Electronic Supplementary Material

Targeted delivery of celastrol to glomerular endothelium and podocytes for chronic kidney disease treatment

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Section 1 Supporting methods

Section 1.1 Preparation CLT-phospholipid lipid nanoparticles (C-PLNs)

C-PLNs were prepared by a solvent evaporation method. Briefly, 1.00 mg CLT powder, 15.60 mg E80, 2.00 mg Chol and 4.40 mg DSPE-PEG2000 were co-dissolved in 5 mL solvent (DCM: EA: $CHCl_3$ = 57: 110: 59) under gently stirring at room temperature for 1 h. Then, the organic solvent was removed by rotary evaporation under reduced pressure to form CLT-phospholipid complex (C-PC). Then added 3 μL soybean oil and 3 mL solvent (DCM: EA: $CHCl_3$ = 57: 110: 59) into C-PC, under gently stirring at room temperature for 10 min. After that, the organic solvent was removed by rotary evaporation again, and the film was hydrated in 4 mL 0.02M Hepes Buffer. Next, sonicated above formulation about 7 min in a sonicator (210 W) to obtain the C-PLNs. As for negative charged CLT-loaded PLNs (NC-PLNs), it was prepared same as C-PLNs, except no DOTAP added.

Section 1.2 Preparation DiD-loaded PLNs (D-PLNs)

They were prepared as same as CLT-loaded PLNs, except that CLT was replaced by DiD.

Section 1.3 Preparation D-PLNs with different potentials

Briefly, similarly to the above preparation method, only the amount of DOTAP needs to be adjusted to obtain phospholipid lipid nanoparticles with different potentials: (1) 0.68 mg DOTAP for negative potential D-PLNs (Neg-D-PLNs); (2) 1.08 mg DOTAP for neutral potential D-PLNs (Neu-D-PLNs); (3) 1.20 mg for positive potential D-PLNs (Pos-D-PLNs); (4) 1.32 mg for high positive potential D-PLNs (HPos-D-PLNs).

Section 1.4 Determination of peptide grafting rate

According to the sulfhydryl determination kit (KeyGEN BioTech, KGT019) to determine the free sulfhydryl content in free peptide solution, PC-PLNs solution and C-PLNs solution (as a control). The free sulfhydryl content is the amount of free peptide. The peptide content of the free peptide solution is regarded as the total peptide amount. The free peptide content in the PC-PLNs solution is the amount of polypeptide that has not been successfully grafted. After calculating the difference between the total amount of peptide and the amount of unsuccessfully grafted peptide, the grafting rate is the ratio of the difference and the total.

Section 1.5 Repeated low-dose cisplatin induced chronic progressive nephropathy (CPN)

Many studies have shown that repeated low-dose cisplatin treatment can induce chronic kidney disease. Here, we refered to the successful examples and induced the CPN model in Balb/c mice. Because Balb/c mice were more susceptible than C57 mice, we made some changes. Therefore, the method was as follows: the modeling lasted three weeks. The mice were intraperitoneally injected with 5 mg·kg–1 of cisplatin for once a week in the first and third weeks, in the second week, 8 mg·kg–1 cisplatin was injected intraperitoneally, once a week. A few mice were sacrificed randomly in order to examine whether the model was successful.

Section 1.6 TUNEL assay

The 5-μm paraffin sections were analyzed by TUNEL staining using In Situ Cell Death Detection Kit (Roche Basel, Switzerland) to measure renal cell apoptosis. They were photographed by a microscope camera (Leica DM 4000B), magnified by 4 times and 100 times, and calculated the percentage of apoptotic cells. All analyses were performed by a qualified pathologist.

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Section 2 Supporting figures and tables

(a)

Size (nm)	PDI	Zeta potential (mV)	EE (%)	DL (%)
114.0±1.85	0.207±0.015	11.50±1.03	94.83±0.87	2.83±0.025

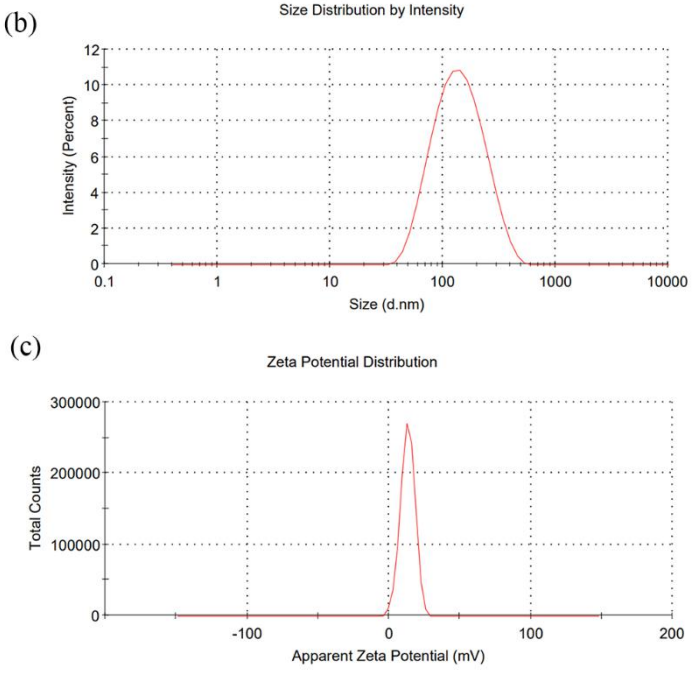

Figure S1 Characterizations of PC-PLNs. (a) Size, polydispersity index (PDI), and zeta-potential of PC-PLNs were measured using dynamic light scattering. Encapsulated and drug loading efficiency of PC-PLNs were measure by HPLC. Data are mean ± SD (n = 3), results are representative of three independent experiments; (b) Size distribution of PC-PLNs, as determined by dynamic ligh scattering; (c) Zeta potential of PC-PLNs, as determined by dynamic light scattering.

Table S1 Characteristics of PLNs (mean ± SD，n=3).

Sample	Size (nm)	PDI	Zeta potential (mV)
C-PLNs	109.5±2.82	0.207±0.024	11.47±1.24
NC-PLNs	108.4±2.90	0.222±0.025	-12.20±1.27
Neg-D-PLNs	109.6±3.21	0.197±0.020	-13.33±0.80
Neu-D-PLNs	110.4±4.45	0.205±0.012	-1.00±0.36
Pos-D-PLNs	110.4±2.65	0.218±0.009	6.20±0.32
HPos-D-PLNs	110.4±4.57	0.206±0.013	11.60±0.92

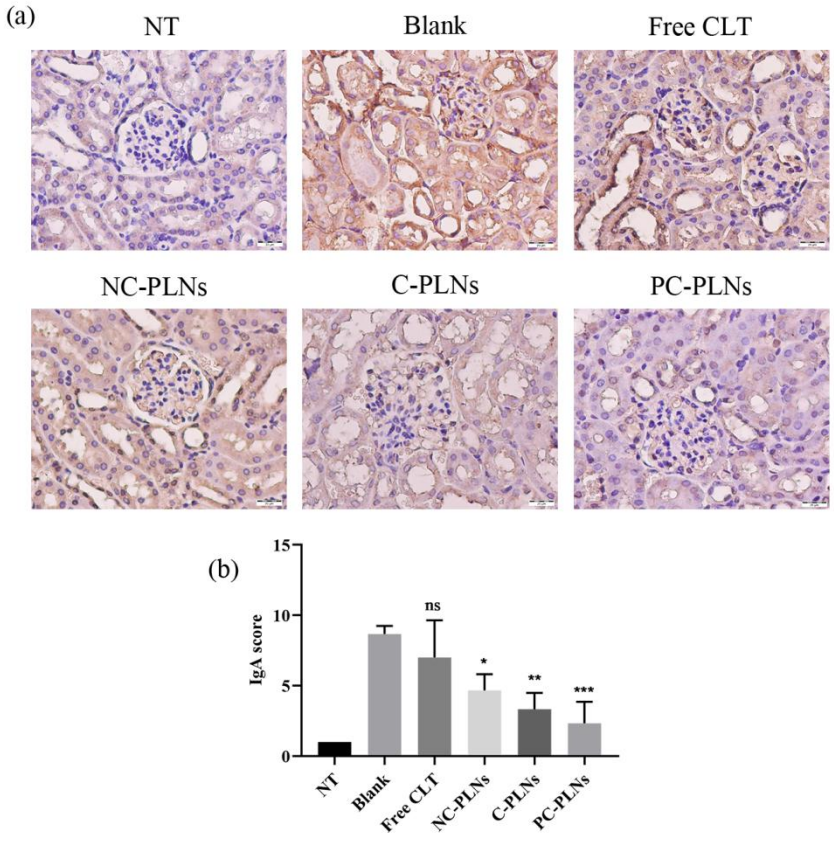

Figure S2 PC-PLNs effectively reduced IgA deposition in model mice (n = 5). (a) Representative images of IgA-stained kidney tissues. Scale bars, 20 μm; (b) The levels of IgA were semi-quantitatively scored, all data are shown as mean ± SD, *P<0.05, **P < 0.01, *** P < 0.001, ns, not significant vs. Blank group.

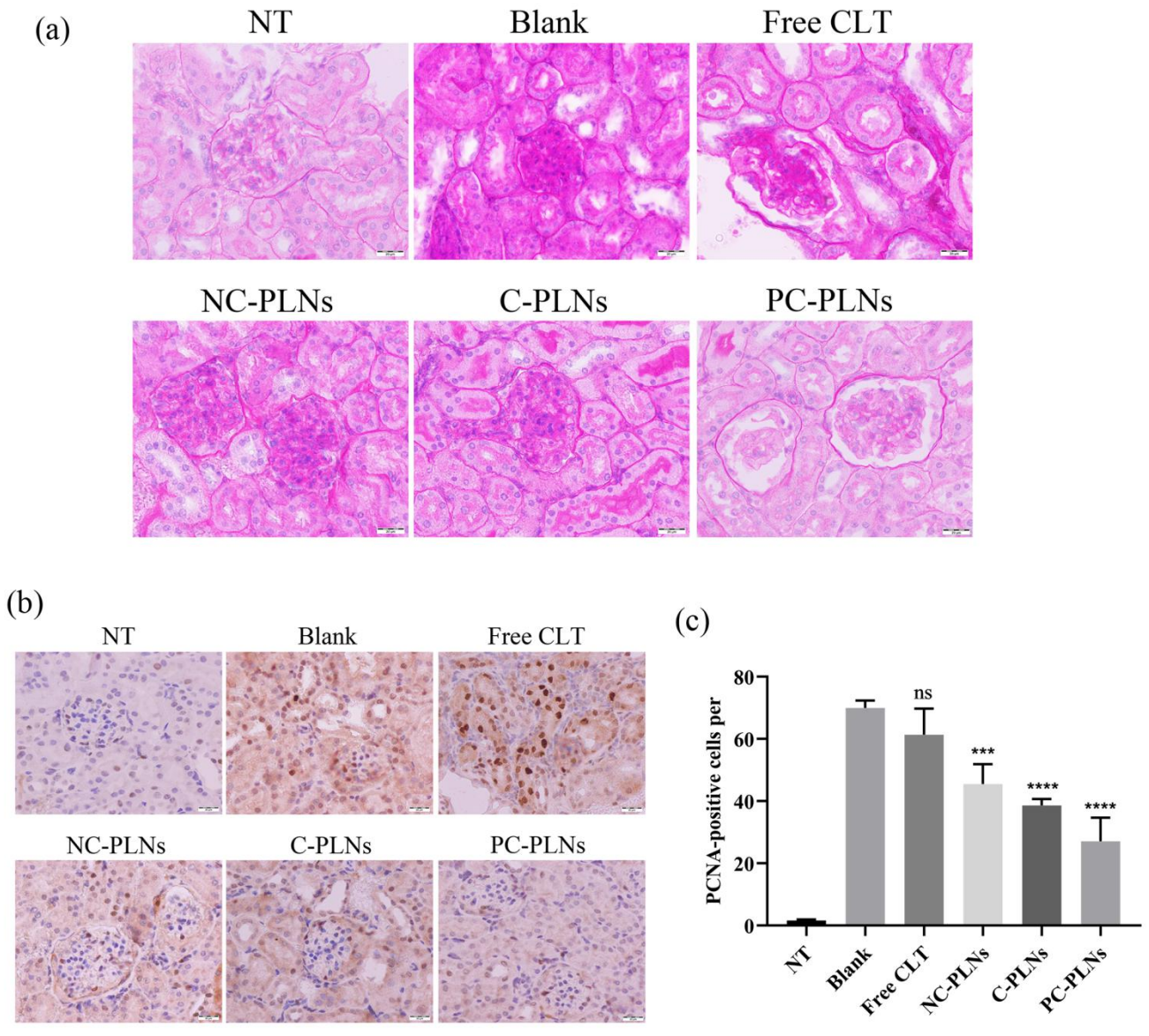

Figure S3 PC-PLNs effectively alleviated glomerular proliferation in IgAN model mice (n = 5). (a) Representative images of PAS-stained kidney tissues. Scale bars, 20 μm; (b) Representative images of PCNA-stained kidney tissues. Scale bars, 20 μm; (c) The levels of PCNA were semi-quantitatively scored, all data are shown as mean ± SD, ***P < 0.001, **** P < 0.0001, ns, not significant vs. Blank group.

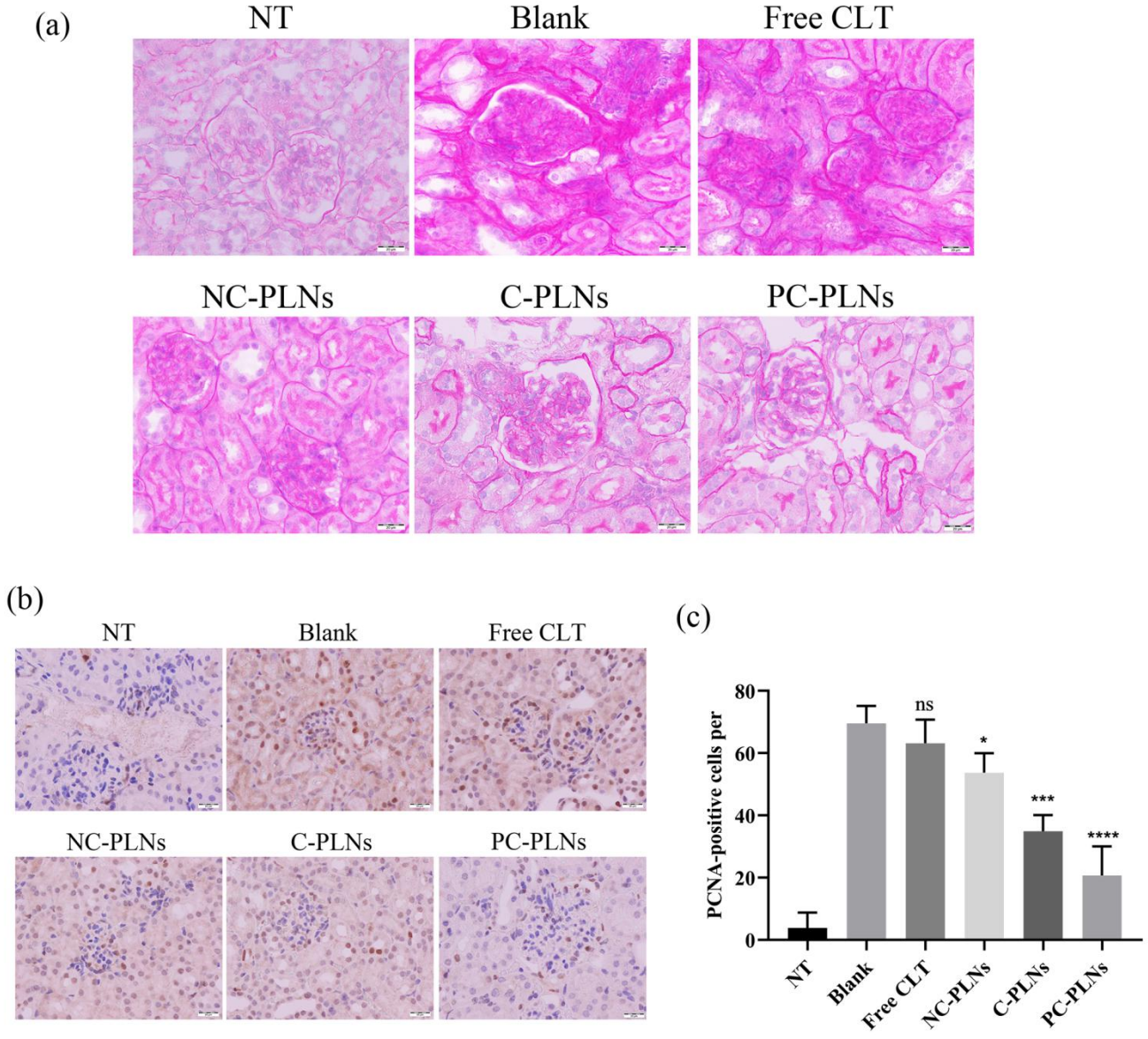

Figure S4 PC-PLNs effectively alleviated glomerular proliferation in CPN model mice (n = 5). (a) Representative images of PAS-stained kidney tissues. Scale bars, 20 μm; (b) Representative images of PCNA-stained kidney tissues. Scale bars, 20 μm; (c) The levels of PCNA were semi-quantitatively scored, all data are shown as mean ± SD, *P < 0.5, ***P < 0.001, **** P < 0.0001, ns, not significant vs. Blank group.

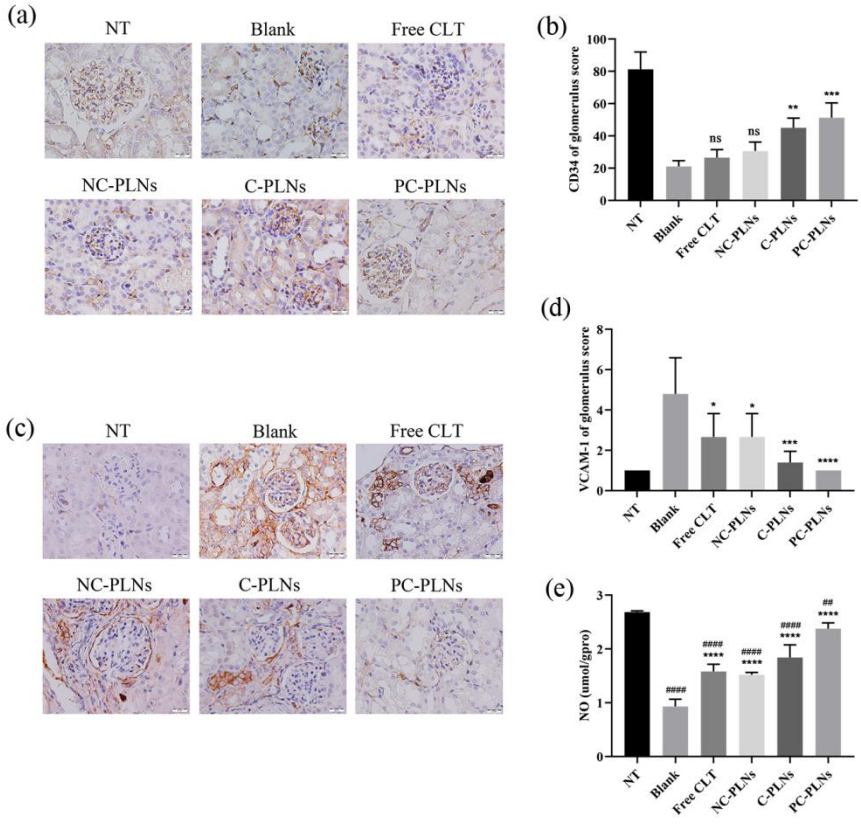

Figure S5 PC-PLNs alleviated renal endothelium injury in CPN mice (n=5). (a) Representative images of CD34-stained kidney tissues. Scale bars, 20 μm; (b) The levels of CD34 were semi-quantitatively scored, all data are shown as mean ± SD, **P < 0.01, *** P < 0.001, ns, not significant vs. Blank group; (c) Representative images of VCAM-1-stained kidney tissues. Scale bars, 20 μm; (d) The levels of VCAM-1 were semi-quantitatively scored, all data are shown as mean ± SD, *P < 0.05, *** P < 0.001, ****P < 0.0001, vs. Blank group; (e) The levels of renal NO, all data are shown as mean ± SD, ****P < 0.0001, vs. Blank group, ##P <0.01, ####P <0.0001, vs. NT group.