Supplementary Information for

Nanoscale coordination polymers co-deliver carboplatin and gemcitabine for highly effective treatment of platinum-resistant ovarian cancer

Christopher Poon,[†] Xiaopin Duan,[†] Christina Chan, Wenbo Han, and Wenbin Lin[*]

Department of Chemistry, University of Chicago, 929 E 57th Street, Chicago, IL 60637, USA; E-mail: wenbinlin@uchicago.edu; Tel: 1 773 834 7163

Supplementary Methods

Preparation of NCP-Carbo

DOPA-NCP-Carbo was synthesized in reverse microemulsions. 25 mg/mL cis,trans-[Pt(1,1-cyclobutanedicarboxylato)$(NH_3)_2$ $(OCONHP(O)(OH)_2)_2$] (8.6 μmol), and DOPA (11 μmol) were added to a 5 mL aliquot of Triton-X-100 (0.3 M in 1.5 M hexanol/cyclohexane) solution to form a *W*=7.4 microemulsion. Another microemulsion of 5 mL Triton-X-100 (0.3 M, 1.5 M hexanol/cyclohexane) containing $Zn(NO_3)_2 \cdot 6H_2O$ aq. (131 mmol) was also prepared. The two microemulsions were separately stirred vigorously for 15 min at room temperature and then combined and stirred for an additional 30 min at room temperature. NCP-Carbo particles were washed once with ethanol, once with 50% (v/v) ethanol/cyclohexane, twice with 50% (v/v) ethanol/THF, and then redispersed in THF. DOPA-capped NCP-Carbo nanoparticles were coated with DSPC, cholesterol, and DSPE-PEG2k at a 2:1:0.75 mol ratio.

Synthesis of Rhodamine B-Doped NCP-Carbo/GMP

A *W*=7.4 microemulsion was prepared with the addition of 25 mg/mL cis,cis,trans-$[Pt(NH_3)_2Cl_2(OCONHP(O)(OH)_2)_2]$ sodium salt solution (7.6 μmol), 15 mg/mL GMP sodium salt solution (1.3 μmol), 23 mg/mL rhodamine B sodium salt solution (0.8 μmol), and DOPA (22 μmol) to a 5 mL aliquot of Triton-X-100 (0.3 M in 1.5 M hexanol/cyclohexane) solution. Another microemulsion of 5 mL Triton-X-100 (0.3 M, 1.5 M hexanol/cyclohexane) containing $Zn(NO_3)_2 \cdot 6H_2O$ aq. (131 mmol) was also prepared. The two microemulsions were stirred vigorously for 15 min at room temperature, after which they were combined. The resulting microemulsion was stirred for 30 min at room temperature. After the addition of 20 mL ethanol, RhB-NCP-Carbo/GMP particles were washed once with ethanol, once with 50% (v/v) ethanol/cyclohexane, twice with 50% (v/v) ethanol/THF, and then redispersed in THF.

Supplementary Figures

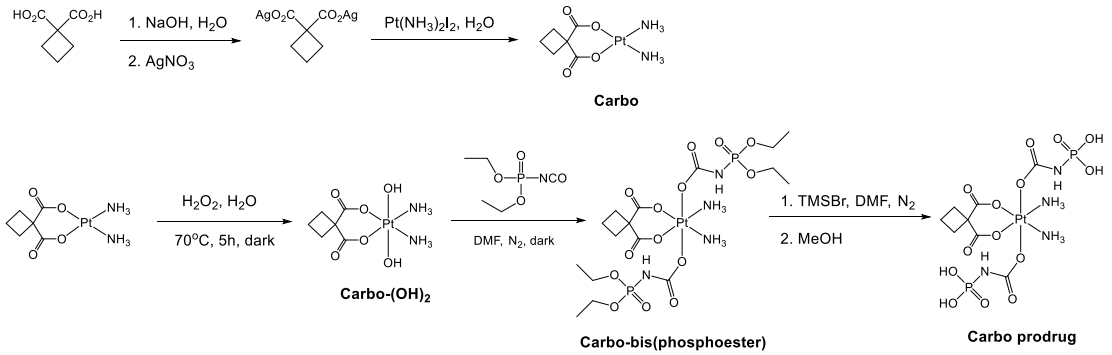

Figure S1. Scheme of Carbo prodrug synthesis.

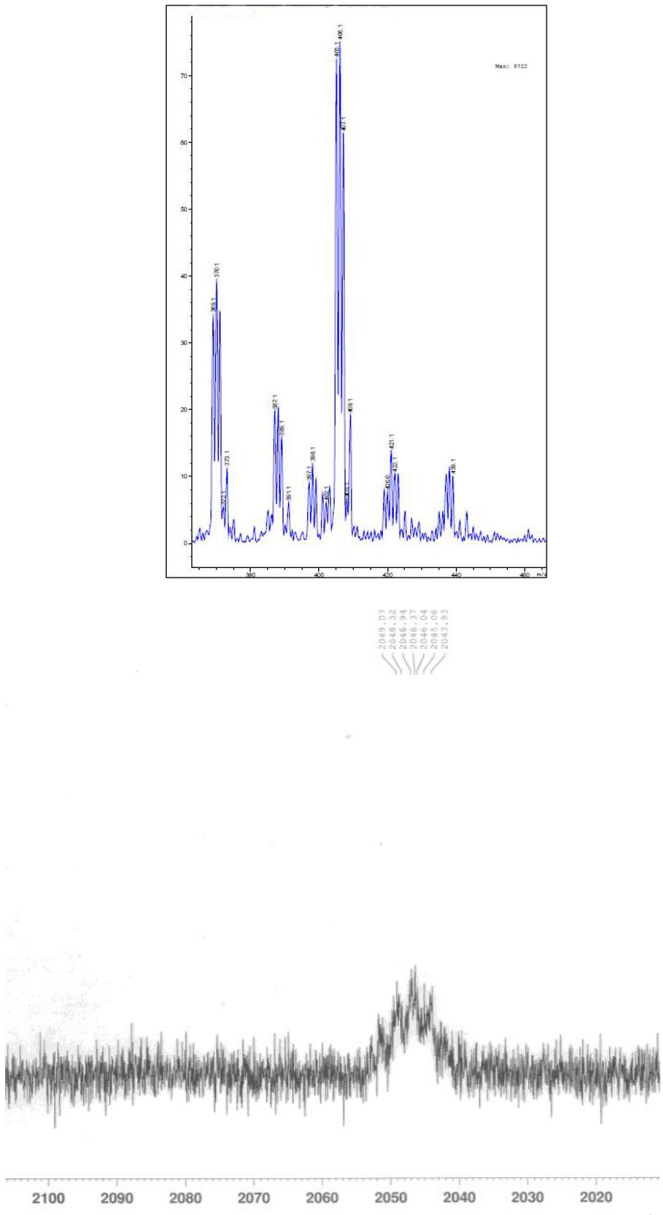

Figure S2. Top, ESI-MS of Carbo-$(OH)_2$ showing the peak for $[M+H]^+$ at M/Z=406.1 (expected 406.1). Bottom, ^{195}Pt (bottom) NMR spectrum of Carbo-$(OH)_2$.

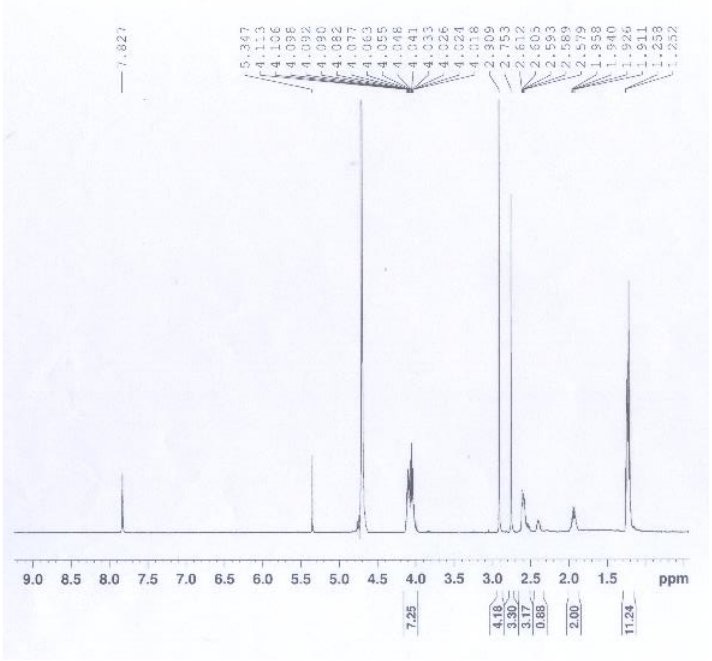

Figure S3. The ^{1}H NMR spectra of Carbo-bis(phosphoester).

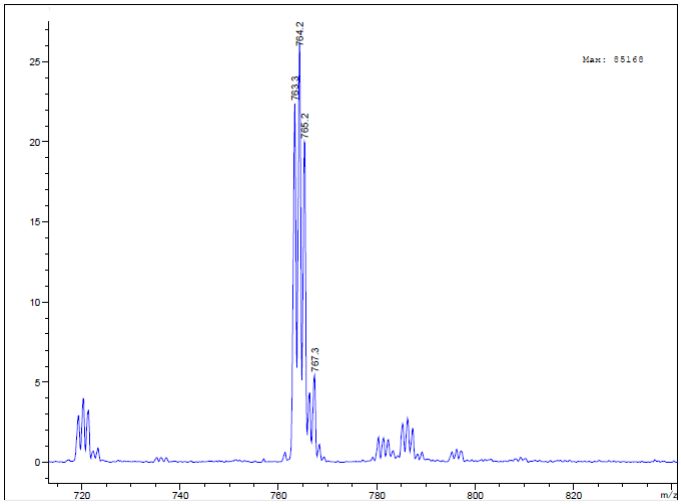

Figure S4. ESI-MS of Carbo-bis(phosphoester) showing the peak for $[M+H]^{+}$ at M/Z=764.2 (expected 764.1).

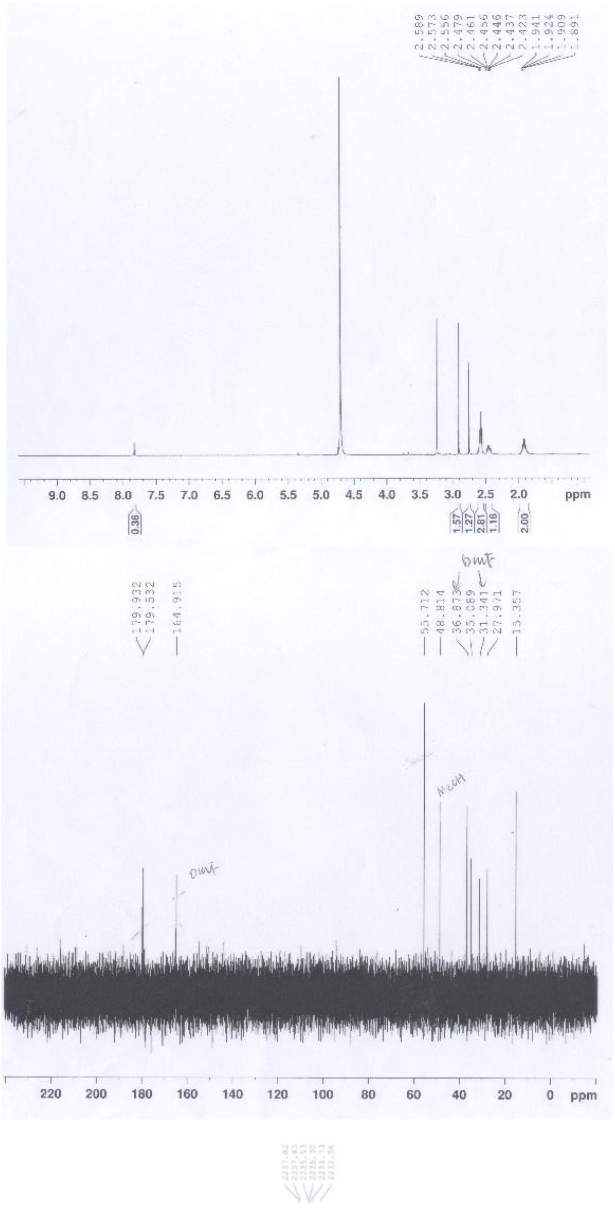

Figure S5. The ^{1}H (top), ^{13}C (middle), and ^{195}Pt (bottom) NMR spectra of carbo prodrug.

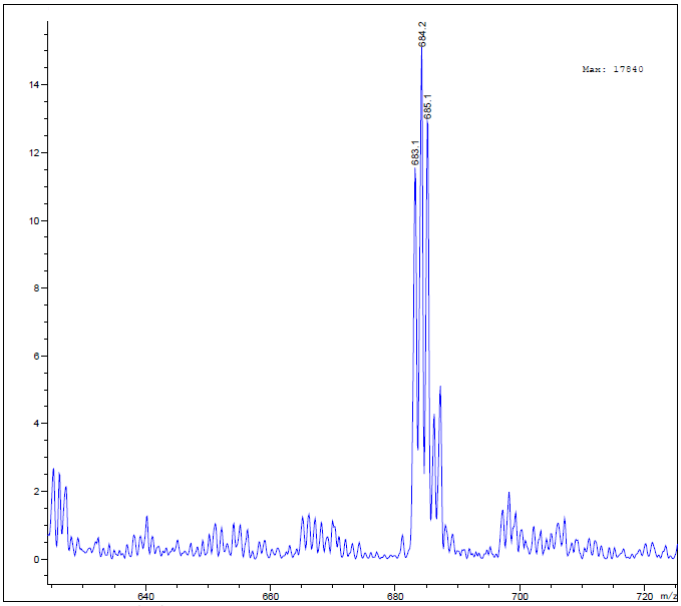

Figure S6. ESI-MS of Carbo prodrug showing the peak for $[M+H]^+$ at M/Z=648.2 (expected 648.0).

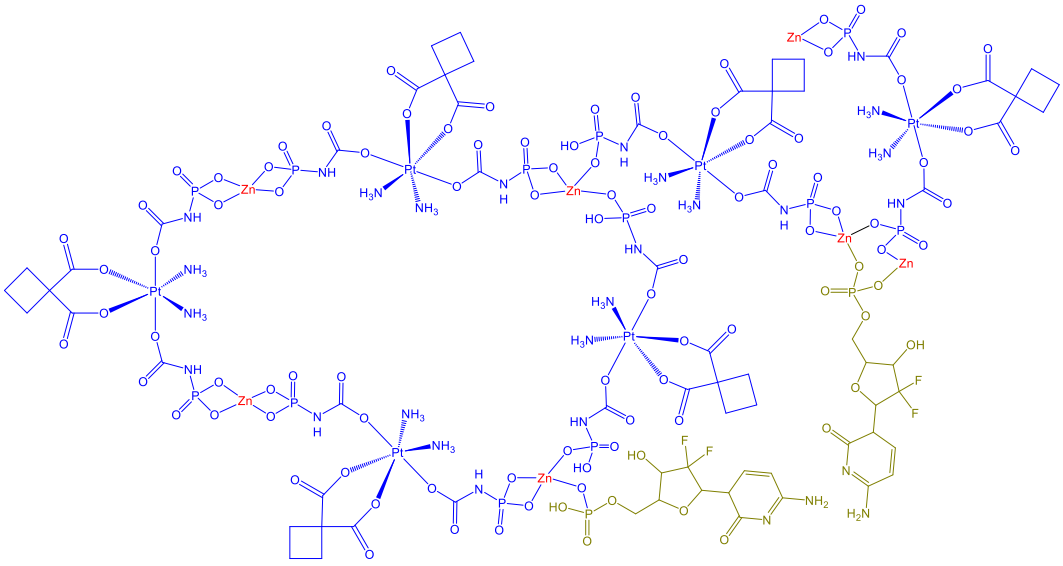

Figure S7. Schematic showing the core of NCP-Carbo/GMP nanoparticles.

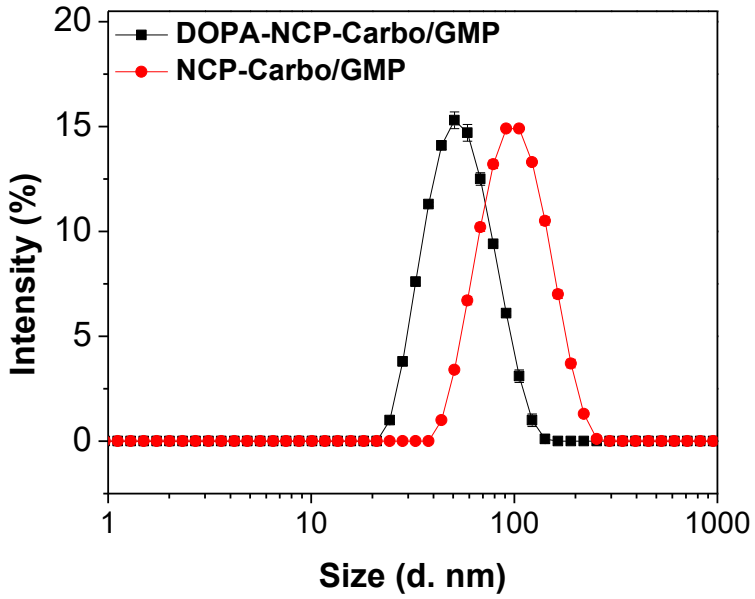

Figure S8. Intensity-average size distribution of NCP-Carbo/GMP particles. Bare and lipid-coated particles were measured in THF and PBS, respectively.

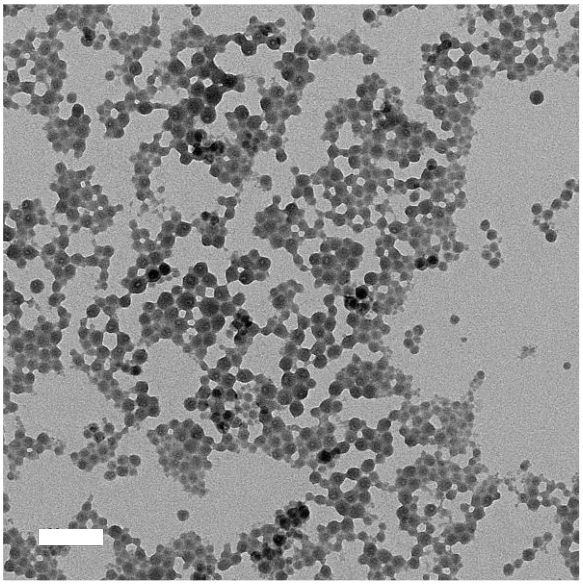

Figure S9. TEM micrographs of DOPA-NCP-Carbo/GMP. Scale = 100 nm.

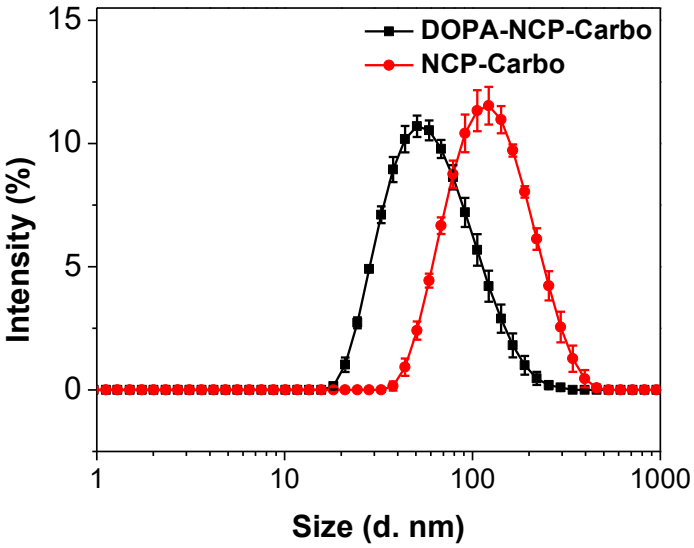

Figure S10. Intensity-average size distribution of NCP-Carbo particles. Bare and lipid-coated particles were measured in THF and PBS, respectively.

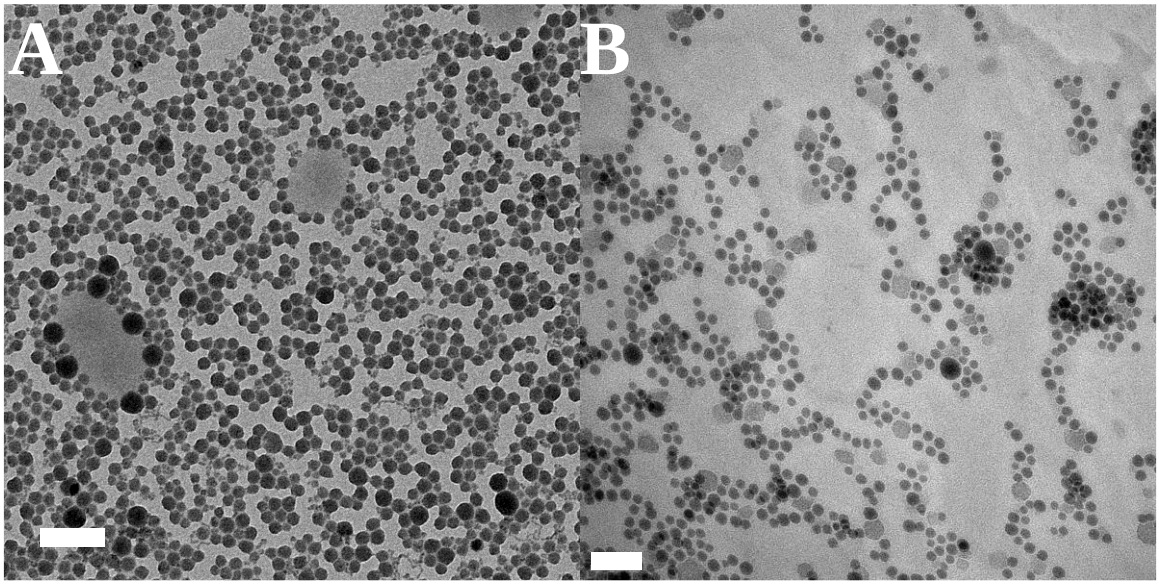

Figure S11. TEM micrographs of (A) DOPA-NCP-Carbo and (B) NCP-Carbo. Scale = 100 nm.

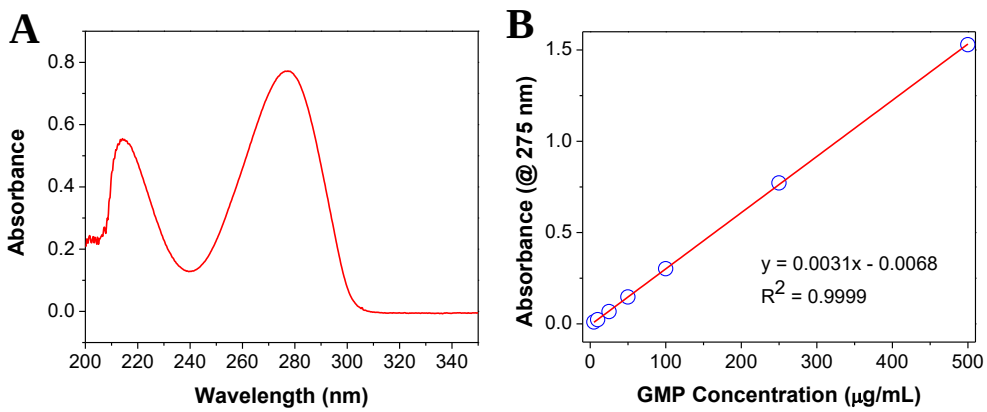

Figure S12. UV-Vis spectrum (A) and standard curve (B) of DOPA-NCP-Carbo/GMP to determine GMP wt.% loading.

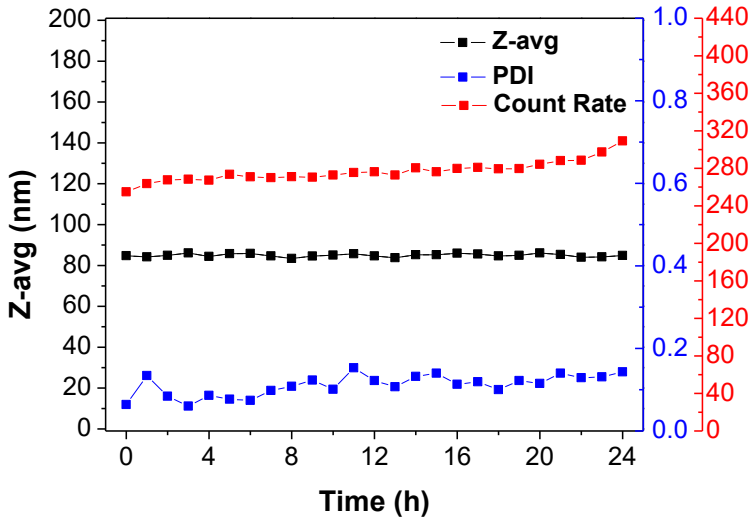

Figure S13. Stability test of NCP-Carbo/GMP after PEGylation in PBS buffer with BSA at 37 ^{o}C. Z-avg diameters were evaluated over a 24 h-period.

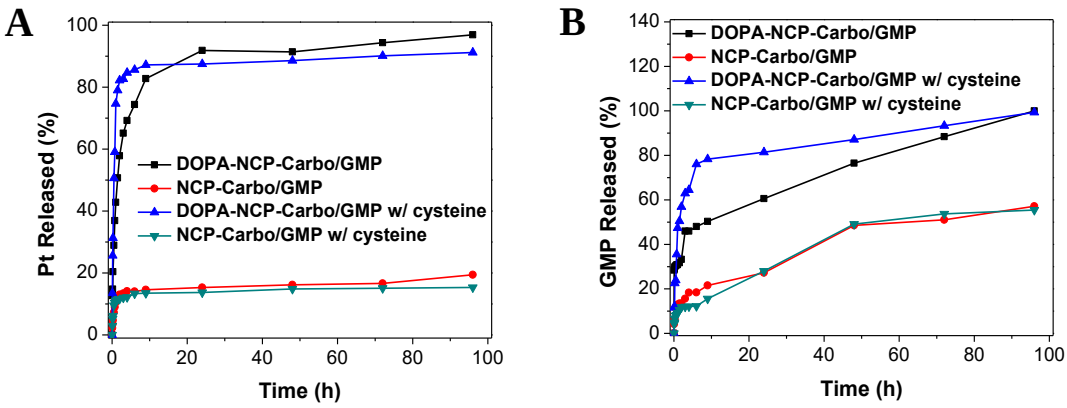

Figure S14. Pt (A) and GMP (B) release profiles of DOPA-NCP-Carbo/GMP and NCP-Carbo/GMP in 1x PBS buffer with or without 5 mM cysteine at 37 ºC.

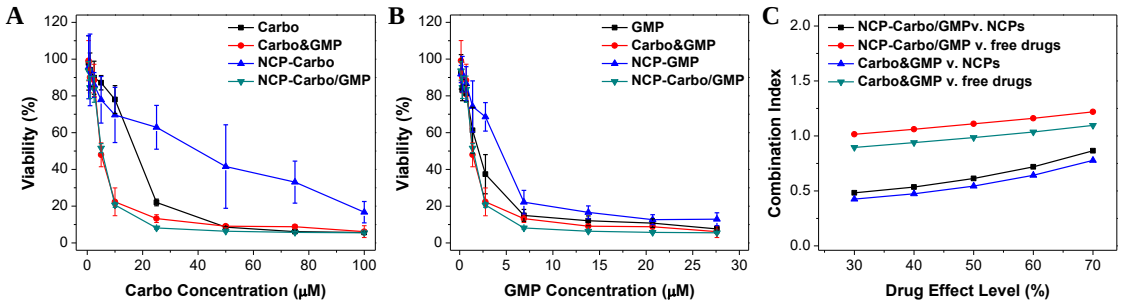

Figure S15. *In vitro* cytotoxicity plots and combination indices (CI) of carbo/GMP combinations on A2780/CDDP. A2780/CDDP cells were exposed to NCP-Carbo, NCP-GMP, NCP-Carbo/GMP, or free drugs (carbo, or GMP) for 4 h. The cell media were replenished with fresh media, and the cell viability was measured after an additional 44 h incubation. Data are mean ± S.D. (n=6).

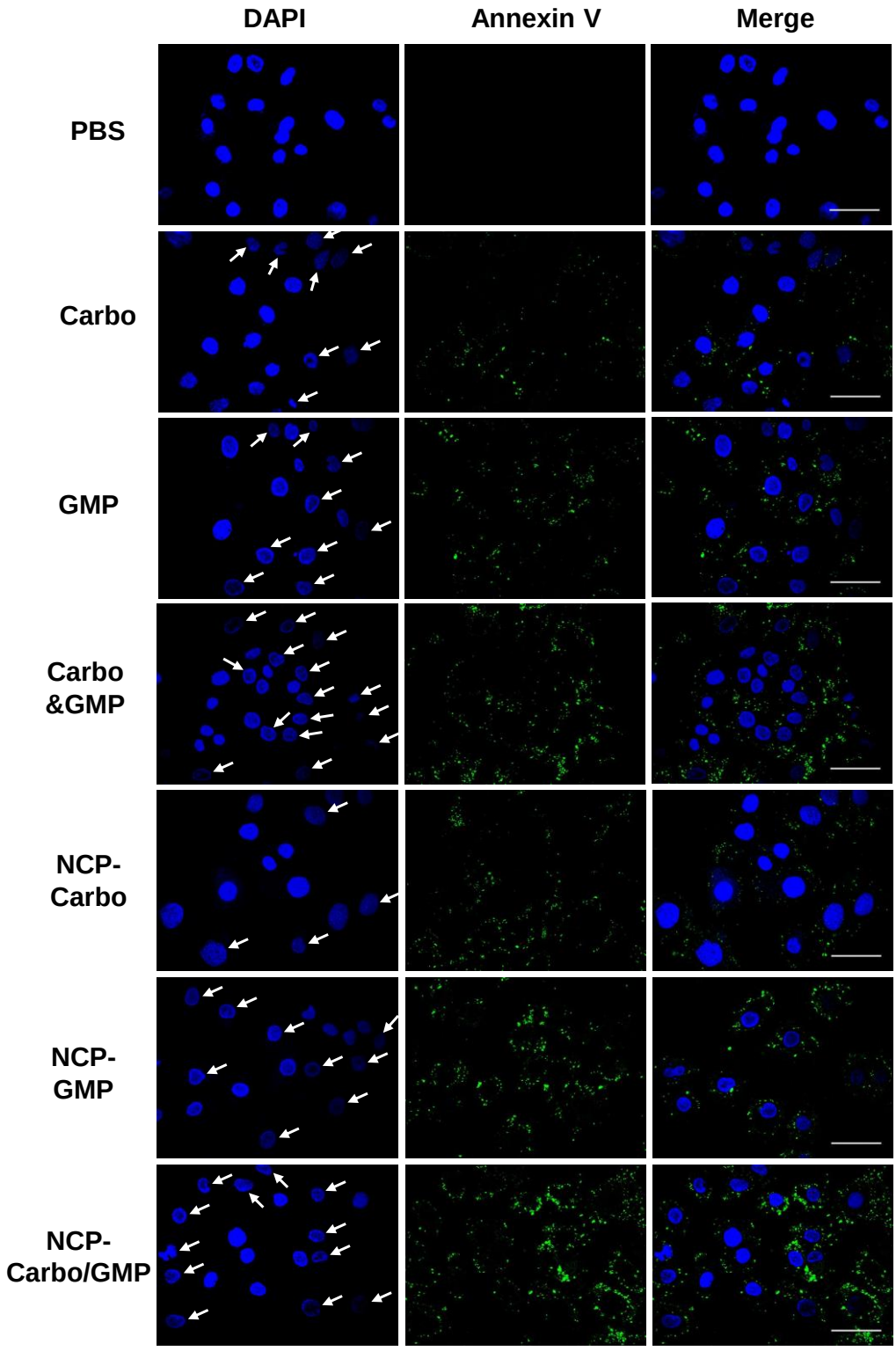

Figure S16. CLSM images showing cell apoptosis in SKOV-3 cells after incubation with free drugs or particles for 24 h. Cells were stained with Alexa Fluor 488 conjugated Annexin V and the nuclei were stained with DAPI. Scale bars: 50 μm. While arrows represent the apoptotic nuclei.

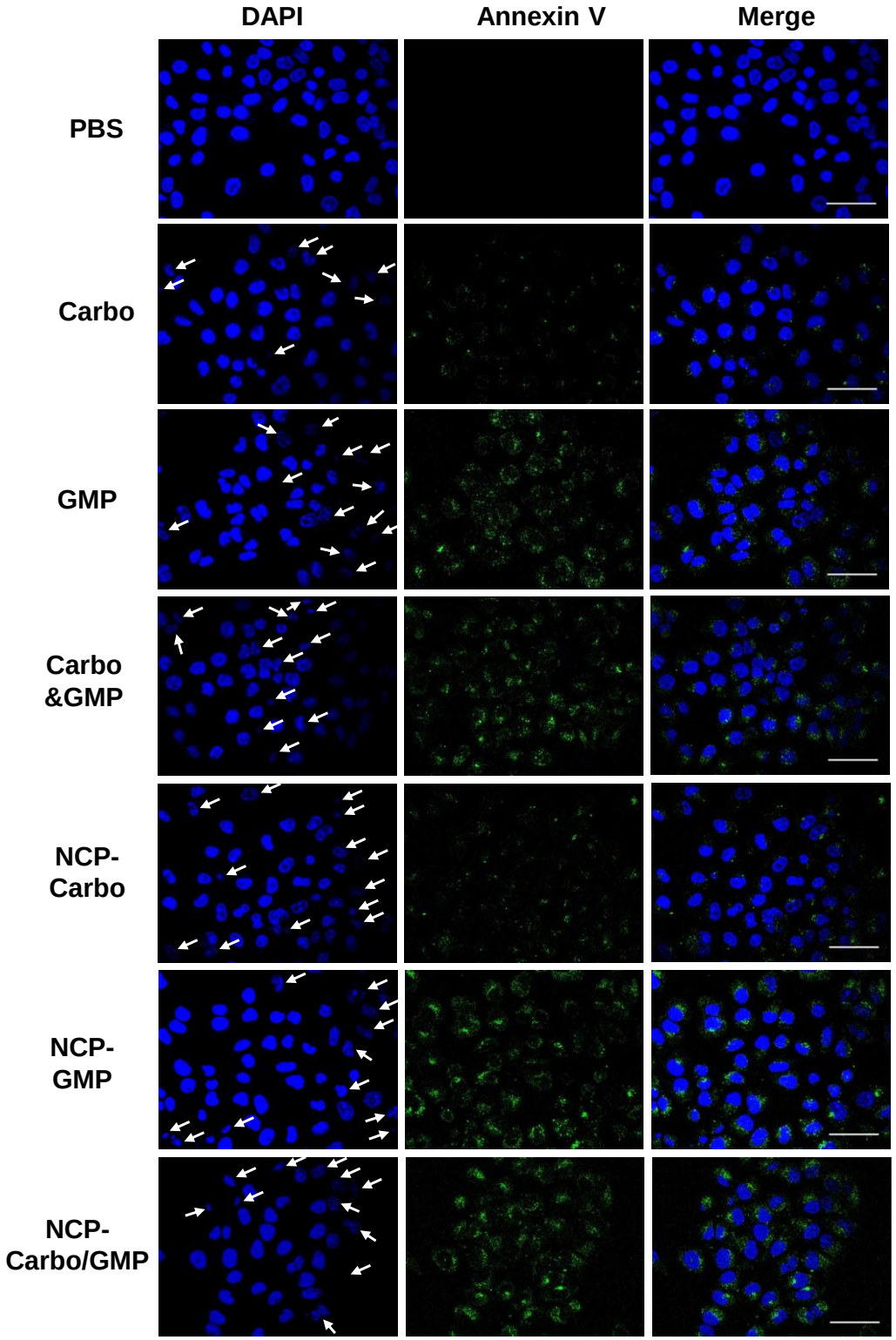

Figure S17. CLSM images showing cell apoptosis in A2780/CDDP cells after incubation with free drugs or particles for 24 h. Cells were stained with Alexa Fluor 488 conjugated Annexin V and the nuclei were stained with DAPI. Scale bars: 50 μm. While arrows represent the apoptotic nuclei.

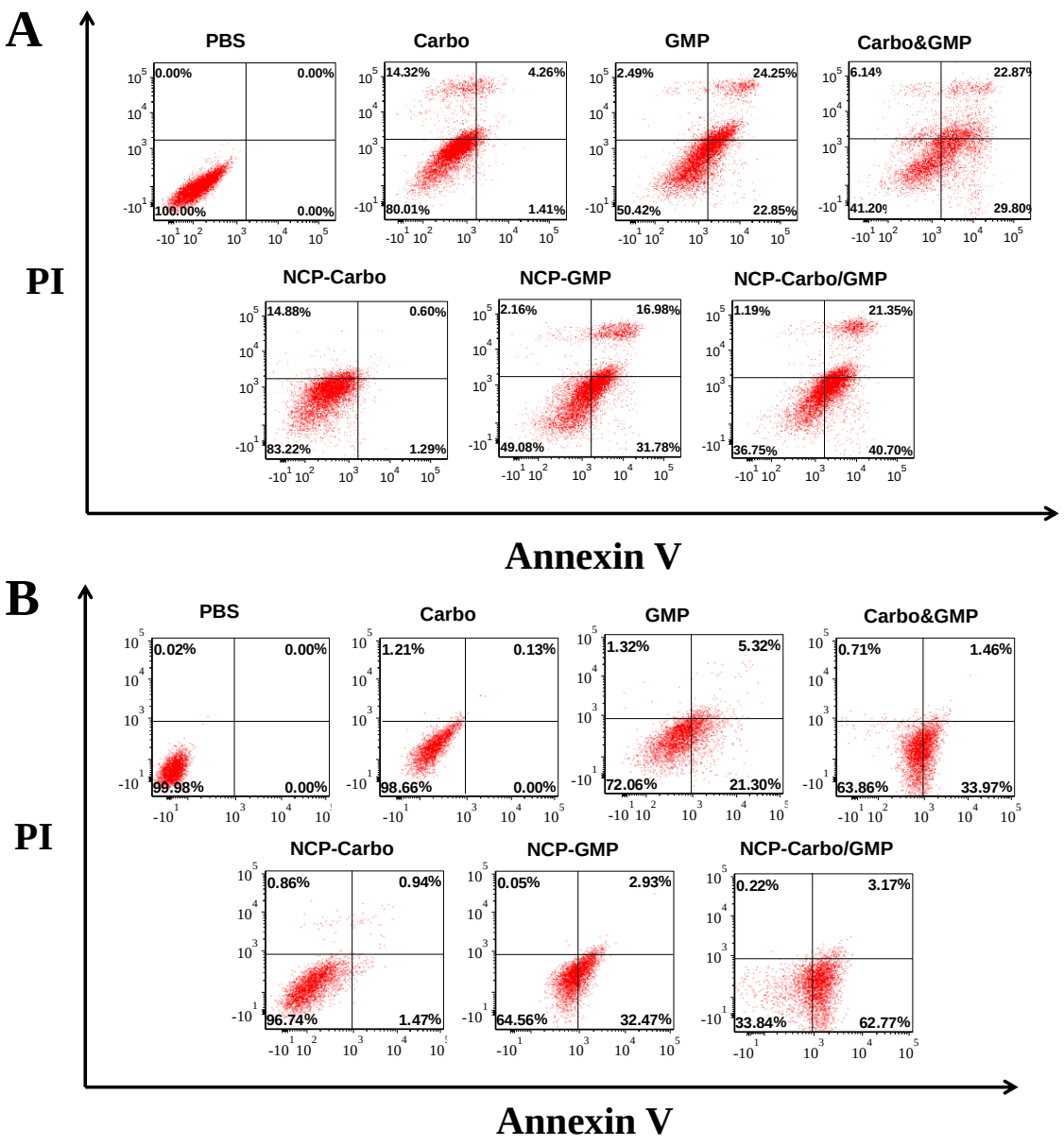

Figure S18. Flow cytometry analysis of PBS, Carbo, GMP, Carbo/GMP, NCP-Carbo, NCP-GMP, and NCP-Carbo/GMP in SKOV-3 (A) and A2780/CDDP (B) ovarian cancer cells.

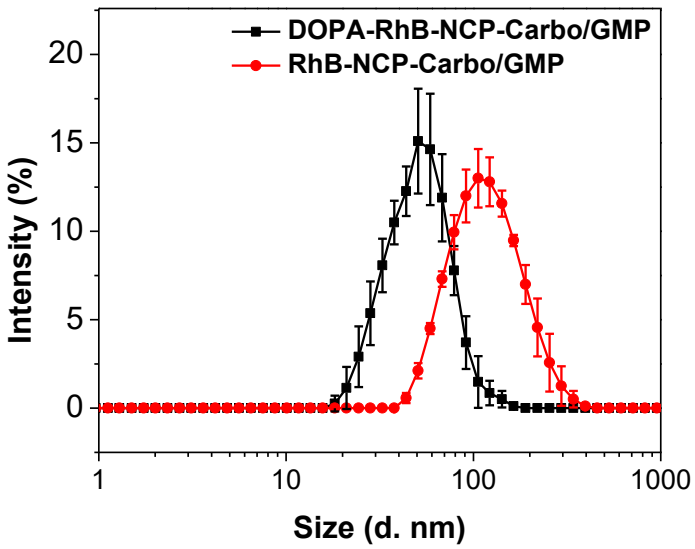

Figure S19. Intensity-average size distribution of RhB-NCP-Carbo/GMP particles. Bare and lipid-coated particles were measured in THF and PBS, respectively.

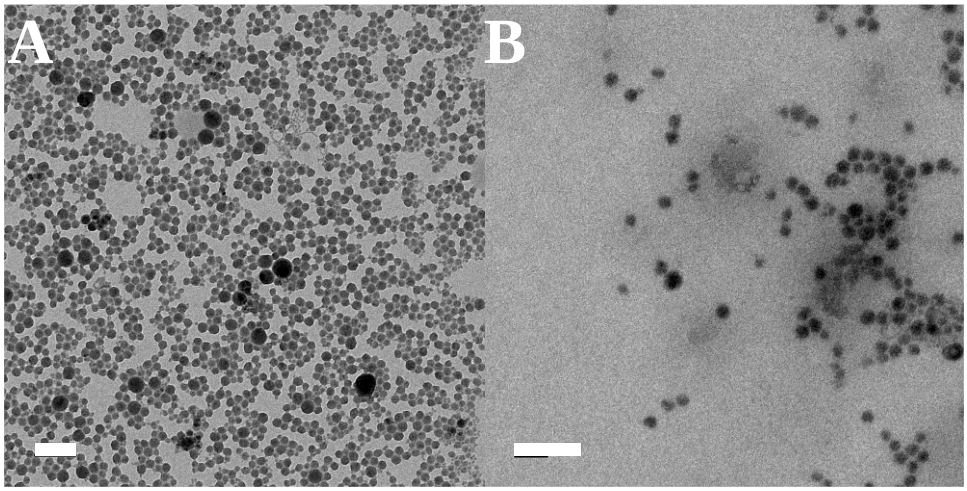

Figure S20. TEM micrographs of (A) DOPA-RhB-NCP-Carbo/GMP and (B) RhB-NCP-Carbo/GMP. Scale = 100 nm.

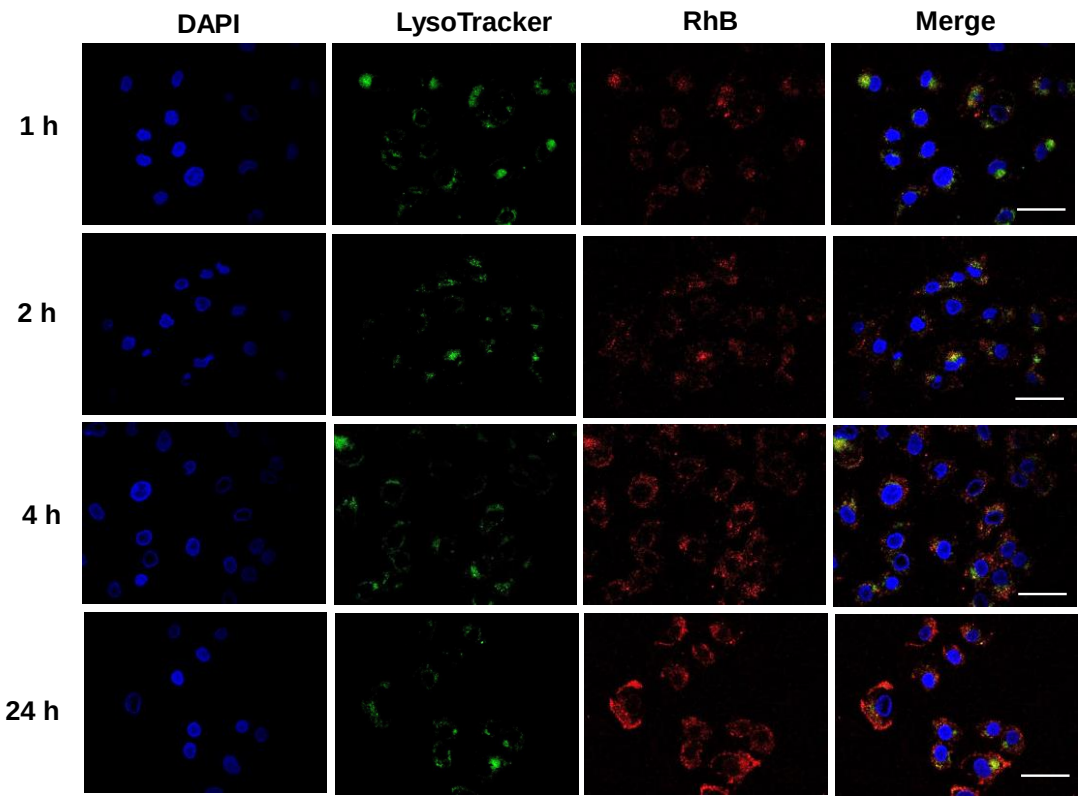

Figure S21. CLSM images of co-localization of RhB (red) from Carbo/GMP particles with a late endosome and lysosome marker, LysoTracker (green), in SKOV-3 cells after incubation for varying lengths of time. Scale bars: 50 μm.

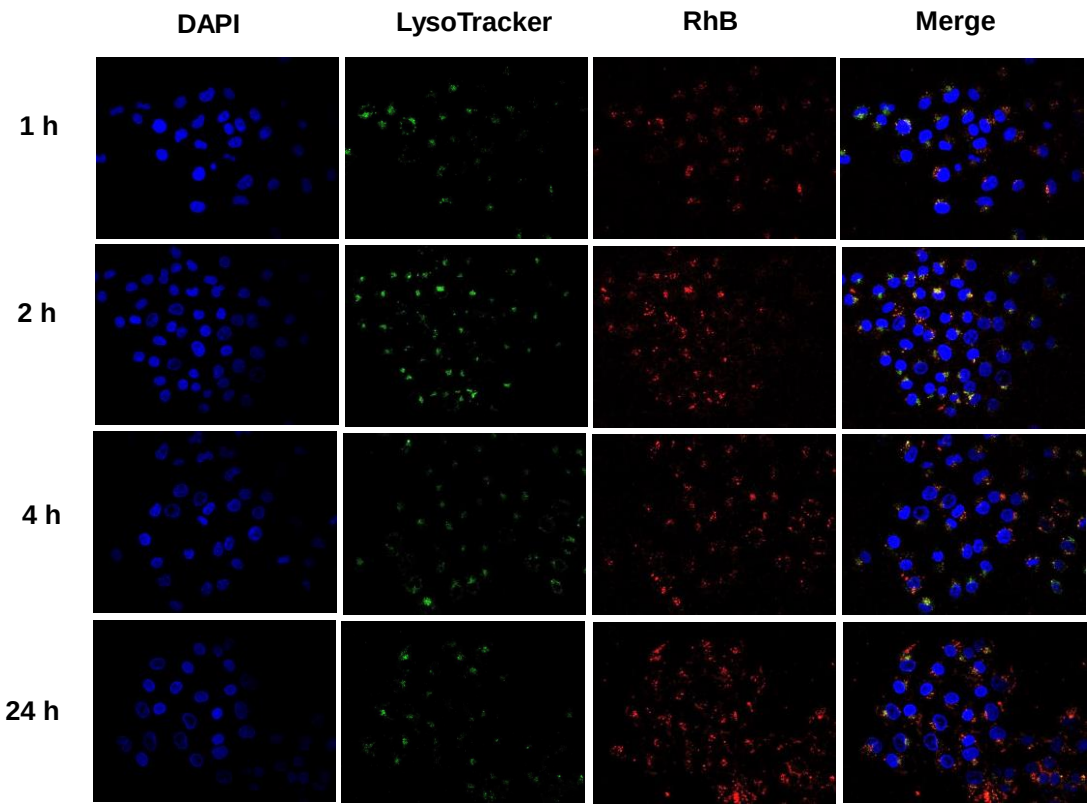

Figure S22. CLSM images of co-localization of RhB (red) from Carbo/GMP particles with a late endosome and lysosome marker, LysoTracker (green), in A2780/CDDP cells after incubation for varying lengths of time. Scale bars: 50 μm.

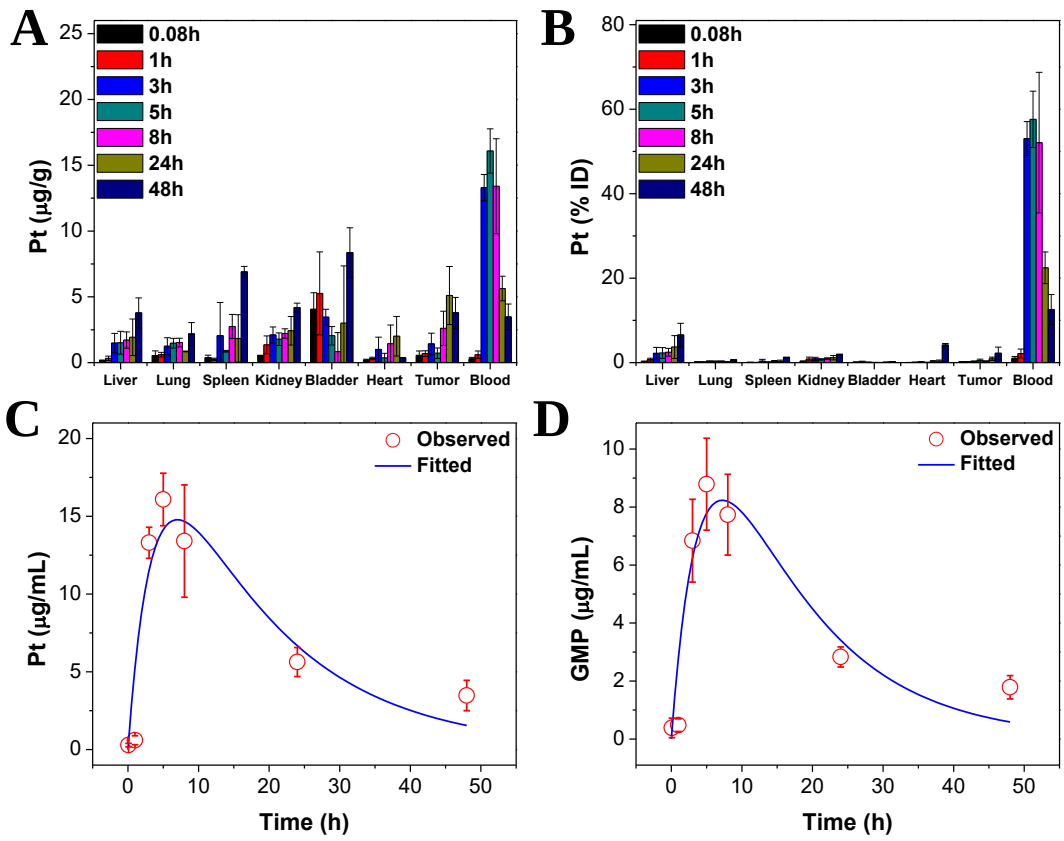

Figure S23. Concentration (μg/g) (A), and percentage injected dose (% ID) (B) of Pt distribution in tissues and blood 5 min, 1 h, 3 h, 5 h, 8 h, 24 h, and 48 h after intraperitoneal administration of NCP-Carbo/GMP in CT26 tumor-bearing mice at time points. Observed and fitted time-dependent Pt μg/mL (C) and GMP μg/mL (D) in blood. Data are mean ± S.D. (n=3).

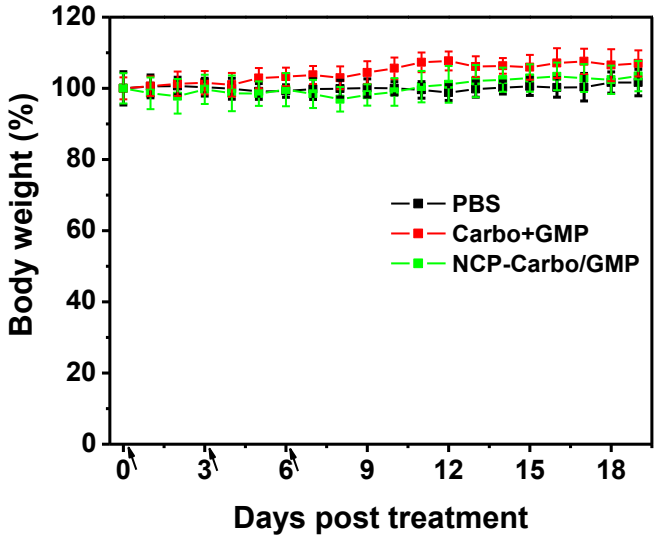

Figure S24. Body weight evolution of SKOV-3 tumor-bearing athymic mice treated with NCP-Carbo/GMP (10 mg carboplatin/kg and 2.4 GMP mg/kg).

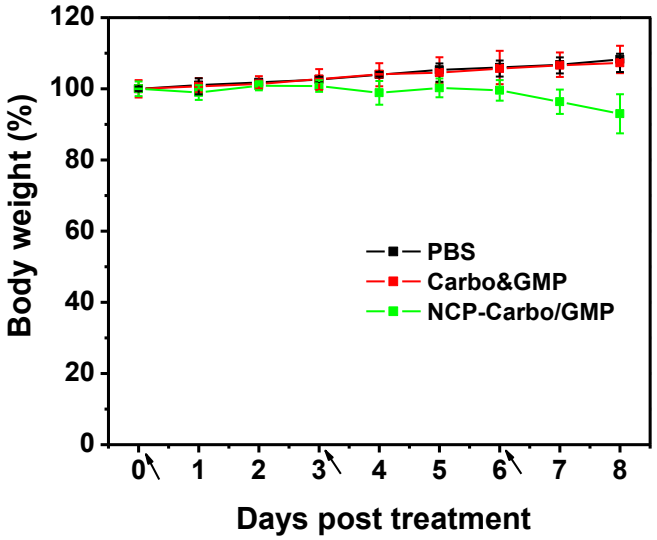

Figure S25. Body weight evolution of A2780/CDDP tumor-bearing athymic mice treated with NCP-Carbo/GMP (10 mg carboplatin/kg and 2.4 GMP mg/kg).

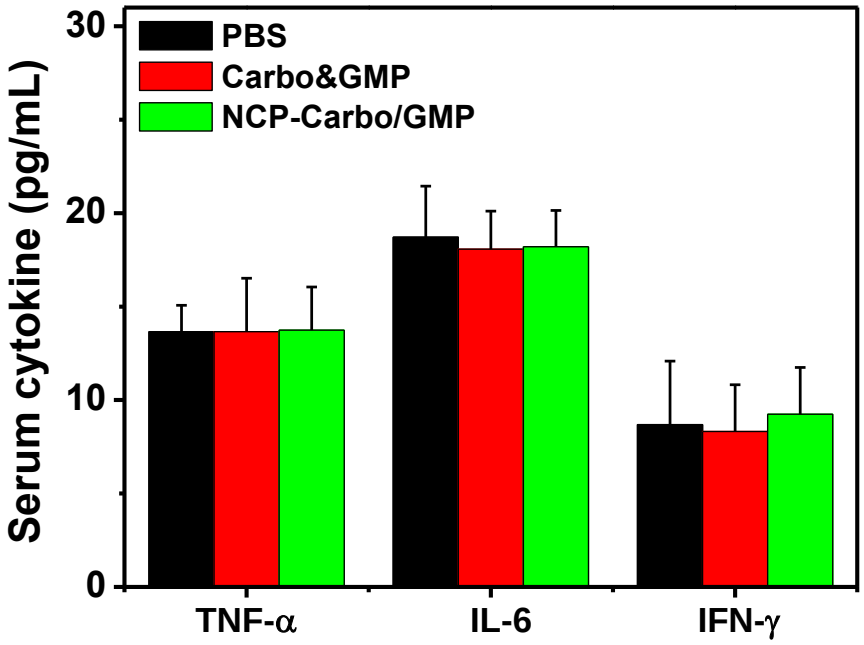

Figure S26. Immunogenic response and hypersensitivity induced by saline, Carbo&GMP, and NCP-Carbo/GMP in A2780/CDDP tumor-bearing mice.

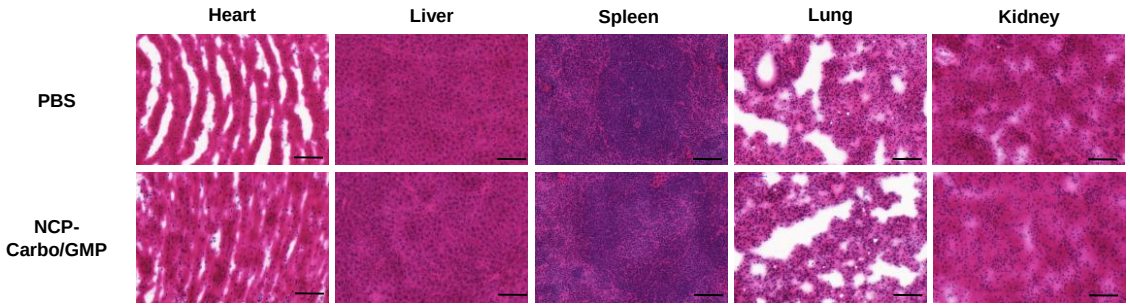

Figure S27. Histology images of resected organs (with H&E staining) of A2780/CDDP tumor-bearing mice treated with saline or NCP-Carbo/GMP. Bar = 100 μm.

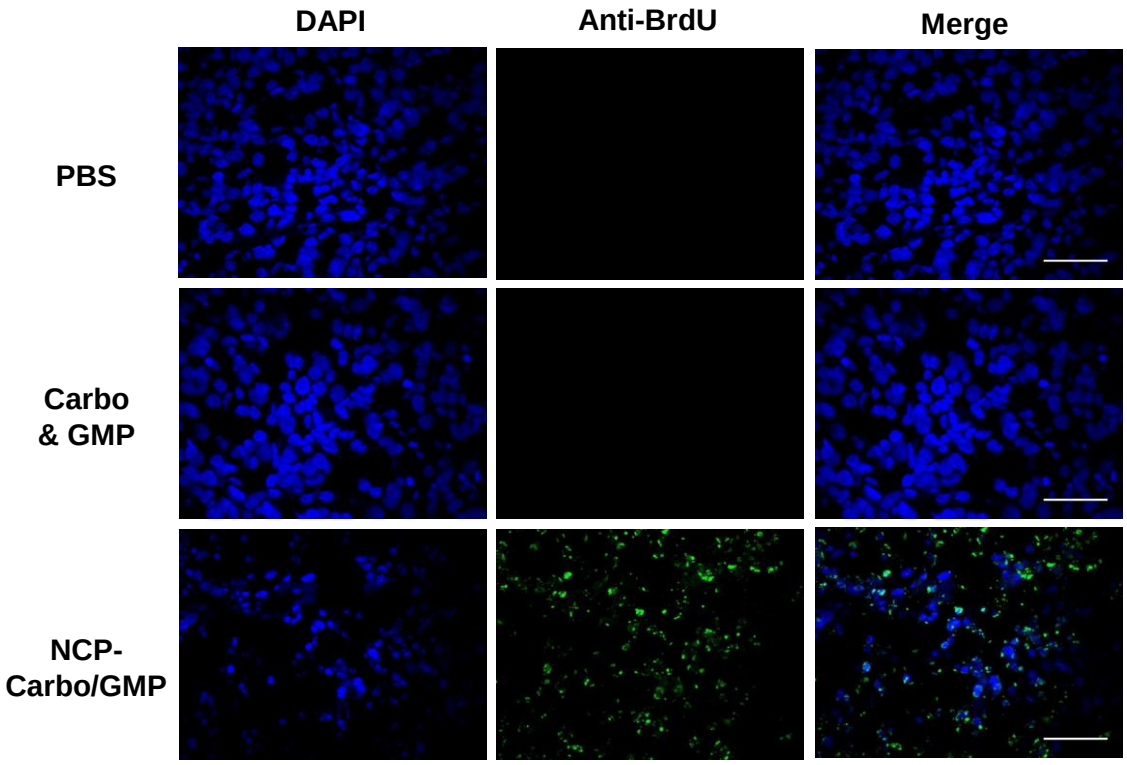

Figure S28. TUNEL analysis of apoptosis induction by different treatments on SKOV-3 tumors. The first columns from left to right represent DAPI-stained nuclei, Anti-BrdU stained cells, and merged images. Bar = 50 μm.

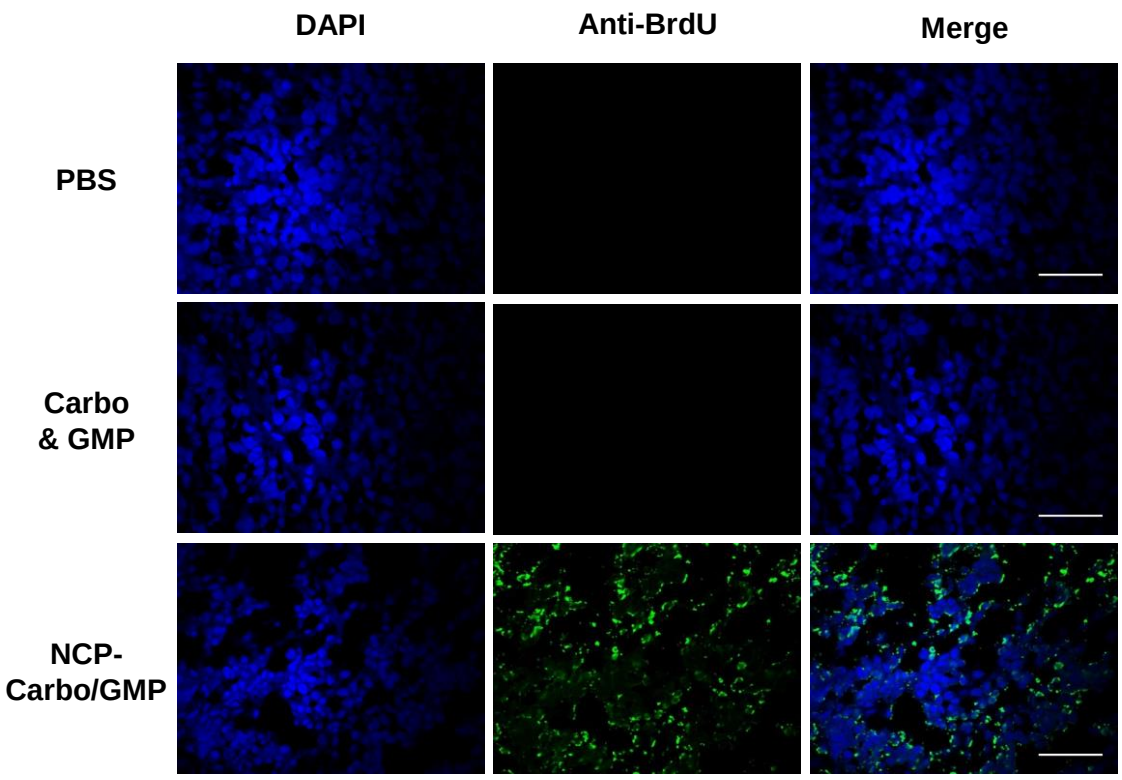

Figure S29. TUNEL analysis of apoptosis induction by different treatments on A2780/CDDP tumors. The columns from left to right represent DAPI-stained nuclei, Anti-BrdU stained cells, and the merged images. Bar = 50 μm.

Supporting Tables

Table S1. Particle sizes NCPs.

NCPs	Z-Ave diameter (nm)	Number-Ave diameter (nm)	PDI	Zeta Potential (mV)
DOPA-NCP-Carbo	54.1±0.2[#]	27.3±1.3[#]	0.203±0.006	NA
NCP-Carbo	89.7±1.1[$]	40.4±3.8[$]	0.191±0.012	-0.02±0.01
DOPA-NCP-GMP	54.1±0.2[#]	27.3±1.3[#]	0.203±0.006	NA
NCP-GMP	108.6±1.9[$]	63.1±2.9[$]	0.172±0.018	-5.87±0.40

[#]Measured in THF. [$]Measured in PBS buffer. Data are expressed as means±SD.

Table S2. Carbo IC_{50} values of carbo, GMP, NCP-Carbo, NCP-GMP, NCP-Carbo/GMP against A2780/CDDP cells incubated for 4 h (the numbers in parenthesis refer to GMP concentrations).

	Carbo(μM)	GMP (μM)	Carbo&GMP (μM)	NCP-Carbo (μM)	NCP-GMP (μM)	NCP-Carbo/GMP (μM)
A2780/CDDP	15.9±0.4	(1.9±0.5)	4.8±1.1 (1.3±0.3)	26.3±7.5	(3.7±0.4)	5.4±0.1 (1.5±0.1)

Data are expressed as means±SD.

Table S3. Particle sizes RhB-NCP-Carbo/GMP.

NCPs	Z-Ave diameter (nm)	Number-Ave diameter (nm)	PDI	Zeta Potential (mV)
DOPA-RhB-NCP-Carbo/GMP	59.9±0.3[#]	27.17±1.8[#]	0.204±0.004	NA
RhB-NCP-Carbo/GMP	108.6±1.9[$]	63.1±2.9[$]	0.172±0.018	-12.9±3.2

[#]Measured in THF. [$]Measured in PBS buffer. Data are expressed as means±SD.