Supporting information

Metabolic reprogramming-targeted albumin nanoparticles combined with phototherapy for suppressing triple-negative breast cancer

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Supplementary Notes

1. Synthesis of CDM

CDM was synthesized as previously described with slight modifications.[1]

*1.1. Synthesis of compound **3***

We dissolved 2-chloromethylpyridine hydrochloride (368.2 mg, 2.2 mmol), Na_2CO_3 (471.1 mg, 4.4 mmol), and NaI (374.7 mg, 2.5 mmol) in 20 mL anhydrous acetonitrile (ACN) and the sample was stirred at room temperature. After 2 h, N-boc-ethylenediamine (163.5 mg, 1 mmol) was added to the vessel and the reaction mixture was heated at 70°C for 48 h under argon atmosphere. The solvent was removed under reduced pressure. The residue was suspended in NaOH 1N, and the organic product was extracted with dichloromethane (DCM). The DCM phase was collected and dried using anhydrous Na_2SO_4. After evaporation, a yellow liquid, compound **2**, was obtained. The Boc protection group of compound **2** was removed by adding trifluoroacetic acid (TFA) (5 ml) on an ice bath and further stirring for 3 h at room temperature. NaOH at 1 N was added to adjust the pH of solution up to 10. After a similar extraction process and evaporation as described above, a yellow liquid, compound 3, was obtained.

1.2. Synthesis of CDM

Compound **2** (72.7 mg, 0.3 mmol), IR780 iodide (105.3 mg, 0.15 mmol) and Na_2CO_3 (16.1 mg, 0.15 mmol) were dissolved in anhydrous ACN. The mixture was refluxed to 80°C for 5 h under argon atmosphere in the dark. ACN was then removed by a rotor-evaporator and DCM was added to suspend the residue. The inorganic compound was removed by washing with DW and brine three times. Anhydrous Na_2SO_4 was applied to dry the organic layer. The blue residue after removing DCM was further purified using silica column chromatography.

The solvent was evaporated under reduced pressure and washed with water and brine for three times. After adding DCM, the organic layer was extracted and dried by anhydrous sodium sulfate. The crude product was purified by silica column chromatography (DCM/ammonia 4% in MeOH/TEA = 92:8:2.5) to yield the final product CDM.

2. Synthesis of TPPLND

Briefly, lonidamine (LND) (169.0 mg, 0.5 mmol), DCC (208.4 mg, 1.0 mmol), NHS (117.4 mg, 1.0 mmol), and DMAP (124.7 mg, 1 mmol) were dissolved in 5 mL of DMSO and stirred for 24 h at room temperature. DCU precipitation was removed by centrifugation at 14,000 rcf in 30 min. The product of the first reaction, LND-NHS, in the DMSO layer was added to the solution containing (6-aminohexyl)triphenylphosphonium bromide hydrobromide (279.4 mg, 0.6 mmol) and TEA (84 μL, 0.6 mmol) in DMSO (2.5 mL) and stirred at room temperature. After another 24 h, 30 mL of DW was added to the reaction vessel and HCl 1 N was used to neutralize the mixture; the sample was held overnight at 2–8^{o}C. The white precipitation was collected by centrifugation at 14,000 rcf for 30 min and then dissolved in DCM. After 3 washes with DW and brine, the DCM layer was dried by anhydrous Na_2SO_4. After removing the organic solvent by rotor evaporator, the final product was used for further experiments without any further purification step.

3. Preparation of FITC-LIR-CCM NPs and FITC-CCM NPs

BSA-FITC has been previously described. In 5 mL of PBS solution (10 mM, pH 7.4), 50 mg of BSA and BSA-FITC at a ratio of 9:1 (w/w) were dissolved. Solutions of TPPLND 10 mM, IR780 10 mM, and curcumin (CCM) 10 mM in DMSO were also prepared. TPPLND, IR780, and CCM (at 112.5 μL (6 equiv), 37.5 μL (2 equiv), and 18.75 μL (1 equiv), respectively) were added to the albumin-containing solution under vigorous stirring. The remained step to acquire FITC-LIR-CCM NPs followed the washing protocol as described in the main text. For the preparation of FITC-CCM NPs, CCM 168.75 μL (9 equiv) was used instead of TPPLND and IR780.

4. Preparation of Cy5-LIR NPs.

Firstly, LIR was fabricated using a similar protocol as the preparation of LCIR NPs, but CDM was replaced by LNDTPP (n_{LNDTPP}:n_{IR780} = 7:2) . After stirring overnight, an aliquot of 50 μL Cy5.5-NHS ester was quickly added to 5 mL of LIR NPs solution. The reaction was held for 2 hours. The mixture was centrifuged at 2,500 g for 3 min to remove unbound hydrophobic compound and filtered using an Amicon Ultra Centrifugal Filter (MWCO: 100kDa) and DW 4 times to completely remove organic solvent and unreacted dyes. The remaining residue was lyophilized and stored for use in *in vivo* imaging experiments.

5. Thermal stability of metabolism inhibitors

CDM and LNDTPP were dispersed into DMEM containing 1% FBS + 1% PS. These solutions were incubated at 50^{o}C for 30 min before treating to cells. The MTT assay was applied to evaluate the cytotoxicity of mild-heated CDM and LNDTPP to 4T1 cells.

6. *In vivo* ATP assay.

Mice at day 2 of treatment period was randomly sacrificed to collect tumors. These tumors were homogenized with lysis buffer from Luminescent ATP Detection Assay Kit at low temperature overnight. Intratumor ATP level was measured by using the similar protocol with Luminescent ATP Detection Assay Kit as described in *in vitro* experiments. The ATP level was normalized by tumor weight.

References

[1] L. Cui, A.M. Gouw, E.L. LaGory, S. Guo, N. Attarwala, Y. Tang, J. Qi, Y.-S. Chen, Z. Gao, K.M. Casey, A.A. Bazhin, M. Chen, L. Hu, J. Xie, M. Fang, C. Zhang, Q. Zhu, Z. Wang, A.J. Giaccia, S.S. Gambhir, W. Zhu, D.W. Felsher, M.D. Pegram, E.A. Goun, A. Le, J. Rao, Mitochondrial copper depletion suppresses triple-negative breast cancer in mice, Nat. Biotechnol., 39 (2021) 357-367.https://doi.org/10.1038/s41587-020-0707-9.

Supplementary figure legends.

Fig S1. Synthesis of CDM.

Fig S2. Synthesis of TPPLND.

Fig S3. Characterization of CDM.

A. TLC results of CDM after reaction (stationary phase: silica GF_{254}, mobile phase: DCM/ammonia 4% in MeOH/TEA = 92:8:2.5); **B.** Absorption and emission spectra of CDM in PBS solution containing BSA 10 mg/mL and DMSO 10%; **D.** Mass spectroscopy of CDM.

Fig S4. Characterization of TPPLND.

A. TLC results of TPPLND after reaction (stationary phase: silica GF_{254}, mobile phase: DCM:MeOH:TEA = 100:5:1); **B.** UV absorption spectra of TPPLND; **C.** Mass spectroscopy of TPPLND.

Fig S5. Cytotoxicity of TPPLND, CDM, IR780 and their combination on 4T1 cancer cells.

Fig S6. Cytotoxicity of TPPLND and CDM after mild-hyperthermia treatment.

Fig S7. Intratumor ATP level of 4T1-bearing mice.

Fig S8. Body weight graph of mice during treatment.

Fig S9. Histological observation of main organs including heart, lung, liver, spleen, and kidney of mice sacrificed after 21 days of different treatments. The scale bars were 200 μm.

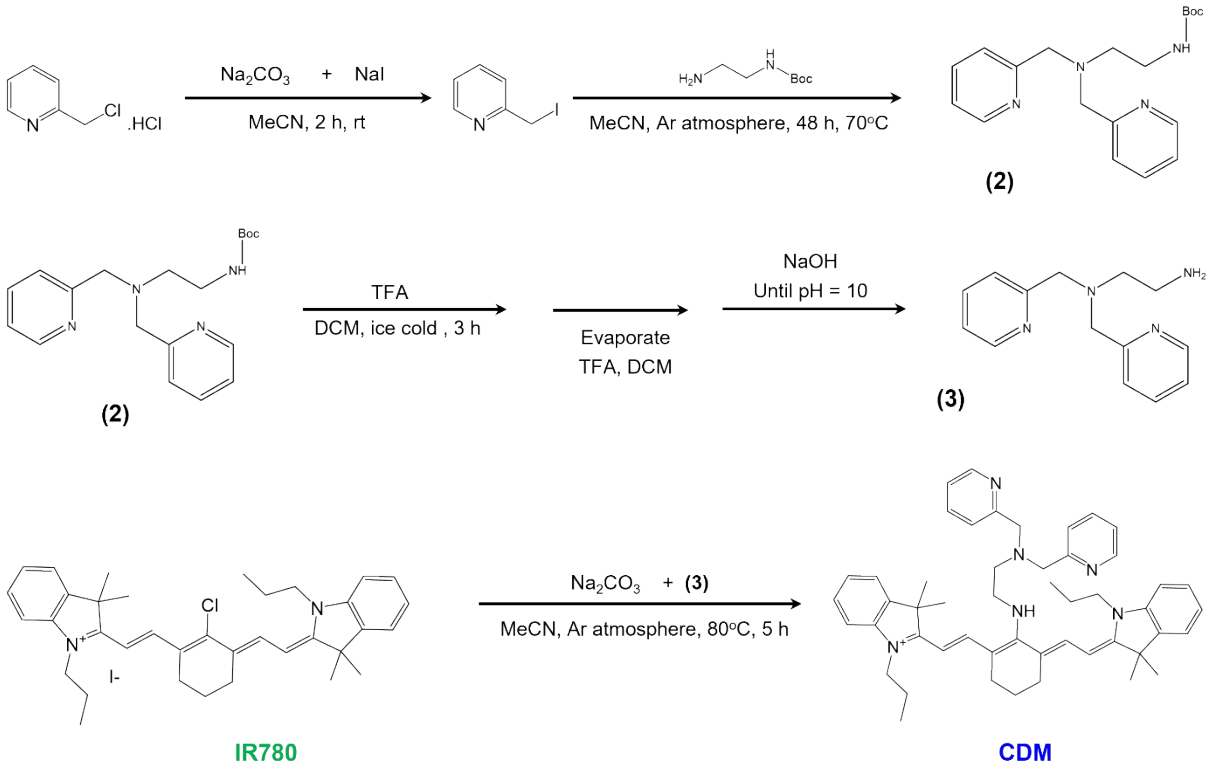

Na2CO3 + NaI
MeCN, 2 h, rt
MeCN, Ar atmosphere, 48 h, 70°C
(2)
TFA
DCM, ice cold , 3 h
Evaporate
TFA, DCM
NaOH
Until pH = 10
(3)
Na2CO3 + (3)
MeCN, Ar atmosphere, 80°C, 5 h
IR780
CDM

Figure S1.

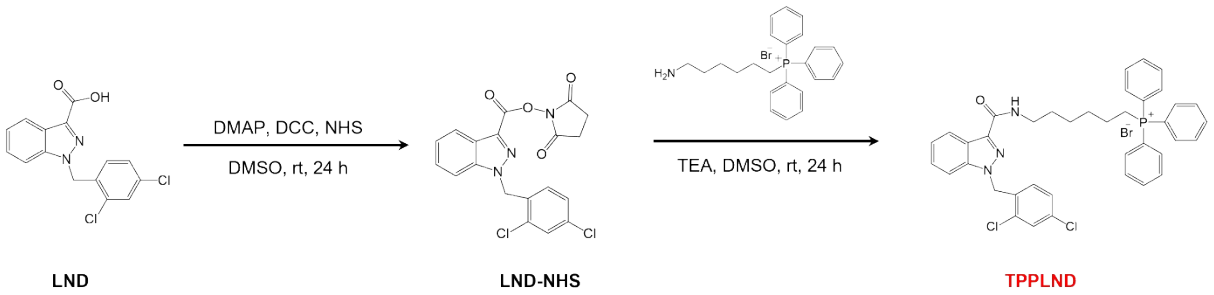


Figure S2.

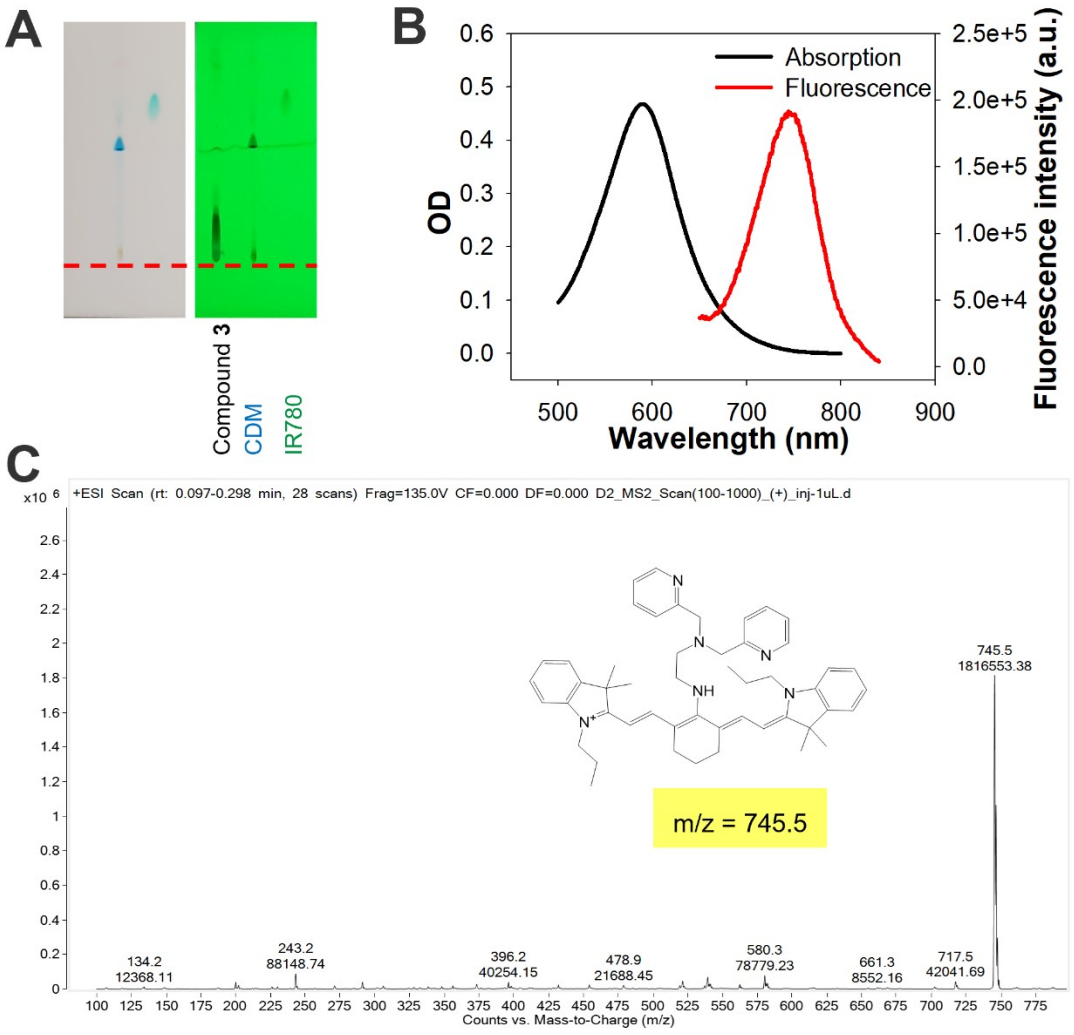

A
Compound 3
CDM
IR780
B
Absorption
Fluorescence
OD
Fluorescence intensity (a.u.)
Wavelength (nm)
C
+ESI Scan (rt: 0.097-0.298 min, 28 scans) Frag=135.0V CF=0.000 DF=0.000 D2_MS2_Scan(100-1000)_(+)_inj-1uL.d
m/z = 745.5
745.5
1816553.38
134.2
12368.11
243.2
88148.74
396.2
40254.15
478.9
21688.45
580.3
78779.23
661.3
8552.16
717.5
42041.69
Counts vs. Mass-to-Charge (m/z)

Figure S3.

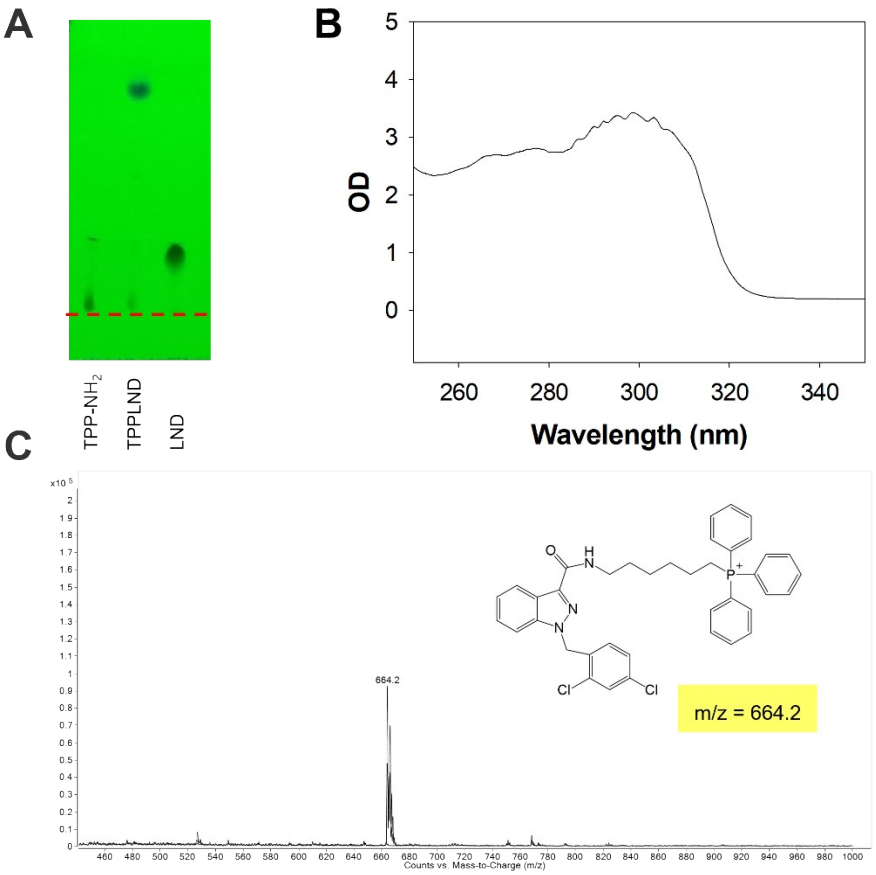
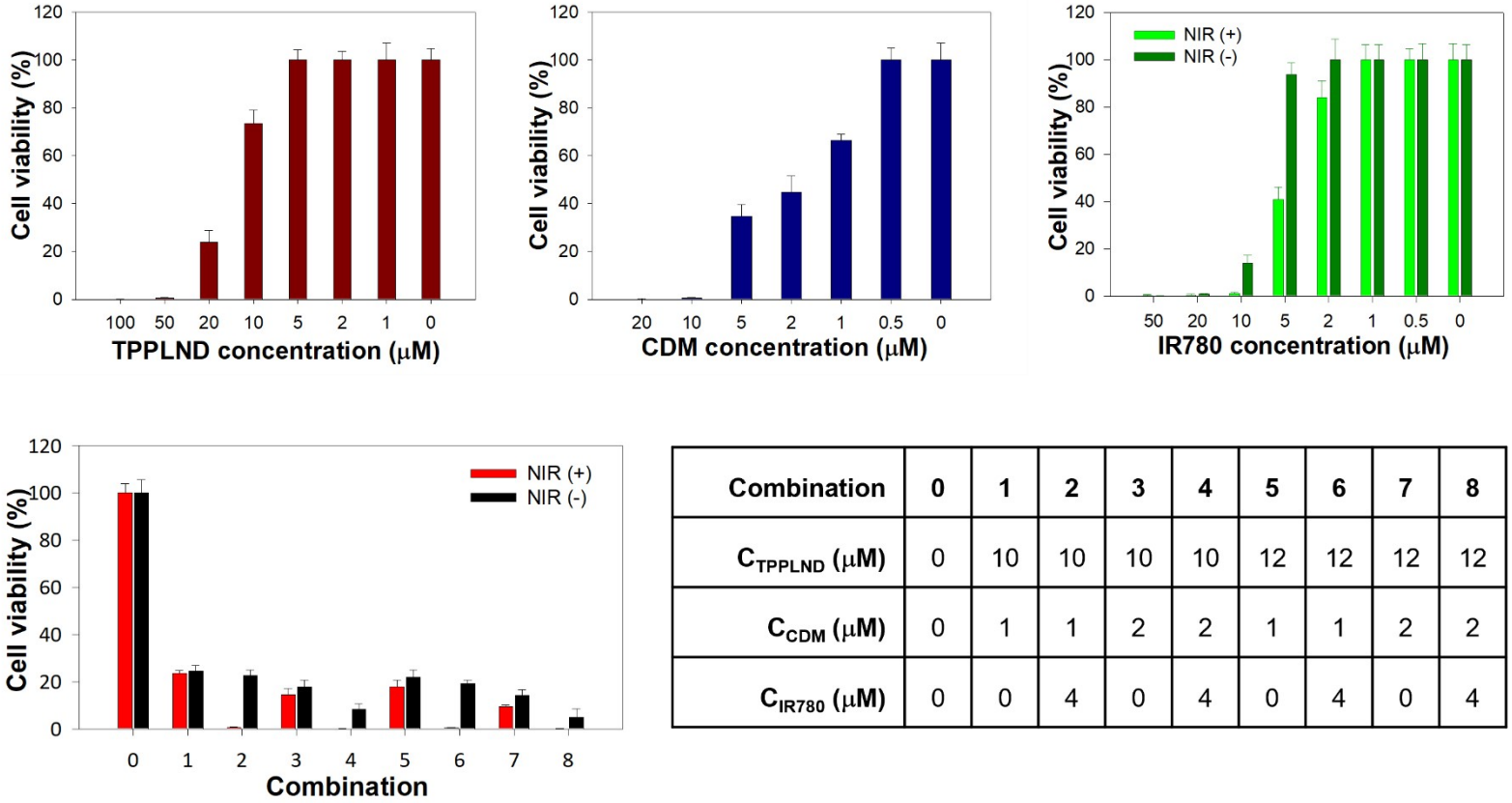

A
TPP-NH2
TPPLND
LND
B
OD
Wavelength (nm)
C
664.2
m/z = 664.2
Counts vs. Mass-to-Charge (m/z)

Figure S4.



Combination	0	1	2	3	4	5	6	7	8
C_{TPPLND} (μM)	0	10	10	10	10	12	12	12	12
C_{CDM} (μM)	0	1	1	2	2	1	1	2	2
C_{IR780} (μM)	0	0	4	0	4	0	4	0	4

Figure S5.

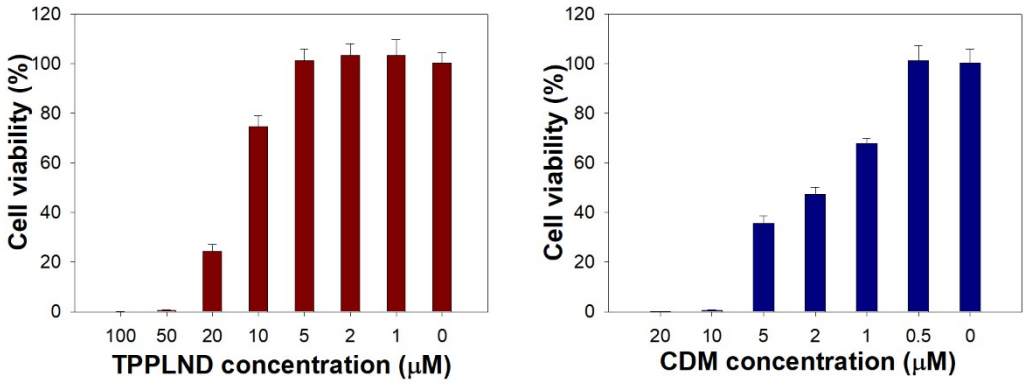
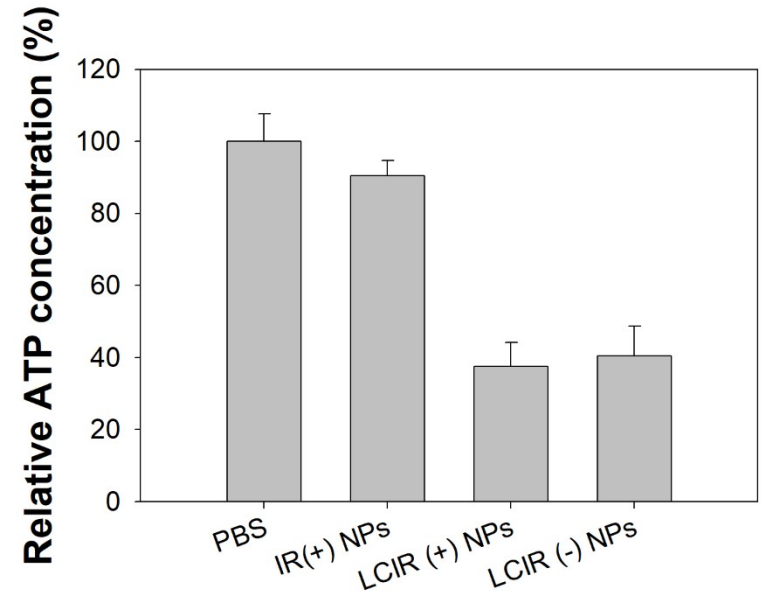

Cell viability (%)
120
100
80
60
40
20
0
100 50 20 10 5 2 1 0
TPPLND concentration (μM)
Cell viability (%)
120
100
80
60
40
20
0
20 10 5 2 1 0.5 0
CDM concentration (μM)

Figure S6.


Relative ATP concentration (%)
120
100
80
60
40
20
0
PBS
IR(+) NPs
LCIR (+) NPs
LCIR (-) NPs

g

Figure S7.

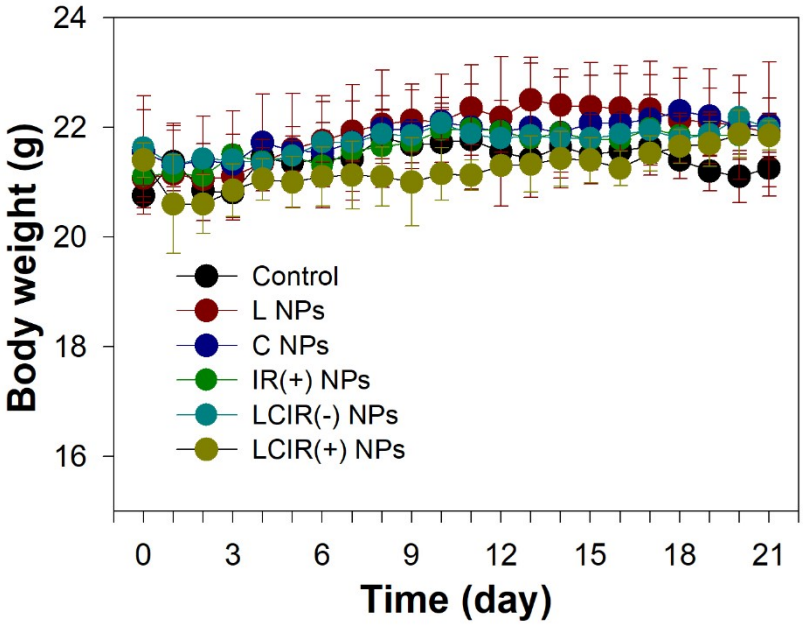

Body weight (g)
24
22
20
18
16
Control
L NPs
C NPs
IR(+) NPs
LCIR(-) NPs
LCIR(+) NPs
0
3
6
9
12
15
18
21
Time (day)

Figure S8.

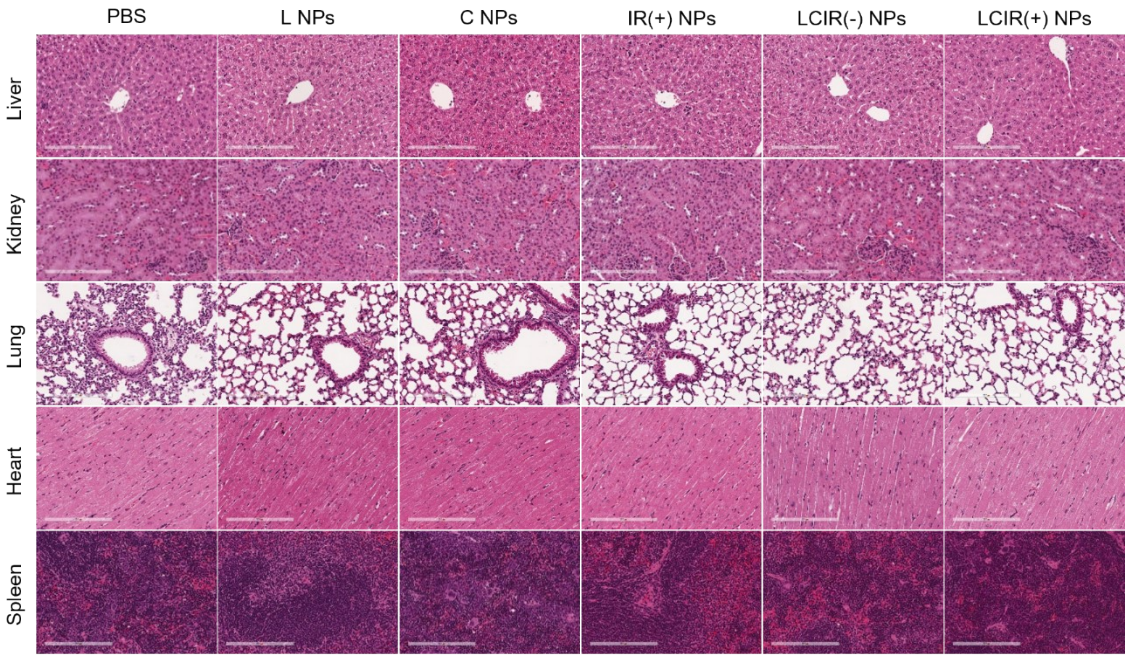

PBS
L NPs
C NPs
IR(+) NPs
LCIR(-) NPs
LCIR(+) NPs
Liver
Kidney
Lung
Heart
Spleen

Figure S9.