

Supporting Information

Ionizable Lipid Nanoparticles Enhance Lung-Delivery of Gold Nanocluster for Improving Acute Lung Injury Alleviation

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Table S1 Compositions and proportion of iLNPs.

Component	MW	w/w
Ionizable lipid	967	33.4%
DSPC	790.2	6.1%
Cholesterol	386.7	14.9%
DMG-PEG2000	2538	4.1%
DOTAP	698.6	41.6%

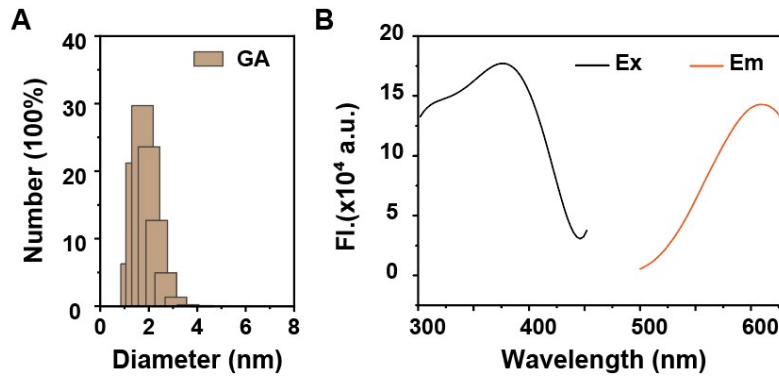


Figure S1. (A) The hydrodynamic diameter distribution of GA. (B) The excitation and emission fluorescence spectra of GA.

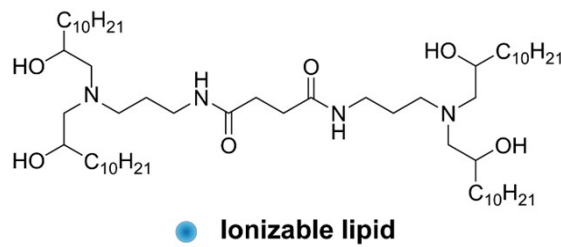


Figure S2. The structure of the ionizable lipid.

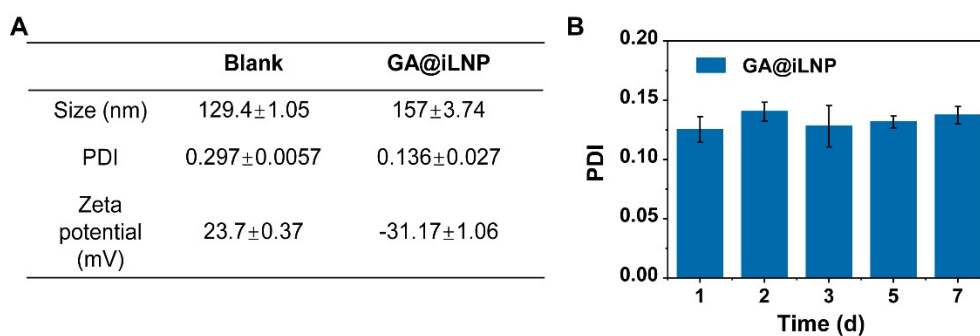


Figure S3. (A) Physicochemical property characterization of iLNP before and after GA encapsulation, which includes the size, PDI and zeta potential. (B) The PDI of GA@iLNP at different times after preparation.

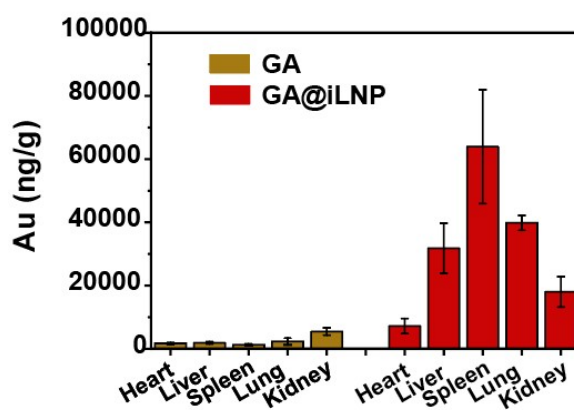


Figure S4. The biodistribution of a single dose of GA (5 mg/kg) and GA@iLNP (containing GA 5 mg/kg) injected intravenically (*i.v.*) in BALB/c mice was compared.

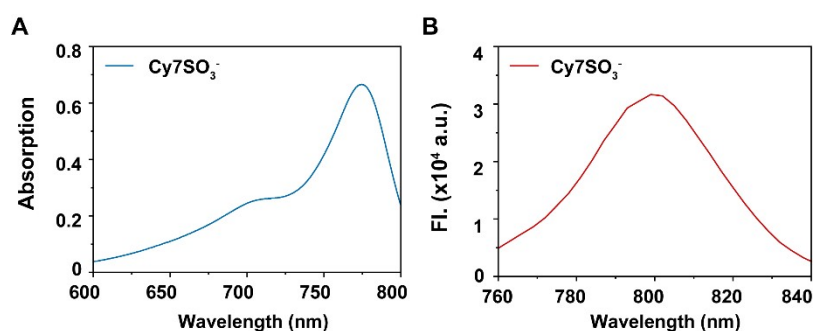


Figure S5. The UV absorption (A) and fluorescence spectra (B) of Cy7SO_3^- .

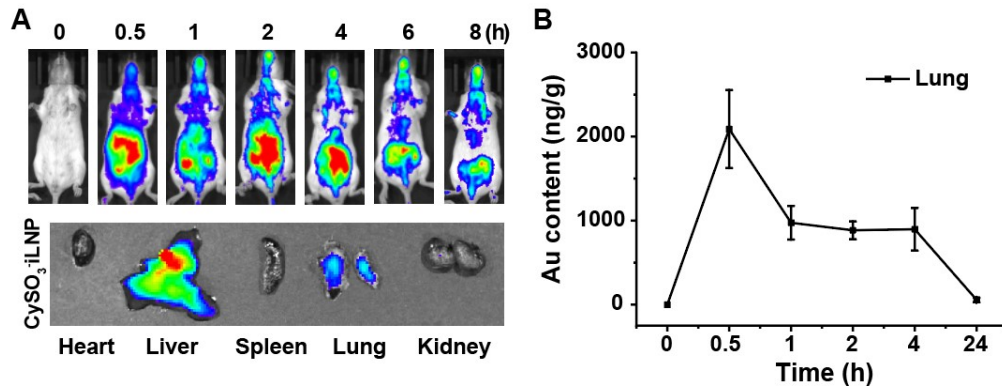


Figure S6. *In vivo* distribution of intravenously injected lung-targeted iLNP. (A) *In vivo* and *ex vivo* fluorescence imaging after intravenous injection of the fluorescent nanoprobe CySO₃@iLNP (100 μM, 100 μL). (B) The Au content distribution in lungs after *i.v.* injection of GA@iLNP (contained GA 5 mg/kg).

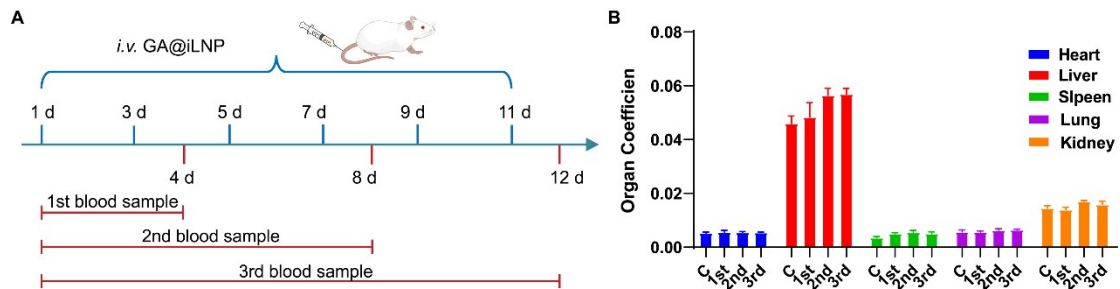


Figure S7. (A) The experimental protocol for long-term safety monitoring and (B) organ coefficient statistics of each group. Data are presented as means ± SD, n = 3.

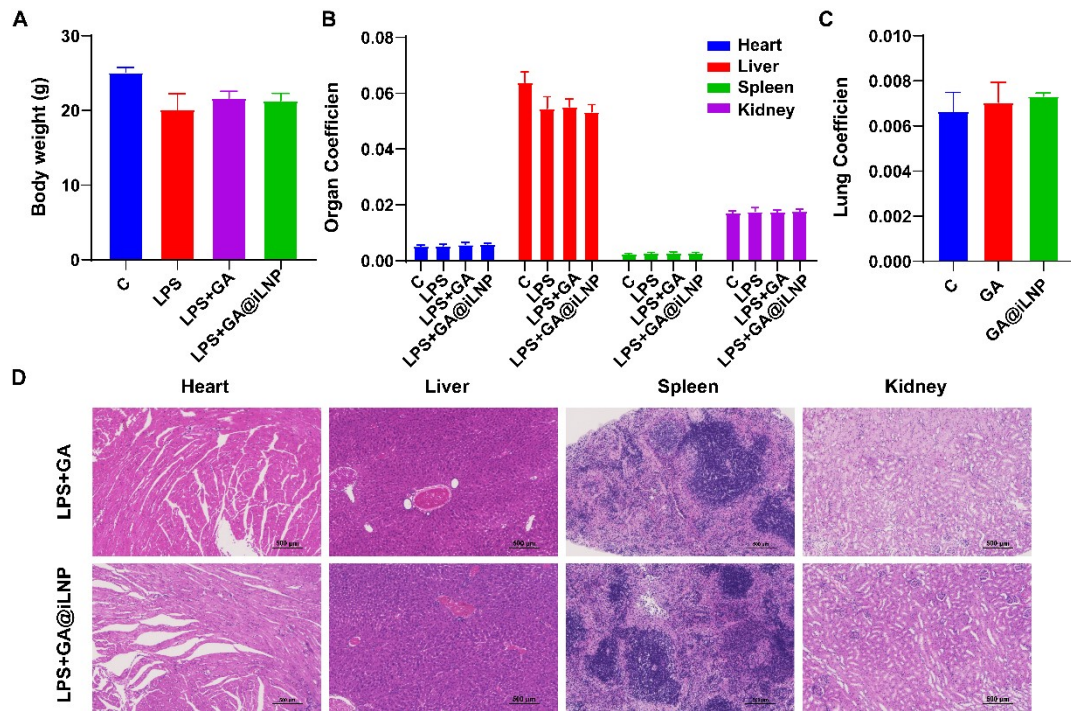


Figure S8. (A) The body weight of each group was compared: Control, LPS, LPS + GA, LPS + GA@iLNP. Data are presented as means \pm SD, n = 5. (B) The organ coefficients of major organs from each group. Data are presented as means \pm SD, n = 5. (C) The lung coefficients from control and GA or GA@iLNP treated normal mice. (D) HE staining of major organs from LPS + GA and LPS + GA@iLNP treated groups.

Table S2 Blood cell indexes of mice treated with GA@iLNP (mean values \pm SD, n=3).

Parameter	Short name	Ctrl	day 4	day 8	day 12	Unit	Reference value range
White blood cell count	WBC	3.59 \pm 0.37	2.74 \pm 0.11	3.54 \pm 0.49	3.27 \pm 0.54	10 ⁹ /L	0.80 - 10.60
Neutrophil count	Neu#	0.72 \pm 0.06	0.68 \pm 0.09	0.63 \pm 0.14	0.76 \pm 0.15	10 ⁹ /L	0.23 - 3.60
Lymphocyte count	Lym#	2.78 \pm 0.34	1.97 \pm 0.13	2.83 \pm 0.39	2.4 \pm 0.39	10 ⁹ /L	0.60 - 8.90
Monocytes count	Mon#	0.067 \pm 0.01	0.05 \pm 0.008	0.053 \pm 0.026	0.073 \pm 0.068	10 ⁹ /L	0.04 - 1.40
Eosinophil granulocyte count	Eos#	0.02 \pm 0.01	0.04 \pm 0.03	0.017 \pm 0.004	0.033 \pm 0.004	10 ⁹ /L	0.00 - 0.51
Basophil granulocyte count	Bas#	0	0	0	0	10 ⁹ /L	0.00 - 0.12
Percentage of neutrophil	Neu%	20 \pm 1.5	24.7 \pm 3.56	17.77 \pm 2.45	22.97 \pm 0.82	%	6.5 - 50.0
Percentage of lymphocyte	Lym%	77.33 \pm 1.73	71.7 \pm 2.77	80.13 \pm 3.24	73.5 \pm 1.87	%	40.0 - 92.0
Percentage of monocytes	Mon%	1.97 \pm 0.55	2.03 \pm 0.26	1.533 \pm 0.76	2.33 \pm 1.96	%	0.9 - 18.0
Percentage of eosinophil granulocyte	Eos%	0.67 \pm 0.3	1.57 \pm 0.94	0.57 \pm 0.09	1.2 \pm 0.45	%	0.0 - 7.5
Percentage of Basophil granulocyte	Bas%	0.03 \pm 0.04	0	0	0	%	0.0 - 1.5
Red blood cell count	RBC	12.3 \pm 0.3	11.92 \pm 0.47	12.29 \pm 0.28	10.36 \pm 2.17	10 ¹² /L	6.50 - 11.50
Hemoglobin	HGB	199 \pm 3.56	191 \pm 9.27	196.33 \pm 2.35	169.67 \pm 35.6	g/L	110 - 165
Hematocrit	HCT	55.47 \pm 0.68	53.37 \pm 2.52	55.53 \pm 1.45	48.07 \pm 9.5	%	35.0 - 55.0
Mean corpuscular volume	MCV	45.1 \pm 0.8	44.73 \pm 0.45	45.17 \pm 0.66	46.47 \pm 1.71	fL	41.0 - 55.0
Mean hemoglobin content of red blood cells	MCH	16.2 \pm 0.32	16 \pm 0.21	15.97 \pm 0.26	16.37 \pm 0.6	pg	13.0 - 18.0
Mean concentration of red blood cell hemoglobin	MCHC	359.33 \pm 1.69	358.33 \pm 2.05	353.33 \pm 4.78	352.67 \pm 4.78	g/L	300 - 360
Coefficient of variation of red blood cell distribution width	RDW-CV	14.3 \pm 0.21	15.73 \pm 1.39	15.03 \pm 0.82	14.1 \pm 0.163	%	12.0 - 19.0
Standard deviation of red blood cell distribution width	RDW-SD	29.03 \pm 0.83	31.53 \pm 2.52	30.4 \pm 2.11	28.931.18	fL	23.0 - 39.0
Platelet count	PLT	572.33 \pm 34.99	579 \pm 114.5	836.33 \pm 198.5	458.33 \pm 45.06	10 ⁹ /L	400 - 1600
Mean platelet volume	MPV	5.9 \pm 0.37	5.93 \pm 0.2	6.13 \pm 0.25	6.37 \pm 0.3	fL	4.0 - 6.2
Width of platelet distribution	PDW	16.9 \pm 0.16	16.47 \pm 0.25	16.63 \pm 0.33	16.6 \pm 0.22	fL	12.0 - 17.5
Thrombocytopenia	PCT	0.337 \pm 0.001	0.347 \pm 0.08	0.509 \pm 0.1	0.294 \pm 0.04	%	0.100 - 0.780

Table S3 Biochemical indexes of mice treated with GA@iLNP (mean values \pm SD, n=3).

Parameter	Short name	Ctrl	day 4	day 8	day 12	Unit	Reference value range
Alanine aminotransferase	ALT	29.4 \pm 3.3	31.1 \pm 1.37	33.4 \pm 1.4	34.7 \pm 4.1	U/L	10.06-96.47
Aspartate aminotransferase	AST	87.9 \pm 13.51	91.1 \pm 9.89	89.3 \pm 3.0	72.9 \pm 4.53	U/L	36.31-235.48
Alkaline phosphatase	ALP	184.3 \pm 9.29	121 \pm 3.86	133.9 \pm 7.39	131.3 \pm 17.9	U/L	22.52-474.35
Urea nitroge	BUN	7.5 \pm 0.51	6.8 \pm 0.55	8.03 \pm 0.64	7.01 \pm 0.13	mmol/L	10.81-34.74
Creatinine	CREA	16.7 \pm 3.18	17.8 \pm 3.31	14.7 \pm 0.49	14.3 \pm 1.63	μ mol/L	10.91-85.09