Electronic Supporting Information for

Metformin-Induced Stromal Depletion to Enhance the Penetration of Gemcitabine-Loaded Magnetic Nanoparticles for Pancreatic Cancer Targeted Therapy

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Experimental Section

Materials

N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC·HCl) and N-Hydroxysuccinimide (NHS) were purchased from Sigma-Aldrich. Gemcitabine hydrochloride (GEM) and metformin hydrochloride (MET) were purchased from Dalian Meilun Biology Technology Co., Ltd. Methyl thiazolyl tetrazolium (MTT) was recived from Beyotime Biotechnology. Prussian Blue Iron Stain Kit was purchased from Solarbio. CA-074Me (cathepsin B inhibitor) was obtained from ApexBio. The pH low insertion peptide (pHLIP) NH_2-AEQNPIYWARYADWLFTTPLLLLDLALLVDADEGT and GEM-GFLG were provided by Shanghai Top-Peptide Biotechnology Co., Ltd (>95%). The carboxyl group ended PEGylated Fe_3O_4 nanoparticles (MNP-PEG-COOH) were synthesized as

previously described.[1] MilliQ Water (18.2 MΩ cm^{-1}) was obtained using a Millipore MilliQ Academic Water Purification System.

Characterization

The size of the nanoparticles was measured using dynamic light scattering (DLS). Measurements were performed using a Zetasizer Nano-ZS from Malvern Instruments equipped with a He-Ne laser with a wavelength of 633 nm at 25°C using a detection angle of 173°. Transmission electron microscopy (TEM) was carried out on a HT7700 TEM (HITACHI, Japan) transmission microscope operated at the accelerating voltage of 100 kV. The fourier transform infrared (FTIR) spectroscopy was obtained from Vector22 spectrometer (Bruker, Germany). Thermogravimetric analysis (TGA) was performed with Q50 (TA, USA). HPLC experiments were carried out on Agilent 1200. Inductively coupled plasma-mass spectrometry (ICP-MS, Ultimate3000-ICAP RQ, Thermo Fisher, USA) was used for the determination of Fe content. The MR relaxivity measurements of the GEM and pHLIP conjugated magnetic Fe_3O_4 nanoparticles (GEM-MNP-pHLIP) were performed on a 3T clinical MRI instrument (GE signa 3.0T HD, Milwaukee, WI). A series of aqueous solutions of GEM-MNP-pHLIP in 2 mL Eppendorf tubes were prepared. The parameters for T1 measurements were set as follows: echo time (TE) = 25.3 ms; repetition time (TR) = 500, 1000, 1500, 2000 ms, respectively. For T2 measurements, the parameters were set as: TR = 2000 ms, and TE = 20, 40, 60, 80, 100 ms, respectively. Fluorescence images were observed using a confocal laser scanning microscope (Leica TCS SP5). *In vivo* body fluorescent imaging was obtained with a CRI Maestro in vivo imaging system which equipping with a tunable liquid-crystal filter and a cooled scientific-grade monochrome CCD camera.

Synthesis of pHLIP and GEM Modified Fe_3O_4 Nanoparticles (GEM-MNP-pHLIP)

MNP-PEG-COOH modified with pHLIP and GEM can be easily obtained by one-pot synthesis. In brief, 10 μmol MNP-PEG-COOH, 0.35mmol EDC·HCl and 0.35mmol NHS were added into 5 mL of phosphate buffer (pH 7.4, 10 mM). The pH of the solution was then adjusted to 5.5 and kept at room temperature for 2 h. 0.3 mmol GEM-GFLG and 50 μmol pHLIPs were then added and stirred for 48 h. Then the

product was concentrated by centrifuge filters and redissolved in PBS for three times. GEM conjugated Fe_3O_4 nanoparticles without pHLIP (GEM-MNP) were obtained at the same condition without adding pHLIP.

In Vitro GEM Release from GEM-MNP-pHLIP Incubated with Cathepsin B

In order to investigate *in vitro* GEM release from MNP-GEM-pHLIP, PBS (10 mM, pH 5.0) and GEM-MNP-pHLIP solution (0.1 mM) were mixed with cathepsin B (10 UN mL^{-1} stock solution) with the final concentrations of 3 μM MNP-GEM-pHLIP and 0.5 UN mL^{-1} cathepsin B. After incubation of the solution at 37 °C for 8 h, 16 h and 24 h 1.0 mL of the solution was taken out and passed through a 0.22 μm Millipore filter. HPLC was employed to determine the concentration of GEM. Another group with the same condition but without adding cathepsin B was used as control.

Cell Lines and Cell Culture

Human pancreatic cancer cell line PANC-1 was obtained from KeyGEN BioTECH. (Nanjing, China). Human pancreatic stellate cells (PSCs) were established from pancreatic cancer surgical specimens using the outgrowth method and PSCs between passages 3 and 8 were used for experiments. PANC-1 cells were cultured in medium of dulbecco modified eagle medium (DMEM) supplemented with penicillin (100 units mL^{-1}), streptomycin (100 units mL^{-1}) and fetal bovine serum (10%) at 37 ºC in a humidified incubator with 5% CO_2 atmosphere. PSCs were cultured with stellate cell medium supplemented with penicillin (100 units mL^{-1}), streptomycin (100 units mL^{-1}) and fetal bovine serum (10%) at 37 ºC in a humidified incubator with 5% CO_2 atmosphere. HUVEC cells (human umbilical vein endothelial cell line) were cultured with RPMI 1640 supplemented with penicillin (100 units mL^{-1}), streptomycin (100 units mL^{-1}) and fetal bovine serum (10%) at 37 ºC in a humidified incubator with 5% CO_2 atmosphere. All experiments were performed when cells were growing in the logarithmic growth phase.

In Vitro Cellular Uptake

Prussian blue staining and ICP-MS were utilized to investigate the internalization of the Fe_3O_4 nanoparticles.

Prussian blue staining: PANC-1 cells were seeded in Φ 20 mm confocal dishes at 3×10^4 cells per well and incubated overnight at 37 °C. The culture medium was subsquently replaced with fresh pH 7.4 or pH 6.5 medium without FBS, and the cells were further incubated for 12 h. Then, GEM-MNP-pHLIP and GEM-MNP (final concentration 10 mg L^{-1} equivalent to Fe) were added, respectively, and incubated for 4 h. The supernatant was decanted and the cells were rinsed with PBS buffer three times to remove the nanoparticles that are not endocytosis. The cells were fixed with 4% formaldehyde for 15 min and incubated with Prussian blue staining solution for 15 min. The cell images were obtained by microscope.

ICP-MS: PANC-1 cells were seeded in 6-well plates at 5×10^5 cells per well in 2 mL of culture medium for 12 h. The culture medium was subsequently replaced with fresh pH 7.4 or pH 6.5 medium without FBS, and the cells were further incubated for 12 h. Then, GEM-MNP-pHLIP and GEM-MNP (final concentration 10 mg L^{-1} equivalent to Fe) were added, respectively, and incubated for 4 h. The supernatant was decanted and the cells were rinsed with PBS buffer three times to remove the nanoparticles that are not endocytosis. The cells were then harvested and eroded with HNO_3. The Fe content was determined using ICP-MS. Data were presented as average ± SD (n = 4).

In Vitro Cytotoxicity Assay

The cell viability was determined by conventional MTT assay.

The biocompatibility of the blank nanocarriers was evaluated at first using HUVEC cells. Briefly, HUVEC cells were seeded in 96-well plates at 5×10^3 cells per well in 180 μL of culture medium for 12 h. Then, the culture medium was replaced with fresh medium containing MNP-pHLIP with different concentrations (final concentrations from 0 to 2 μM equivalent to MNPs) was added and incubated for 72 h. 20 μL MTT (5 mg mL^{-1}) was added to each well and the cells were further cultured at 37 °C for another 4 h. Finally, 150 μL of DMSO was added to each well to replace the culture medium and formazan absorbance was determined by a microplate Bio-Rad reader (Thermo Fisher Scientific, Waltham, MA) at 490 nm. Data were presented as average ± SD (n = 4).

The viability of PANC-1 cells treated with different concentrations of blank nanocarriers MNP-pHLIP was evaluated at pH 7.4 or pH 6.5. Briefly, PANC-1 cells were seeded in 96-well plates at 5×10^3 cells per well in 180 μL of culture medium for 12 h. Then, the culture medium was replaced with fresh medium (pH 7.4 or pH 6.5) containing MNP-pHLIP with different concentrations (final concentrations from 0 to 2 μM equivalent to MNPs) was added and incubated for 72 h. 20 μL MTT (5 mg mL^{-1}) was added to each well and the cells were further cultured at 37 °C for another 4 h. Finally, 150 μL of DMSO was added to each well to replace the culture medium and formazan absorbance was determined by a microplate Bio-Rad reader at 490 nm. Data were presented as average ± SD (n = 4).

The viability of PANC-1 cells treated with different concentrations of GEM-MNP or GEM-MNP-pHLIP was evaluated at pH 7.4 or pH 6.5. Briefly, PANC-1 cells were seeded in 96-well plates at 5×10^3 cells per well in 180 μL of culture medium for 12 h. Then, the culture medium was replaced with fresh medium (pH 7.4 or pH 6.5) containing GEM-MNP or GEM-MNP-pHLIP with different concentrations (final concentrations from 0.05 to 20 μg mL^{-1} equivalent to GEM) was added and incubated for 72 h. 20 μL MTT (5 mg mL^{-1}) was added to each well and the cells were further cultured at 37 °C for another 4 h. Finally, 150 μL of DMSO was added to each well to replace the culture medium and formazan absorbance was determined by a microplate Bio-Rad reader at 490 nm. Data were presented as average ± SD (n = 4).

The viability of PANC-1 cells treated with different drugs was evaluated at pH 6.5. PANC-1 cells were seeded in 96-well plates at 5×10^3 cells per well in 180 μL of culture medium for 12 h. Then, the culture medium was replaced with fresh pH 6.5 medium. GEM-MNP-pHLIP, GEM-MNP and free GEM (final concentrations from 0.05 to 20 μg mL^{-1} equivalent to GEM) were added, respectively, and with incubating for another 72 h. 20 μL MTT (5 mg mL^{-1}) was added to each well and the cells were further cultured at 37 °C for another 4 h. Finally, 150 μL of DMSO was added to each well to replace the culture medium and formazan absorbance was determined by a microplate Bio-Rad reader at 490 nm. Data were presented as average ± SD (n = 4).

The viability of PANC-1 cells treated with different concentrations of GEM-MNP-pHLIP in the presence of cathepsin B inhibitor CA-074Me was evaluated at pH 6.5. Briefly, PANC-1 cells were seeded in 96-well plates at 5×10^3 cells per well in 200 μL

of culture medium for 12 h. Then, the cells were treated with 20 μM CA-074Me for 2 h. After washing cells with PBS, 180μL freash medium at pH 6.5 containing GEM-MNP-pHLIP with different concentrations (final concentrations from 0.05 to 20 μg mL^{-1} equivalent to GEM) was added and incubated for 72 h. 20 μL MTT (5 mg mL^{-1}) was added to each well and the cells were further cultured at 37 °C for another 4 h. Finally, 150 μL of DMSO was added to each well to replace the culture medium and formazan absorbance was determined by a microplate Bio-Rad reader at 490 nm. Data were presented as average ± SD (n = 4).

The viability of HUVEC cells treated with different concentrations of GEM-MNP-pHLIP was evaluated at pH 6.5. Briefly, HUVEC cells were seeded in 96-well plates at 5×10^3 cells per well in 180 μL of culture medium for 12 h. Then, the culture medium was replaced with fresh medium at pH 6.5 containing GEM-MNP-pHLIP with different concentrations (final concentrations from 0.05 to 20 μg mL^{-1} equivalent to GEM) was added and incubated for 72 h. 20 μL MTT (5 mg mL^{-1}) was added to each well and the cells were further cultured at 37 °C for another 4 h. Finally, 150 μL of DMSO was added to each well to replace the culture medium and formazan absorbance was determined by a microplate Bio-Rad reader at 490 nm. Data were presented as average ± SD (n = 4).

Establishment of stroma-rich PANC-1/PSCs Subcutaneous Xenograft Models and Orthotopic Pancreatic Tumor Models

All animal experiments were performed according to the "Principles of Laboratory Animal Care" (NIH publication NO. 86-23, revised 1985) and the guidelines for Animal Care and Use Committee, Zhejiang University. Healthy BALB/c nude mice (4-5 weeks old, weight around 15 g) were provided by the animal center of Zhejiang Academy of Medical Sciences. To obtain pancreatic subcutaneous xenograft models, PANC-1 cells (2×10^6) and PSCs cells (1×10^6) in 0.1 mL of PBS/Matrigel (1:1, v/v) were injected subcutaneously into the right rear flank area of male nude BALB/c mice of weight 20 g. To establish bioluminescent orthotopic pancreatic tumor models, PANC-1 cells were transfected with a luciferase gene at first. 50 μL of DMEM/Matrigel (1:1, v/v) containing PANC-1 cells (4×10^5) and PSCs cells (2×10^5) was injected into the tail of the pancreas of male BALB/c nude mice of weight 20 g.

In Vivo MRI Assessment

MR properties of Fe_3O_4 nanoparticles in orthotopic xenografts were carried out on a NOVA 9.0T/110 MRI System (Time Medical Systems, USA) equipped with a mouse coil. The MR images pre- and post-contrasted with Fe_3O_4 nanoparticles were acquired using a spin echo sequence. The imaging parameters are set as follows: repetition time (TR) = 500 ms, echo time (TE) = 7.7 ms. The Fe_3O_4 nanoparticles were administrated *via* tail intravenous injection at the dose of 10 mg Fe kg^{-1} and the signal variation was recorded before and after the injection of 4 h.

Sircol Soluble Collagen Assay

In order to qualitatively analysis the stromal suppression by MET, PANC-1/PSCs xenografts mice were randomly divided into seven groups (five mice per group). Two groups were intraperitoneally administrated with 100 mg kg^{-1} MET daily for a consecutive week. Then one group was intravenously injected with GEM-MNP-pHLIP (6 mg kg^{-1} GEM equivalents). Meanwhile, other five groups without intraperitoneally administration with MET were intravenously injected with PBS, 6 mg kg^{-1} GEM (GEM6), 15 mg kg^{-1} GEM (GEM15), GEM-MNP (6 mg kg^{-1} GEM equivalents), GEM-MNP-pHLIP (6 mg kg^{-1} GEM equivalents). The tumor tissues were harvested after 24 h. The tumor collegan content was measured using sircol collagen assay kit (Biocolor, UK) according to the manufacturer's instructions. Data were presented as average ± SD (n = 5).

In Vivo Distributions of Fe_3O_4 Nanoparticles in the Tumor Site

ICP-MS was further used to quantitatively evaluate the suppression of stromal density by MET and the targeting ability of MNP by pHLIP polypeptide. PANC-1/PSCs xenografts mice were randomly divided into three groups (fifteen mice per group). The mice from three groups were received GEM-MNP, GEM-MNP-pHLIP, and MET/GEM-MNP-pHLIP, respectively. After different time intervals, five mice from each group were sacrificed. Tumors were rapidly excised, rinsed in saline and weighed. Then the Fe content was determined by ICP-MS after the collected tumor tissues were eroded with 3 M HNO_3. Data were presented as average ± SD (n = 5).

In Vivo Antitumor Efficacy Study

PANC-1/PSCs xenografts mice were randomly divided into seven groups (five mice per group). Two groups were intraperitoneally administrated with 100 mg kg^{-1} MET daily for a consecutive week. Then one group was intravenously injected with GEM-MNP-pHLIP (6 mg kg^{-1} GEM equivalents) every 3 days for four times (MET/GEM-MNP-pHLIP). Meanwhile, other five groups without intraperitoneally administration with MET were intravenously injected with PBS, 6 mg kg^{-1} GEM (GEM6), 15 mg kg^{-1} GEM (GEM15), GEM-MNP (6 mg kg^{-1} GEM equivalents), GEM-MNP-pHLIP (6 mg kg^{-1} GEM equivalents). Tumor growth and body weight of the mice were checked and recorded. The tumor size was measured using a caliper, and the tumor volume was calculated as 1/2×length ×$width^2$. At day 29, mice were sacrificed and the tumors were harvested with weight data collected.

Luciferase transfected cells were orthotopically implanted into the tail of the pancreas. After 5 days, mice were randomly divided into seven groups (five mice per group) and received PBS, MET, GEM6, GEM15, GEM-MNP, GEM-MNP-pHLIP and MET/GEM-MNP-pHLIP. After intraperitoneal injection with 75 mg/kg D-luciferin, *In vivo* body fluorescent imaging was used to study the tumor growth in mice by collecting bioluminescence data. After day 15, mice were sacrificed and the tumors were harvested with weight data collected.

Metabolism of GEM in tumor tissue

The *in vivo* metabolism of GEM in tumor tissue was performed on PANC-1 xenografts divided into five groups (15 mice per group). The mice were injected with a single dose of GEM6, GEM15, GEM-MNP, GEM-MNP-pHLIP and MET/GEM-MNP-pHLIP (MET: intraperitoneally administrated with 100 mg kg^{-1} daily for a consecutive week) *via* the tail vein. After different time intervals, five mice from each group were sacrificed. Tumors were rapidly excised, rinsed in saline, weighed, homogenized in ammonium acetate buffer (pH 5) at various times (6 h, 12 h and 24 h). Then the tumor samples were centrifuged at a speed of 5000 rpm for 10 min. The obtained tumor homogenate was measured using HPLC. Data were presented as average ± SD (n = 5).

Histological Analysis

In order to evaluate the effect of stromal depletion in the tumor tissue after treatment with MET, the tumor tissues were harvested at 24 h after injection of PBS, MET, GEM6, GEM15, GEM-MNP, GEM-MNP-pHLIP and MET/GEM-MNP-pHLIP. Tissues samples were fixed in 4% neutral buffered formalin, processed routinely into paraffin, sectioned into 4 μm, and stained with α-smooth muscle actin antibody (α-SMA), picroslrius red, and Masson. The samples were examined by an optical microscope.

In order to track the location of Fe_3O_4 nanoparticles *in vivo*, the tumor tissues were harvested at 12 h after injection of GEM-MNP, GEM-MNP-pHLIP and MET/GEM-MNP-pHLIP (10 mg Fe kg^{-1} equivalents). Tissues samples were fixed in 4% neutral buffered formalin, processed routinely into paraffin, sectioned into 4 μm, and stained with prussian blue. The samples were examined by an optical microscope.

In order to investigate the proliferation and apoptosis levels in the tumor tissue after treatment, the tumor tissues were harvested at 24 h after injection of PBS, MET, GEM6, GEM15, GEM-MNP, GEM-MNP-pHLIP, and MET/GEM-MNP-pHLIP. Tissues samples were fixed in 4% neutral buffered formalin, processed routinely into paraffin, sectioned into 4 μm, and stained with hematoxylin and eosin (H&E), Ki67 antibody and TdT-mediated dUTP nick end-labeling (TUNEL). The samples were examined by an optical microscope.

Western Blot Analysis

Firstly, PANC-1 cells were harvested after treatment of PBS, 10 μM of GEM and 5 mM of MET for 24 h. The tumor tissues were harvested at 24 h after injection of PBS, MET, GEM6, GEM15, GEM-MNP, GEM-MNP-pHLIP, and MET/GEM-MNP-pHLIP. Then, the total cellular and tumor tissues proteins were prepared using RIPA lysis buffer and quantified using a BCA kit (Beyotime, Guangzhou, China) according to the manufacturer's instructions. The lysates were centrifuged at 12000 rpm at 4℃ for 15 min, and separated on SDS-polyacrylamide gels. After being transferred to a PVDF membrane (Millipore, USA), the proteins were incubated overnight with antibodies against rabbit P-AMPKα (Cell Signaling Technology, USA), rabbit

AMPKα (Cell Signaling Technology, USA), rabbit TGF-β1 (Abcam, UK), rabbit α-SMA (Abcam, UK), rabbit Collagen I (Abcam, UK) and rabbit β-actin (Santa Cruz, USA). Membranes were then incubated with secondary antibody (Thermo Pierce, USA). Signals were detected using the ECL (West Dura Extended Duration Substrate, Thermo Pierce, USA).

Enzyme-linked immunosorbent assay (ELISA)

PANC-1 and PSCs were serum-starved overnight and subsequently treated with metformin at the concentrations of 5 mM for 72 h. The supernatants were collected and the secretion level of soluble TGF-β1 was determined through an ELISA kit (R&D Systems, USA) according to the manufacturer's protocols. The TGF-β1 concentration of samples was calculated by comparing the OD of the samples to the standard curve.

Immunofluorescence

Indirect co-culture models were established to verify the interactions between PANC-1 and PSCs in the presence of metformin. PANC-1 was serum-starved overnight and treated with PBS, 10 μM of GEM and 5 mM of MET for 24 h. The PANC-1 supernatant (PC) was collected with filtration and then incubated with serum-starved PSCs for 24 h. The immunofluorescence experiments of PANC-1 and PSCs were performed in accordance with manufacturer's instructions. The images were obtained by fluorescent microscope at corresponding excitation.

Determination of blood parameters

ICR mice were treated with PBS, MET, GEM6, GEM15, GEM-MNP, GEM-MNP-pHLIP and MET/GEM-MNP-pHLIP and then the blood samples were collected 24 h post-injection. Serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), creatinine (CREAT) and blood urea nitrogen (BUN) were measured. The changes of white blood cell count (WBC), blood cell count (RBC), red hemoglobin count (HGB), hematocrit (HCT) and platelet count (PLT) in whole blood were also investigated.

Statistical Analysis

The results were expressed as the mean ± standard deviation (SD). Analyses were performed using GraphPad Prism version 8.0 software. Student's t-test was used to make two-group comparisons. Comparisons of parameters among three or more groups were made using one-way analysis of variance (ANOVA) for single-factor variables followed by Tukey post-hoc tests or two-way ANOVA for two-factor variables with repeated measurements over time, followed by Bonferroni post-hoc tests. The difference was considered no significance as n.s., statistically significant as $*p<0.05$ and very significant as $**p<0.01$.

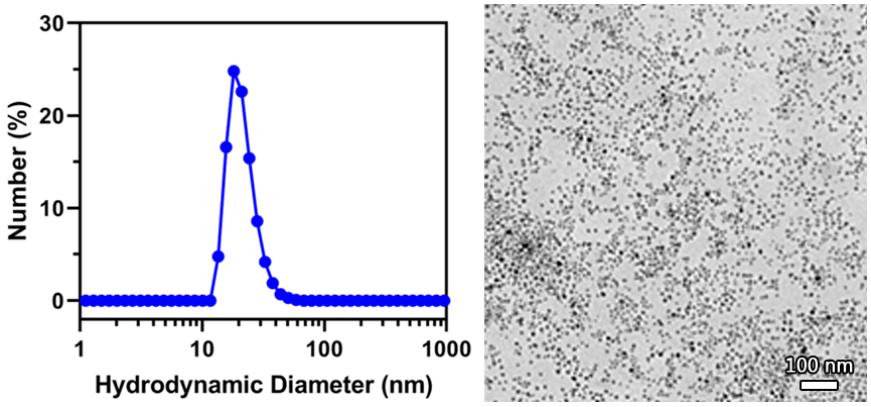

Figure S1. DLS (left) and TEM image (right) of PEGylated MNPs bearing surface carboxyl groups.

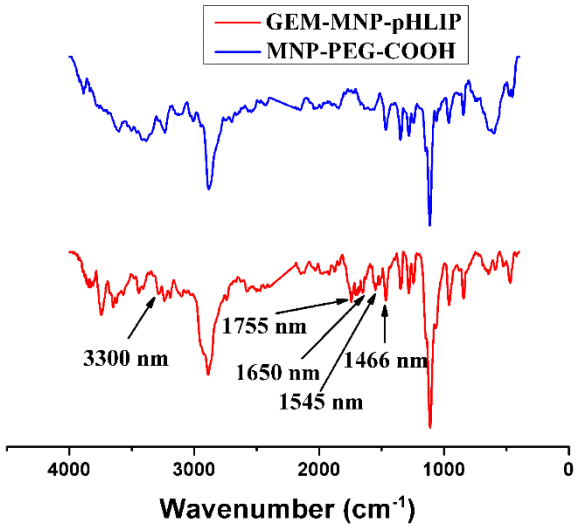

Figure S2. FTIR spectra of carboxyl group ended PEGylated MNPs (MNP-PEG-COOH) and MNP-GEM-pHLIP. The absorbance at 1545 cm^{-1} is usually associated with the amide II band that is attributable to N-H bending and C-N stretching in polypeptide amide groups. The absorbance at 1755 cm^{-1} is due to C=O of amide groups. The band frequency at 3300 nm could be assigned to the stretching vibration of $–NH_2$ groups in GEM. The characteristic absorbance at 1466 cm-1 and 1650 cm-1 could be attributed to C=N and C=C in GEM, respectively.

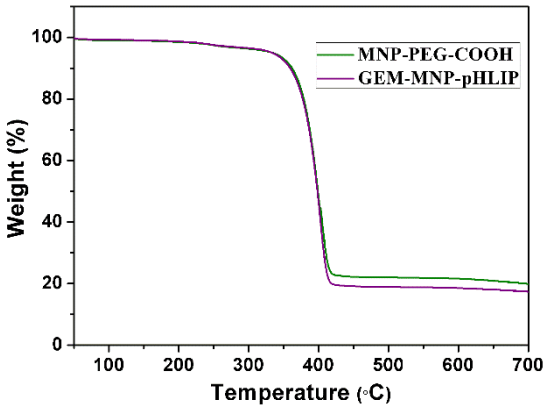

Figure S3. The thermogravimetric analysis of MNP-PEG-COOH and GEM-MNP-pHLIP in the atmosphere of nitrogen.

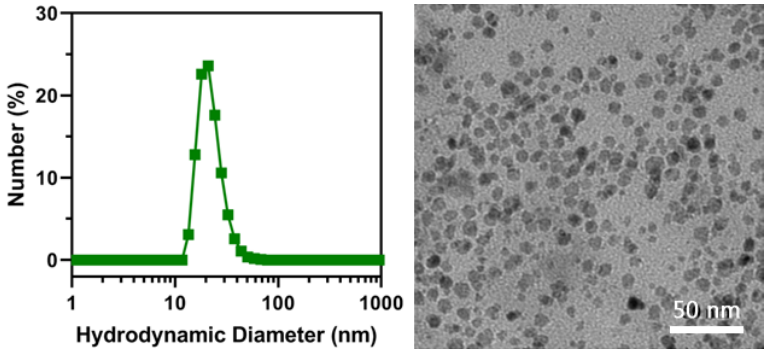

Figure S4. DLS (left) and TEM image (right) of GEM-MNP.

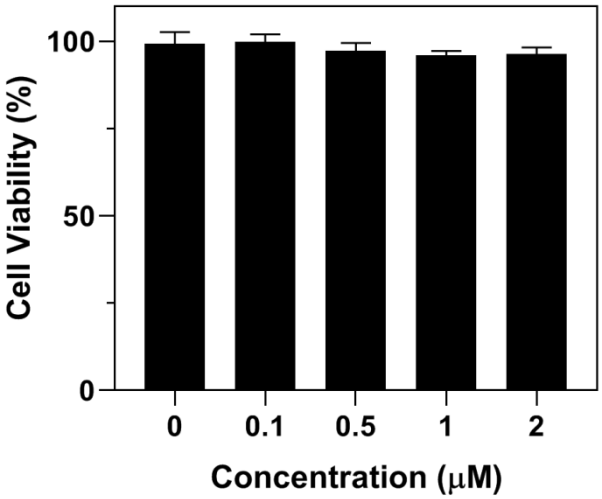

Figure S5. The viability of HUVEC cells treated with different concentrations of blank nanocarriers without GEM conjugation (MNP-pHLIP) for 72 h. (n=4)

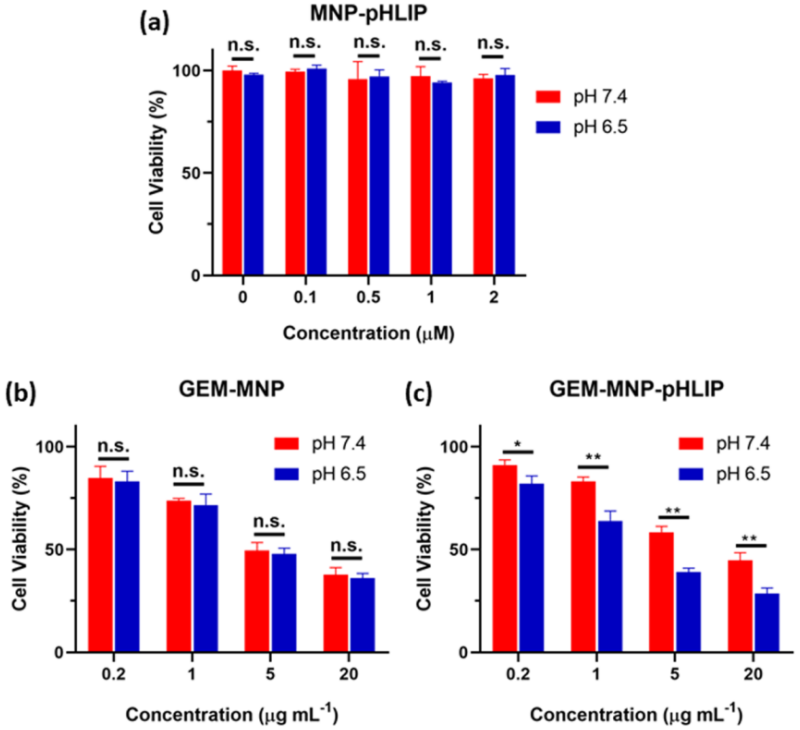

Figure S6. (a) The viability of PANC-1 cells treated with different concentrations of blank nanocarriers without GEM conjugation (MNP-pHLIP) for 72 h at pH 7.4 or pH 6.5. (b) The viability of PANC-1 cells treated with different concentrations of GEM-MNP for 72 h at pH 7.4 or pH 6.5. (c) The viability of PANC-1 cells treated with different concentrations of GEM-MNP-pHLIP for 72 h at pH 7.4 or pH 6.5. (n=4)

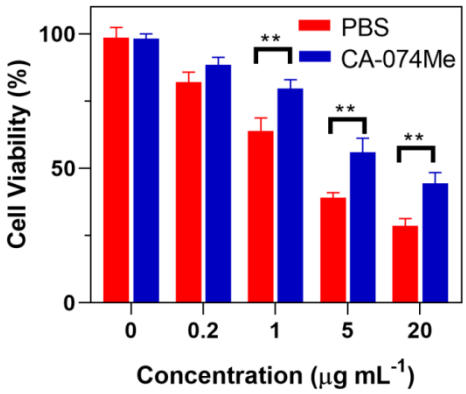

Figure S7. Cell viability of PANC-1 cells treated with GEM-MNP-pHLIP conjugates for 72 h. PANC-1 cells were pretreated with PBS or 20 μM CA-074Me for 2 h to control intracellular cathepsin B level. (n=4)

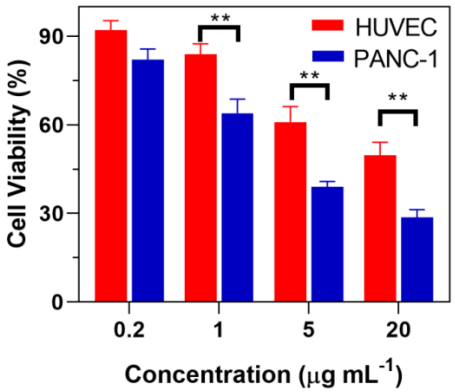

Figure S8. Cell viability of HUVEC or PANC-1 cells treated with GEM-MNP-pHLIP conjugates for 72 h. (n=4)

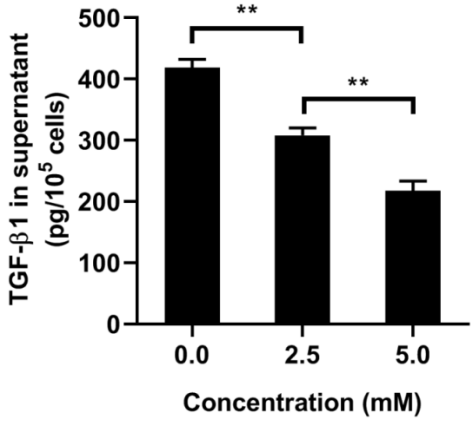

Figure S9. The secretion of TGF-β1 in the supernatants of PANC-1s after treated with MET was evaluated by ELISA.

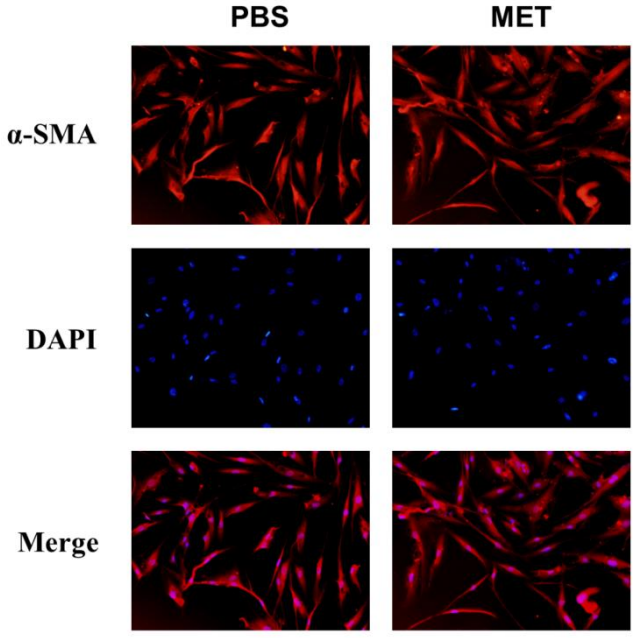

Figure S10. Immunofluorescent images of α-SMA in PSCs after treated with PBS or MET for 24 h.

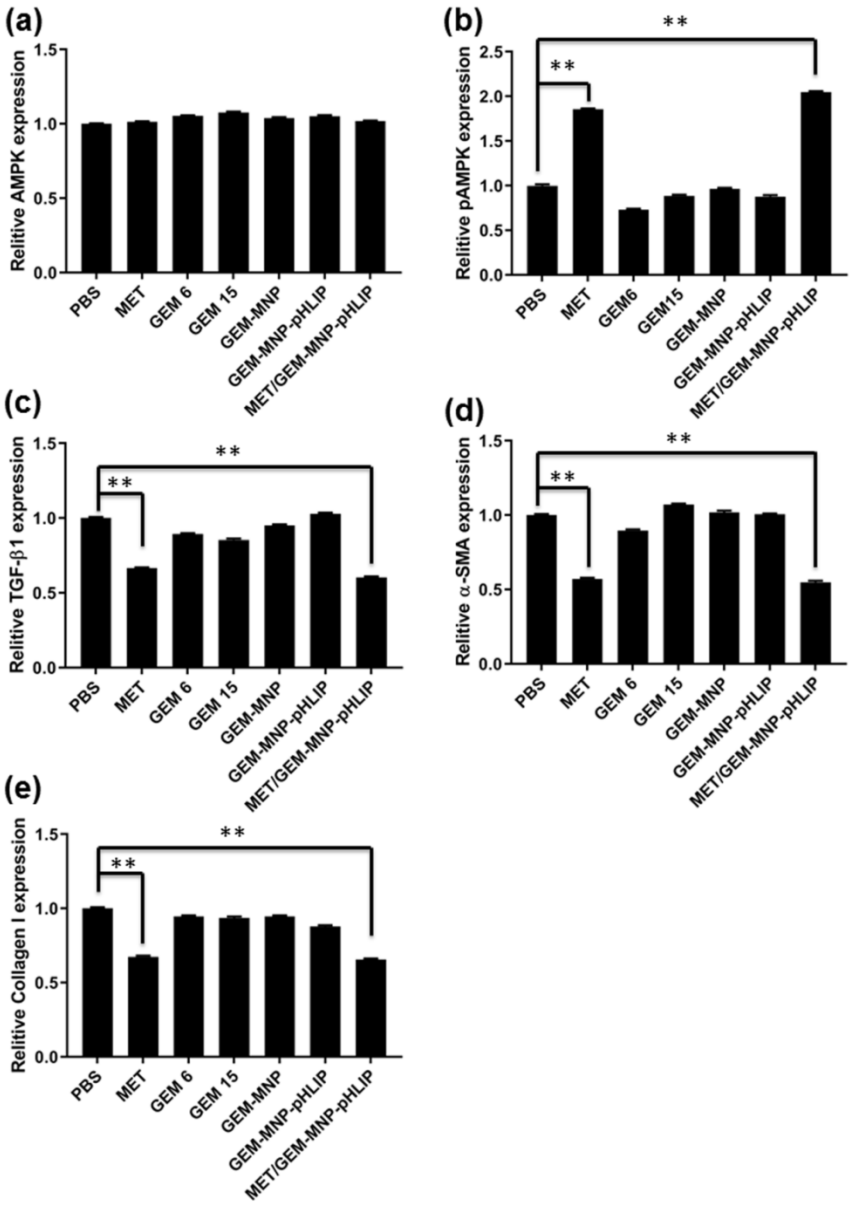

Figure S11. Quantitative analysis of AMKP (a), P-AMKP (b), TGF-β1 (c), α-SMA (d), and collagen I (e) levels from Western blot results in Figure 3b.

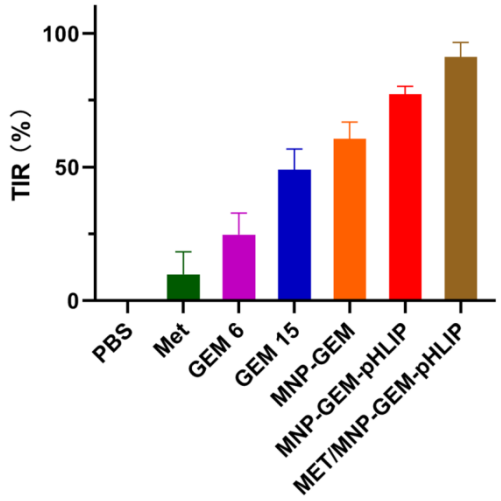

Figure S12. Tumor growth inhibition ratio after different treatments.

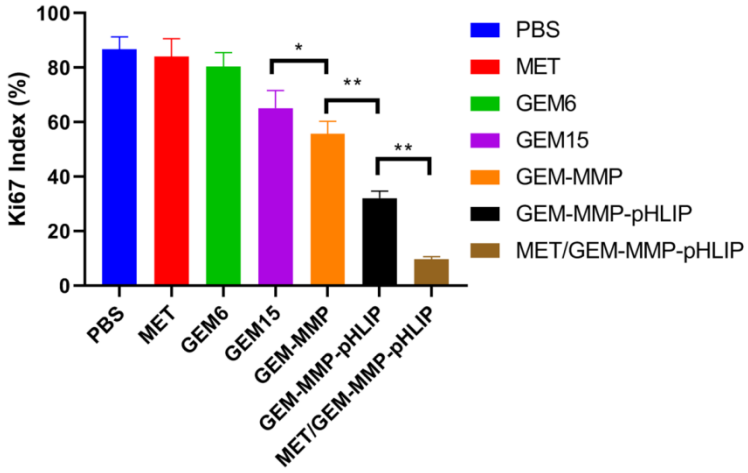

Figure S13. The Ki67 positive cells were counted from the Ki67 immunostaining as shown in Figure 4c.

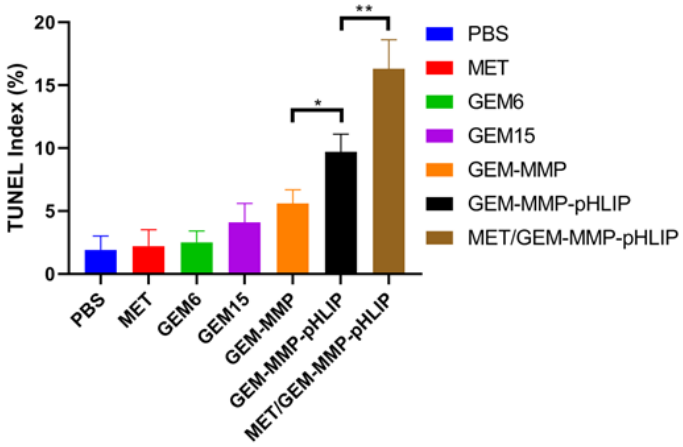

Figure S14. The TUNEL positive cells were counted from the TUNEL immunostaining as shown in Figure 4c.

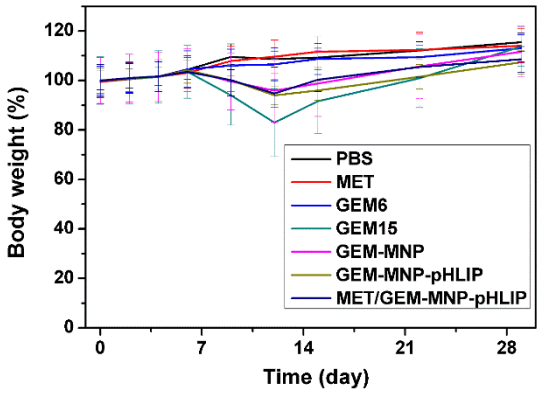

Figure S15. The normalized change of mouse body weight after different treatments.

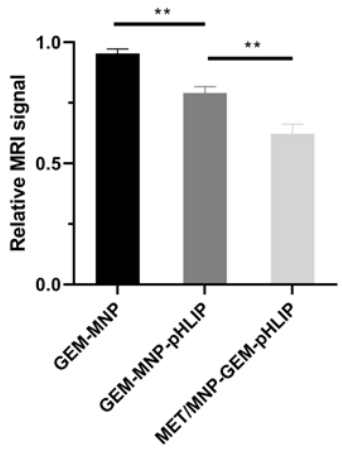

Figure S16. The relative MRI signal of orthotopic PDAC tumors 4 h after receiving different nanodrugs as indicated.

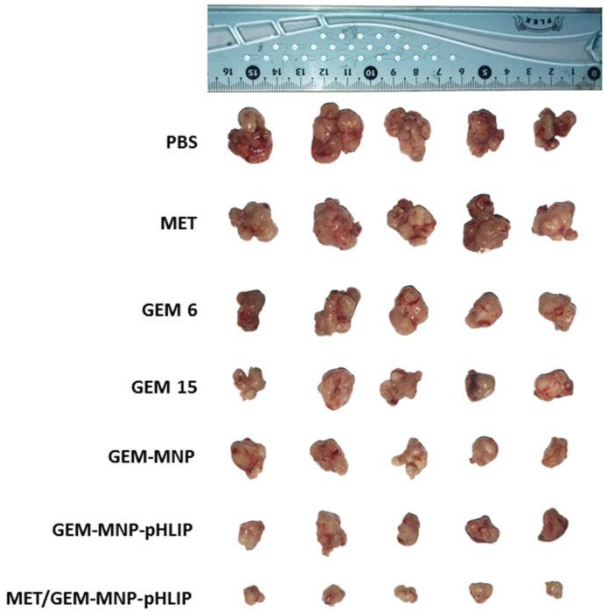

Figure S17. Digital images of excised tumors without spleens after different treatments.

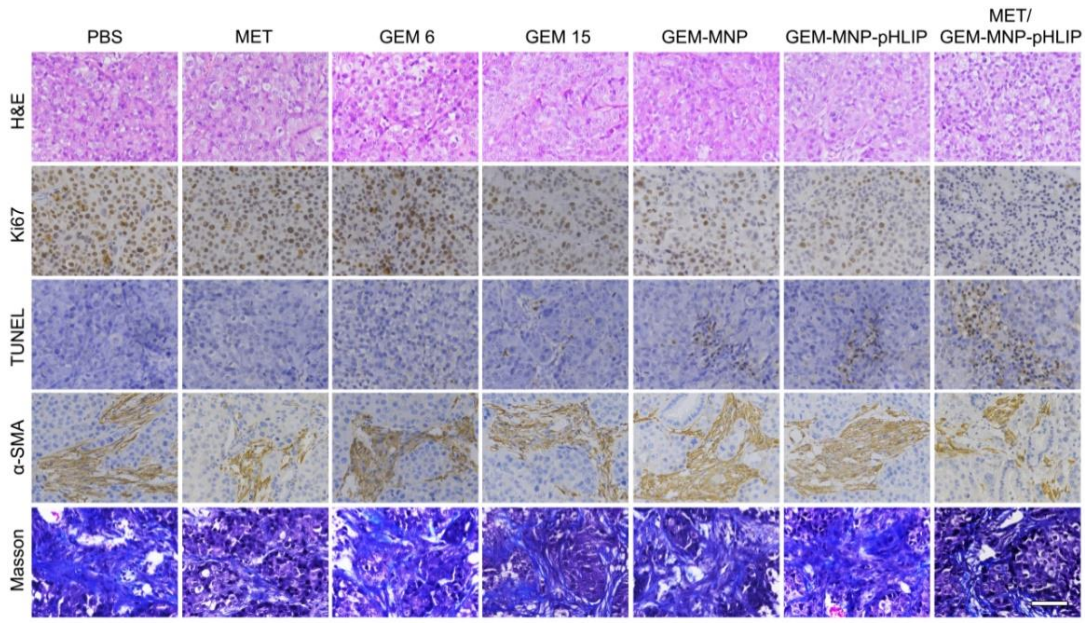

Figure S18. H&E, Ki67, TUNEL, α-SMA, and Masson's trichrome staining of tumor tissues in orthotopic tumor model after treated with various formulations. Scale bar: 50 μm.

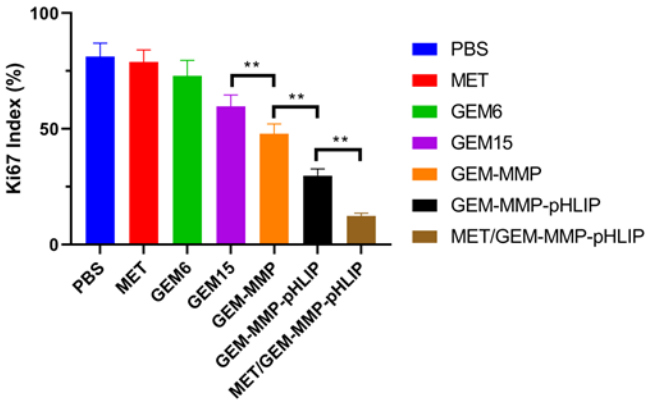

Figure S19. The Ki67 positive cells in orthotopic tumor model were counted from the Ki67 immunostaining as shown in Figure S18.

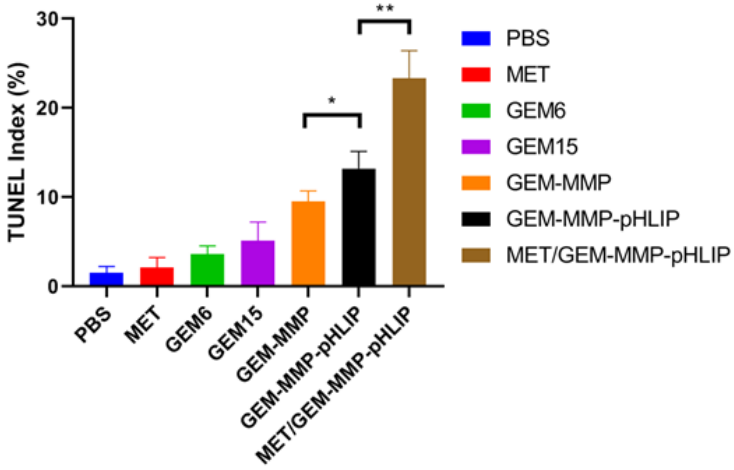

Figure S20. The TUNEL positive cells in orthotopic tumor model were counted from the TUNEL immunostaining as shown in Figure S18.

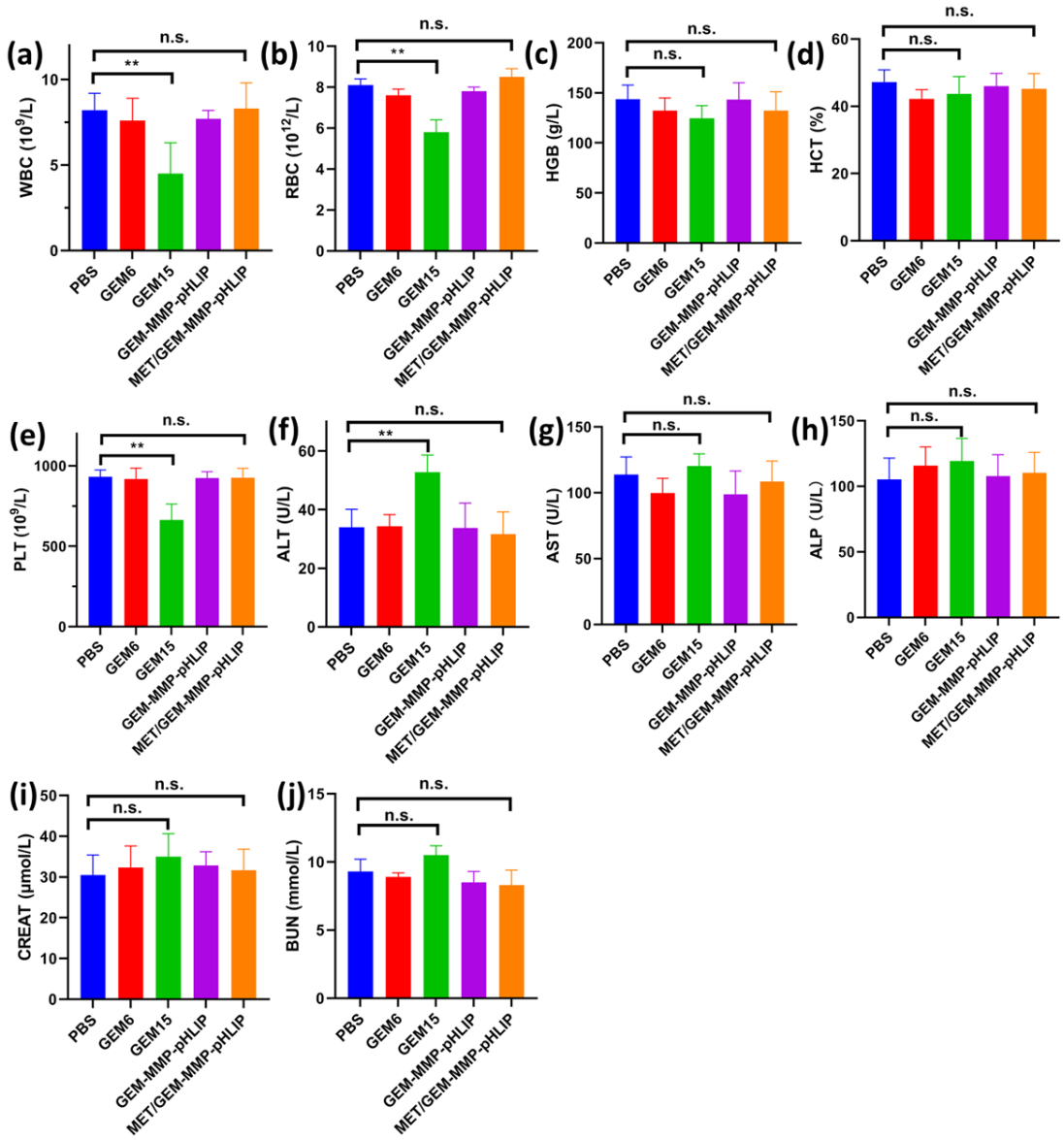

Figure S21. The analysis of biochemical parameters in whole blood, including WBC (a), RBC (b), HGB (c), HCT (d), PLT (e), and in blood serum, including ALT (f), AST (g), ALP (h), CREAT (i), and BUN (j) after treatment with different formulations.

References

1. Qiaojuan Jia, Jianfeng Zeng, Ruirui Qiao, Lihong Jing, Liang Peng, Fenglong Gu, Mingyuan Gao, Gelification: An Effective Measure for Achieving Differently Sized Biocompatible Fe_3O_4 Nanocrystals through a Single Preparation Recipe, *J. Am. Chem. Soc.*, **2011**, *133*, 19512.