal images of HepG2, Hep3B and Huh7 cells treated with empty LNPs or TA/dCPO LNPs, respectively, following by immunofluorescence staining of GPC-3 ($n=3$ independent experiments). Data are presented as mean $\pm$ SD. Statistical significance was determined using Welch's t test.



Supplementary Figure 27. Confocal images of HLF and JHH4 cells treated with empty LNPs or TA/dCPO LNPs, respectively, following by immunofluorescence staining of GPC-3.

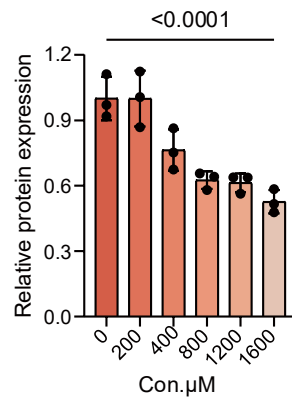


Supplementary Figure 28. The expression levels of GPC-3 proteins in HepG2 cells following treatment with e-LNPs or TA/dCPO LNPs ($n = 3$ independent experiments). Data are presented as mean $\pm$ SD. Statistical significance was determined using one-way ANOVA with Dunnett's multiple comparisons test.

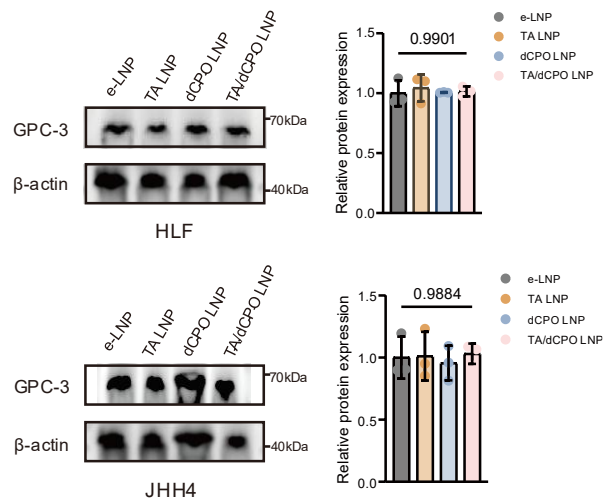


Supplementary Figure 29. The expression levels of GPC-3 proteins in Hep3B cells following treatment with e-LNPs or TA/dCPO LNPs ($n = 3$ independent experiments).

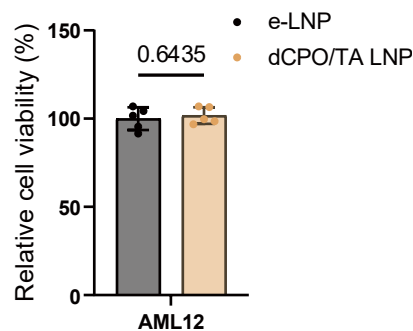
Data are presented as mean $\pm$ SD. Statistical significance was determined using one-way ANOVA with Dunnett's multiple comparisons test.



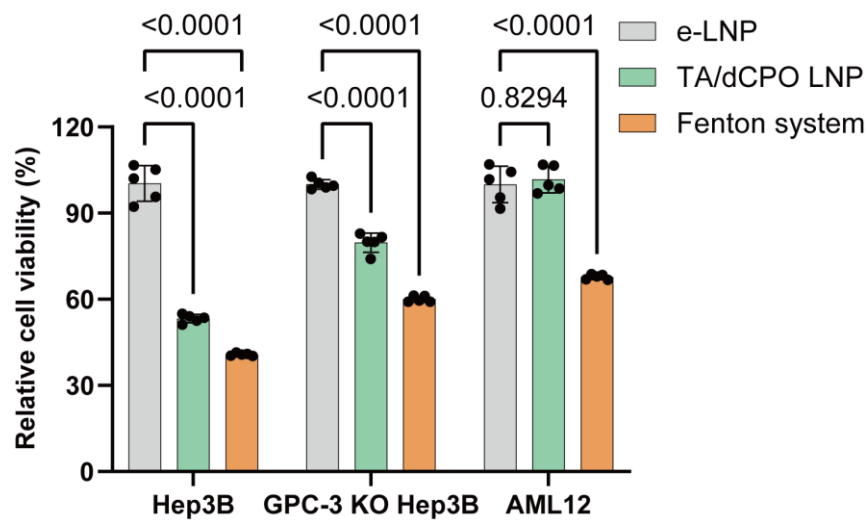
Supplementary Figure 30. The expression levels of GPC-3 proteins in Huh7 cells following treatment with e-LNPs or TA/dCPO LNPs ($n = 3$ independent experiments). Data are presented as mean $\pm$ SD. Statistical significance was determined using one-way ANOVA with Dunnett's multiple comparisons test.



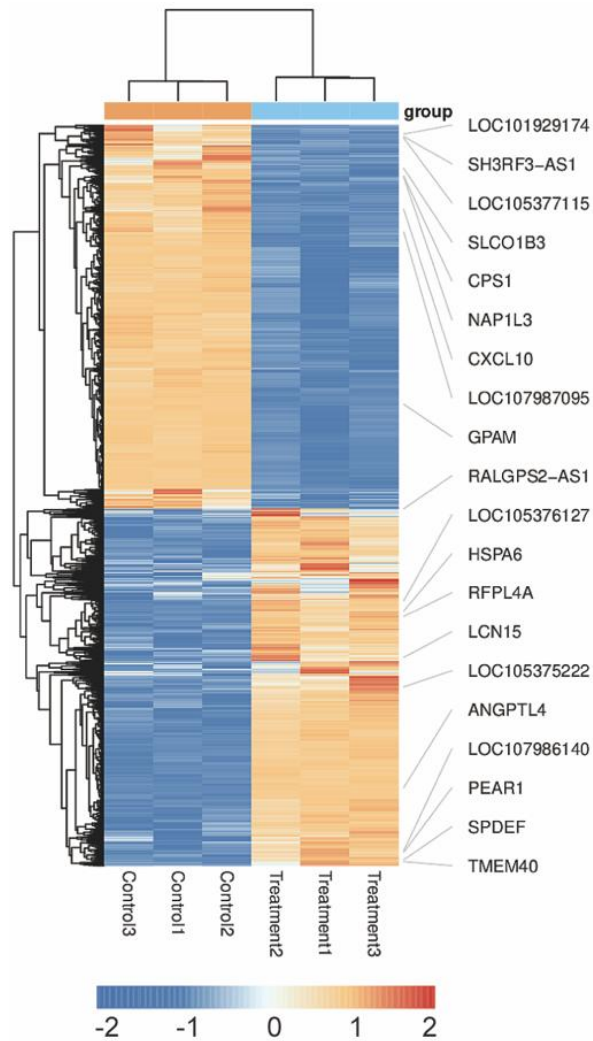
Supplementary Figure 31. Western blotting and quantification analysis in HLF and JHH4 cells after the varied treatments ($n = 3$ independent experiments). Data are presented as mean $\pm$ SD. Statistical significance was determined using one-way ANOVA with Dunnett's multiple comparisons test.



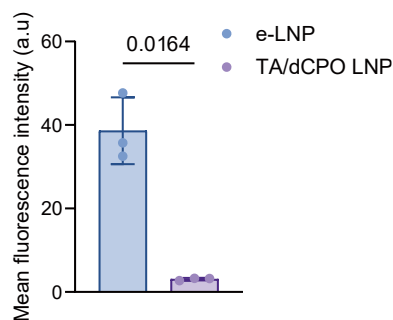
Supplementary Figure 32. Cell viability of AML12 cells after incubation with empty LNP or TA/dCPO LNPs ($n = 5$ independent experiments). Data are presented as mean $\pm$ SD. Statistical significance was determined using Welch's t test.



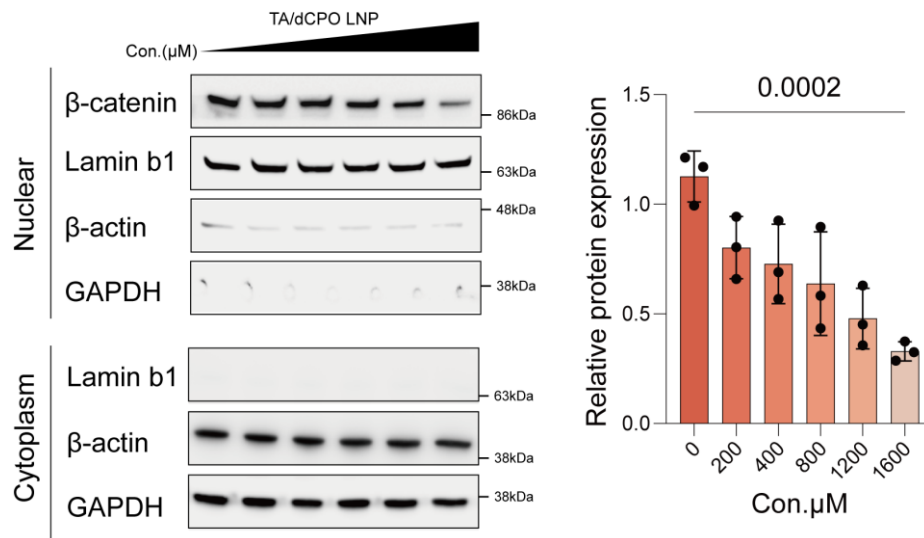
Supplementary Figure 33. Cell viability of Hep3B, GPC-3 KO Hep3B and AML12 cells after incubation with e-LNP, TA/dCPO LNP or Fenton system ($n = 5$ independent experiments). Data are presented as mean $\pm$ SD. Statistical significance was determined using one-way ANOVA with Dunnett's multiple comparisons test.



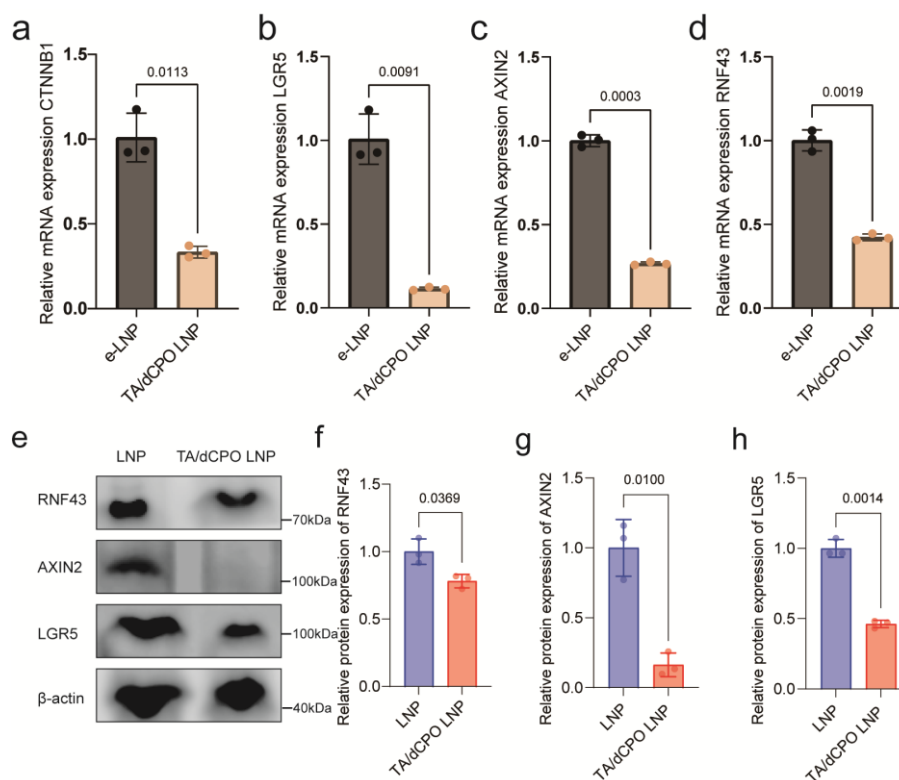
Supplementary Figure 34. Heat map of differentially expressed genes between control group and TA/dCPO LNPs treated group.



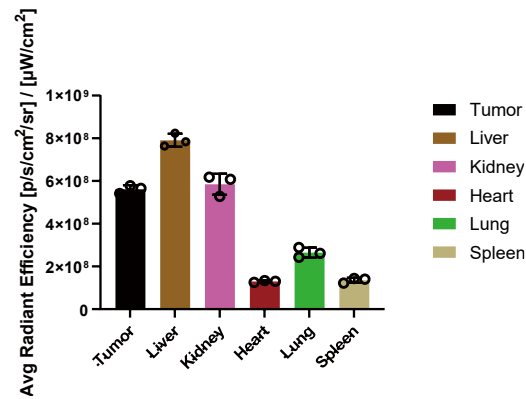
Supplementary Figure 35. Quantification analysis of mean fluorescent intensities of Confocal images of HepG2 cells treated with empty LNPs or TA/dCPO LNPs, respectively, following by immunofluorescence staining of active β -catenin ($n = 3$ independent experiments). Data are presented as mean $\pm$ SD. Statistical significance was determined using one-way ANOVA with Dunnett's multiple comparisons test.



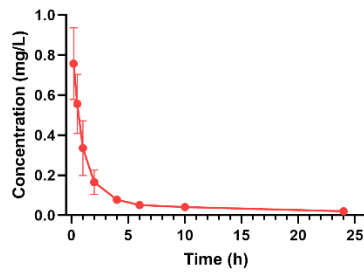
Supplementary Figure 36. Western blot analysis of nuclear β-catenin expression in Hep3B cells following treatment with various concentrations of TA/dCPO LNPs ($n = 3$ independent experiments). Data are presented as mean $\pm$ SD. Statistical significance was determined using one-way ANOVA with Dunnett's multiple comparisons test.



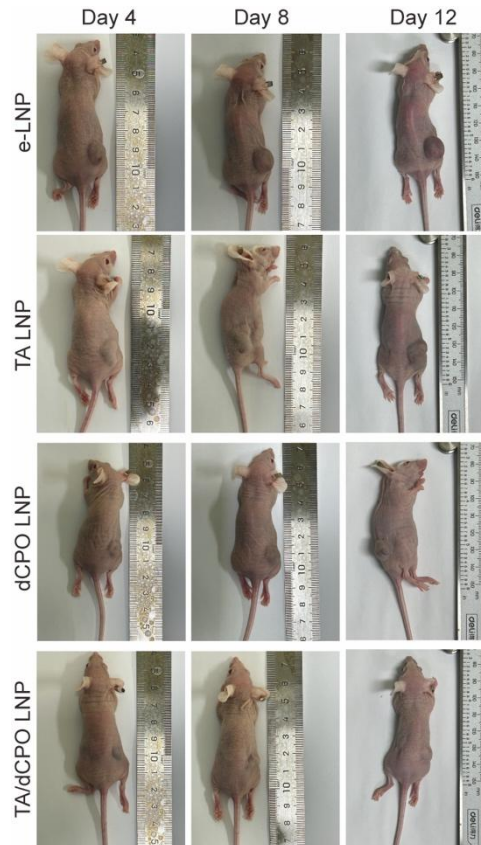
Supplementary Figure 37. a-d) qRT-PCR analysis of CTNNB1, LGR5, AXIN2 and RNF43 mRNA levels in Hep3B cells after TA/dCPO LNP treatment ($n = 3$ independent experiments). e-h) Western Blot and quantification analysis of LGR5, AXIN2, and RNF43 after TA/dCPO LNP treatment ($n = 3$ independent experiments). Data are presented as mean $\pm$ SD. Statistical significance in (a-d, f-h) was determined using Welch's t test.



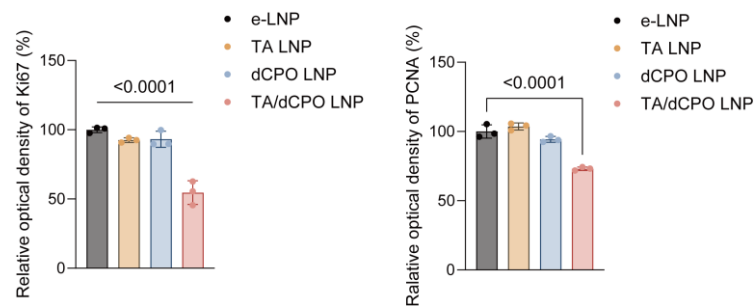
Supplementary Figure 38. Quantification of fluorescence signals from major organs harvested 8 hours after intravenous injection of TA/dCPO LNPs ($n = 3$ independent experiments). Data are presented as mean $\pm$ SD.



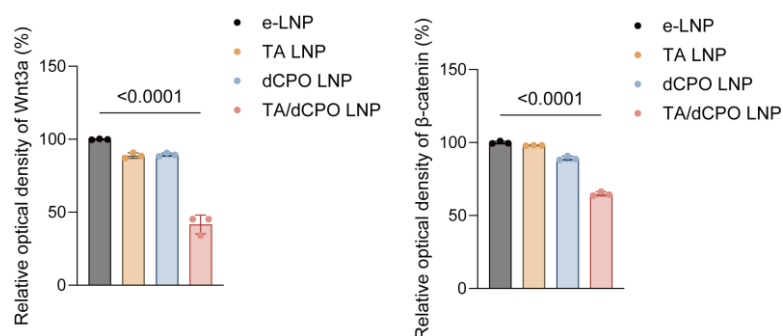
Supplementary Figure 39. Blood half-life of TA/dCPO LNPs in tumor-bearing mice based on Ca levels measured by ICP-OES ($n = 3$ independent experiments). Data are presented as mean $\pm$ SD.



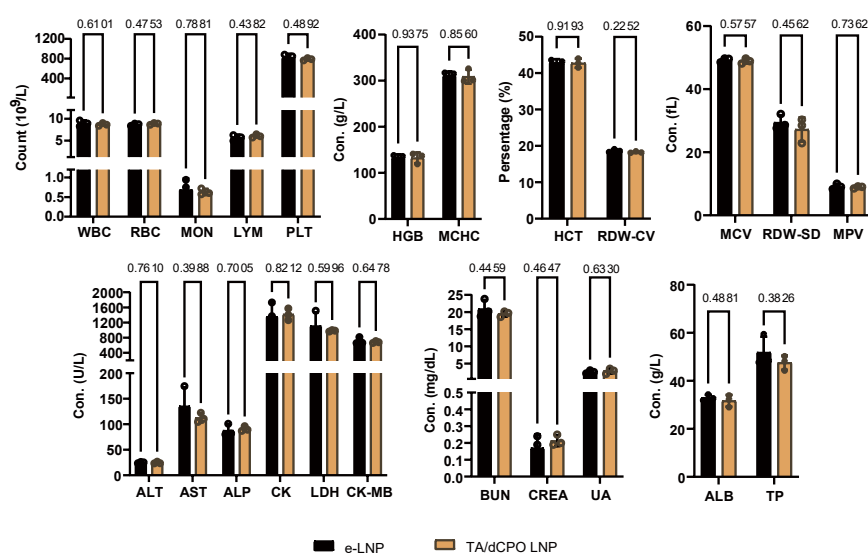
Supplementary Figure 40. Representative photos of mice treated with various LNPs during therapeutic period.



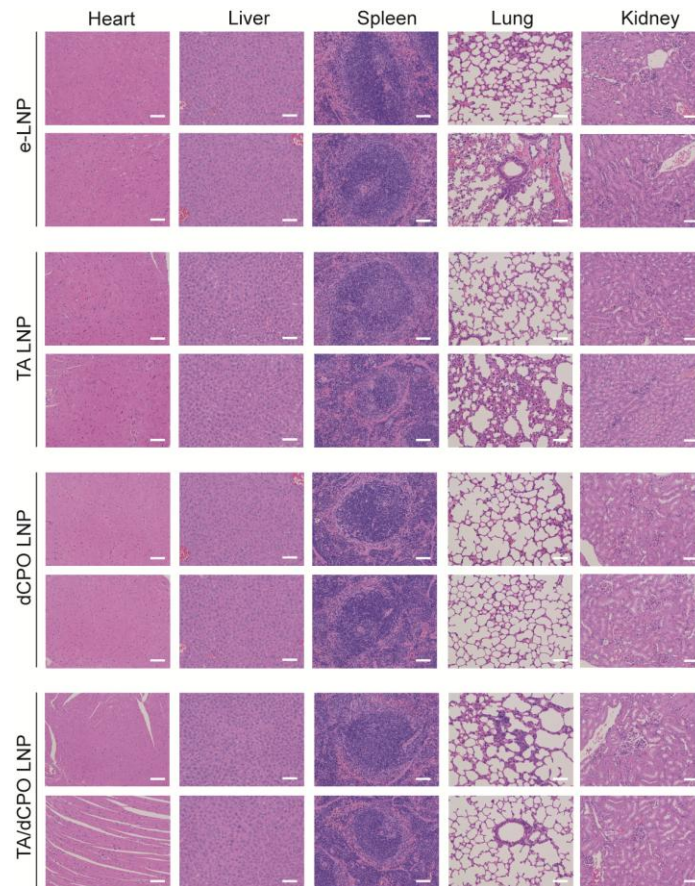
Supplementary Figure 41. Quantification analysis of mean intensities of images of tumor tissues treated with various LNPs, respectively, following by Immunohistochemistry of Ki67 and PCNA ($n = 3$ independent experiments). Data are presented as mean $\pm$ SD. Statistical significance was determined using one-way ANOVA with Dunnett's multiple comparisons test.



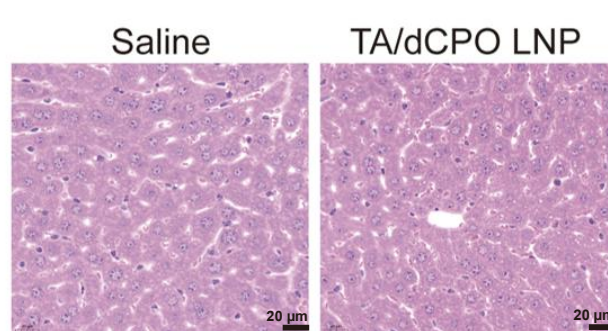
Supplementary Figure 42. Quantification analysis of mean intensities of images of tumor tissues treated with various LNPs, respectively, following by Immunohistochemistry of Wnt3a and β -catenin ($n = 3$ independent experiments). Data are presented as mean $\pm$ SD. Statistical significance was determined using one-way ANOVA with Dunnett's multiple comparisons test.



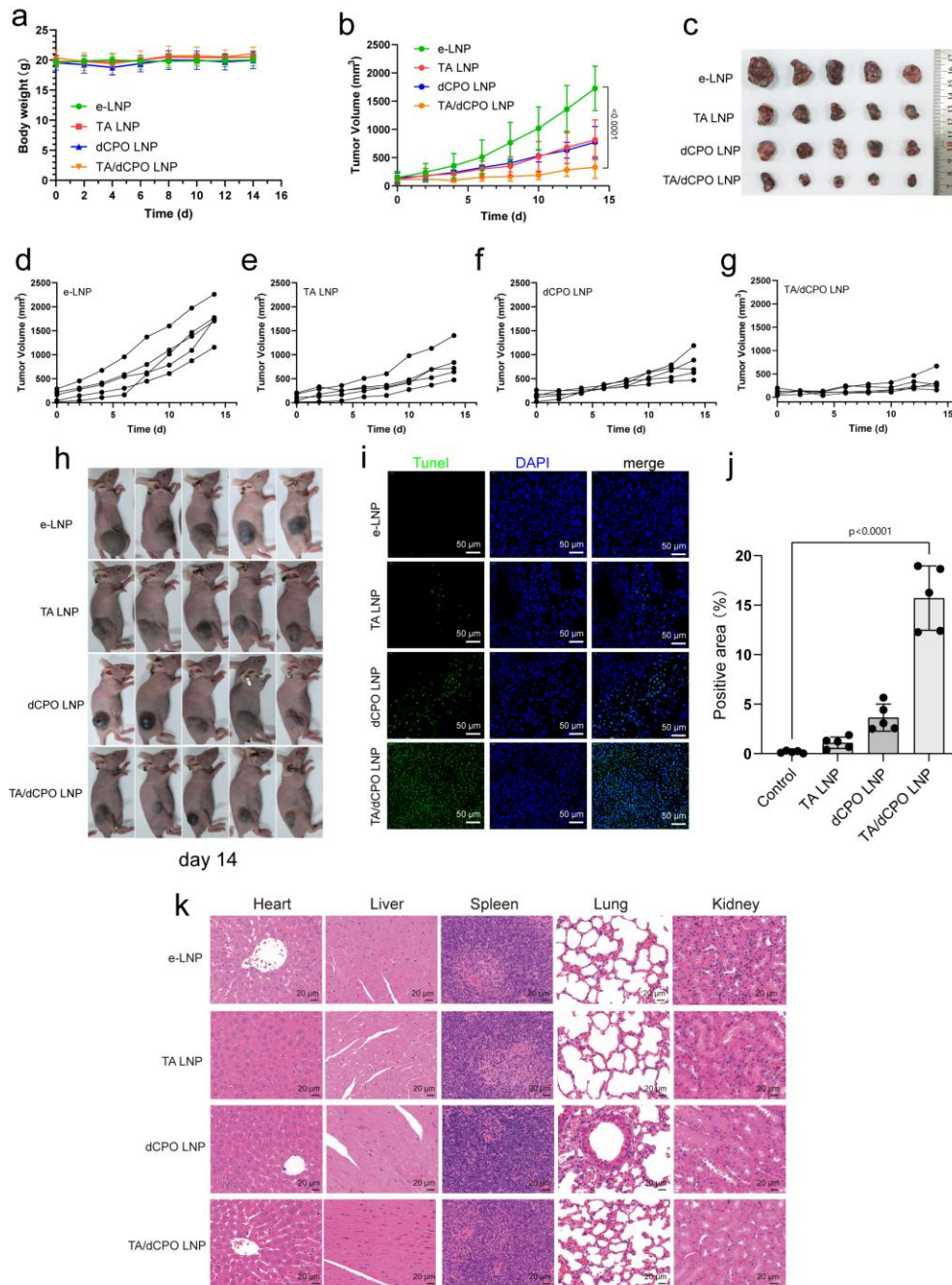
Supplementary Figure 43. Blood-biochemical analysis, levels of serum liver function markers and serum kidney function markers of mice with intravenous injection of TA/dCPO LNPs ($n = 3$ independent experiments). Data are presented as mean $\pm$ SD. Statistical significance was determined using Welch's t test.



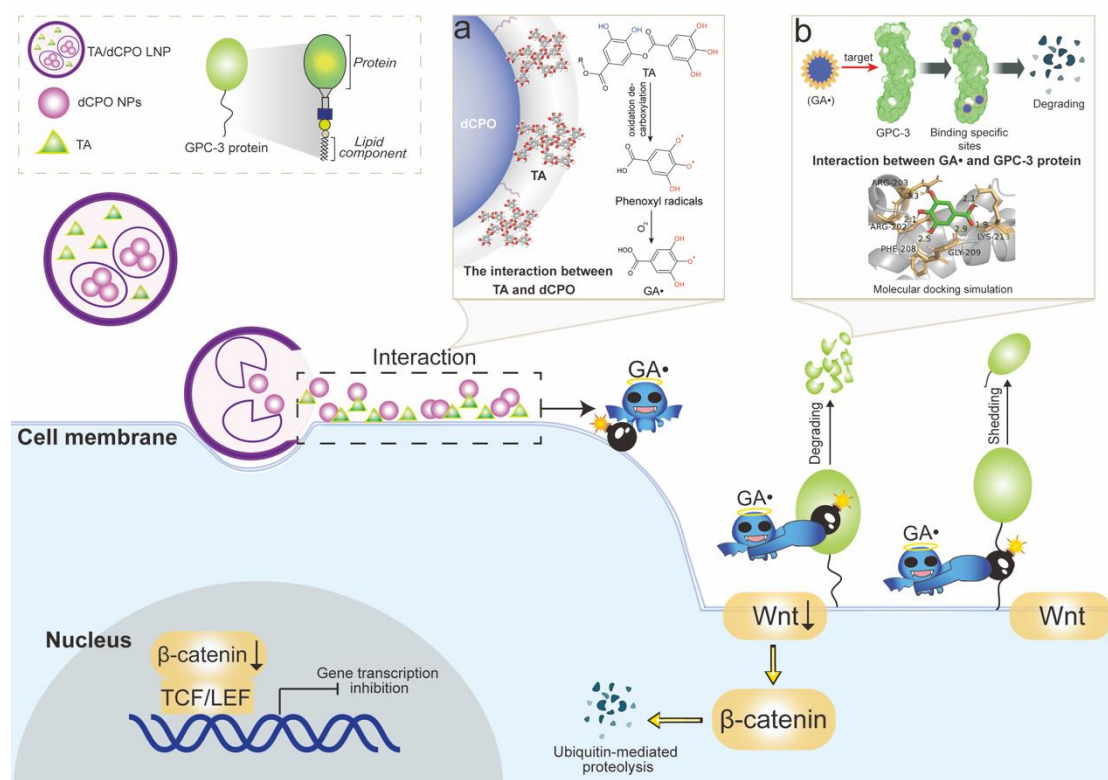
Supplementary Figure 44. H&E staining of major organs from different tissue sections treated with varied LNPs agents. Scale bar, 100 μm .



Supplementary Figure 45. H&E staining of normal liver tissue after treated with saline or TA/dCPO LNP.



Supplementary Figure 46. a) Mice body weight changes during the treatment period. b) Tumor volume monitoring at different time intervals. c) Representative photos of tumor tissue on day 14 after different treatments. d-g) Relative tumor volumes plot with different treatment during the therapeutic process ($n=5$; Ca: 4.5 mg kg^{-1} for i.v. injection). h) Representative photos of mice treated with various LNPs at day 14. i) TUNEL staining images on excised tumor tissues with various LNPs treatments and j) Quantification analysis of mean fluorescent intensities ($n = 3$ independent experiments). Data are presented as mean $\pm$ SD. Statistical significance was determined using one-way ANOVA with Dunnett's multiple comparisons test. k) H&E staining of major organs treated with varied LNPs agents.



Supplementary Figure 47. Generation of GPC-3-Targeted Radicals for Precision Hepatocellular Carcinoma Therapy. During cellular uptake, the components were released into the cellular membrane via membrane fusion, enabling their interaction. dCPO NPs served as oxidizing agents, radicalizing TA and breaking it down into phenoxyl radical-conjugated GA species (e.g. GA•). The in-situ generated GA• further bound to the active pocket of the GPC-3 protein, disrupting critical peptide segments and lipid domains, thereby triggering protein degradation and membrane shedding. This process inhibited downstream signaling pathways, such as Wnt/β-catenin, ultimately suppressing hepatocellular carcinoma proliferation. a) The chemical interaction between TA and dCPO leads to the formation of a relatively stable phenoxyl radical conjugated species (e.g. GA•). b) GA• binds to specific sites of the GPC-3 protein, as evidenced by molecular docking simulations, thereby promoting its degradation (Relative drawing materials were created in BioRender. wanting, H. (2026) <https://BioRender.com/4hk42ct>).