Supplementary information

Zinc Sulfide-Based Hybrid Exosome-Coated Autophagy-Mediated H_2S-sensitized PDT/chemotherapeutic Synergistic Nanoplatform for Targeted Treatment of Glioblastoma Stem-Like Cells in Orthotopic Mouse Glioblastoma Model

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Photocatalytic activity

The photocatalytic performances of ZnS nanoparticles were characterized by degradation of MB in different aqueous media under the irradiation of the 500 W tungsten halogen lamp for a given time. Prior to illumination, the suspension was magnetically stirred for 30 min in the dark to achieve the adsorption equilibrium of the MB on the photocatalyst powders. The absorption spectra of MB solutions in the presence of ZnS sample under different test conditions is shown in Fig. S1.

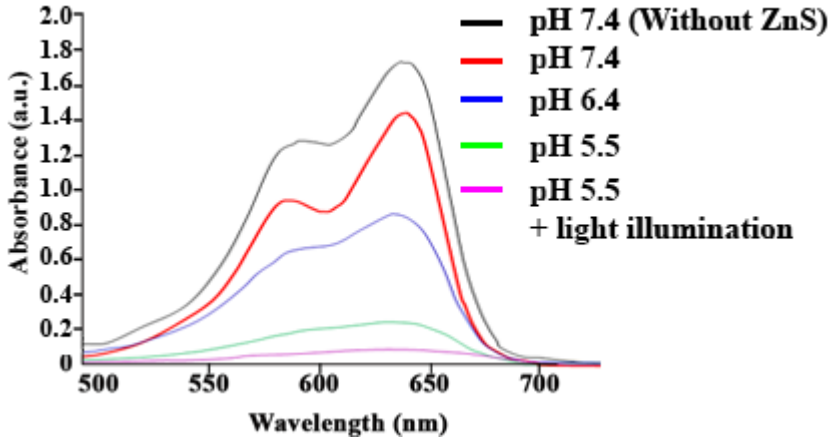
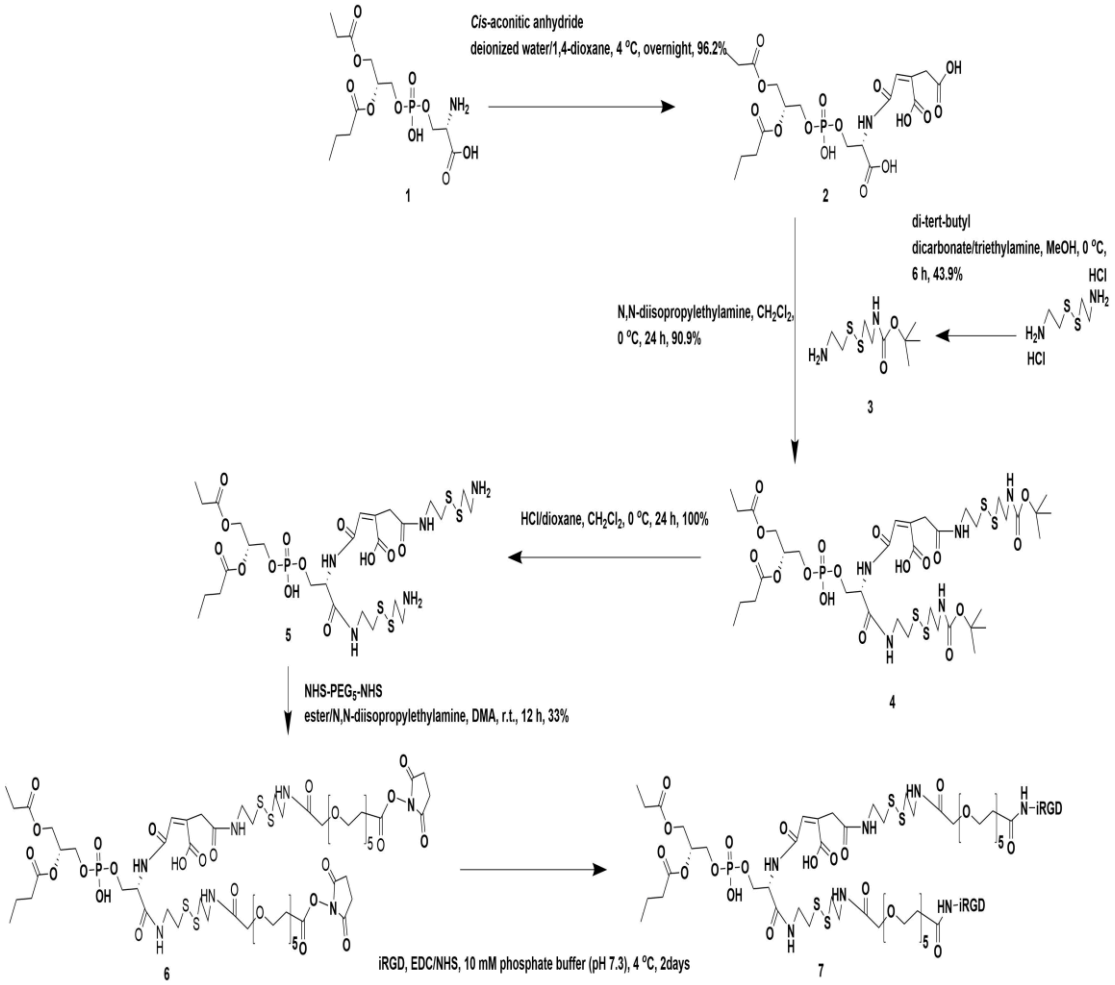


Figure S1. Absorption spectr as of MB solution obtained with/out the presence of ZnS in different test conditions.

Organic Synthesis



Scheme S1. Graphical synthetic routes of iRGD-PEG-ss-aPS.

Phosphatidylserine amidol succinic acid (**2**)

Phosphatidylserine (**1**, 10 g, 25.97 mmol) was dissolved in 4 L of deionized water. *Cis*-aconitic anhydride (4.68 g, 30 mmol) in 100 mL of 1,4-dioxane was slowly added, and the mixture was stirred overnight at 4 °C. The solution was mixed with 500 mL of chloroform and 5 mL of 5 % aqueous sodium bicarbonate. The chloroform phase was decanted, and the residual solution was extracted with ethyl acetate. The extract was concentrated on a rotary evaporator and dried at room temperature under vacuum to get **2** as yellow solid. Yield: 13.56 g (96.2%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.34 (s, 1H, C=CHC=O), 5.58 (quint, 1H, CO-CH-CO), 1.12-1.34 (m, 6H, CH_3).

N-tert-Butoxycarbonyl cystamine (**3**)

Cystamine dihydrochloride (6.756 g, 30.0 mmol) and triethylamine (12.54 mL, 90.0 mmol) were dissolved in MeOH (90 mL) and added to a methanolic solution (60 mL) of di-tert-butyl dicarbonate (3.273 g, 15.00 mmol). The reaction mixture was stirred for 6 h at 0°C, after which the solution was evaporated, and 1 M NaH_2PO_4 was added to the residue. The aqueous solution was washed with diethyl ether to remove di-t-Boc-cystamine. The aqueous solution was adjusted to pH 9 by addition of 1 M NaOH, and the solution was extracted with AcOEt. The organic phase was dried over $MgSO_4$ and

then evaporated. The resulting yellow oil was dried in vacuo to give **3**. Yield: 1.661 g (43.9%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.66 (t, 2H, C(=O)-NHCH_2), 3.33 (t, 2H, CH_2NH), 2.89 (t, 4H, CH_2SSCH_2), 1.56 (s, 9H, CH_3).

Phosphatidylserine-*cis*-aconityl-PEG-ss-Boc (**4**)

Compound **2** (1.624 g, 3 mmol) was conjugated to compound 3 in an EDC/NHS coupling reaction. Compound 3 (2 g, 7.92 mmol) was dissolved in distilled water (200 mL), followed by dilution with 200 mL of methanol. A predetermined amount of compound 2 was dissolved in 200 mL of dimethylformamide, then equal amounts of EDC and NHS (two equivalents of carboxyl group) were added to the solution. After stirring for 15 min, the solution obtained above was added. The resulting solution was stirred for 1 day at room temperature. The solution was dialyzed against excess distilled water. The product was freeze-dried to obtain **4** as yellow solid. Yield: 2.755 g (90.9%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.25 (s, 1H, C=CHC=O), 5.53 (quint., 1H, CO-CH-CO), 4.56 (d, 6H, O-CH_2CH) 3.62 (t, 2H, C(=O)-NHCH_2), 3.40 (t, 8H, CH_2NH), 2.93 (s, 1H, C(=O)-CHC), 2.85 (t, 8H, CH_2SSCH_2), 1.52 (s, 9H, CH_3), 1.15-1.31 (m, 6H, CH_3).

N-Methacryloylcystamine (**5**)

Compound **4** (1.01 g, 1 mmol), dissolved in CH_2Cl_2 (100 mL), was added to 4 N HCl/dioxane (1.5 mL, 5.96 mmol) in CH_2Cl_2 (100 mL), and stirred for 24 h at 0°C. The solution was evaporated to give the crude product as a white solid, which was washed with diethyl ether and dried in vacuo. Yield: 0.779 g (96.2%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.33 (s, 1H, C=CHC=O), 5.53 (quint., 1H, CH_2O-CH-CH_2O), 4.56 (d, 6H, O-CH_2CH) 3.62 (t, 2H, C(=O)-NHCH_2), 3.40 (t, 8H, CH_2NH), 2.93 (s, 1H, C(=O)-CHC), 2.85 (t, 8H, CH_2SSCH_2), 1.15-1.31 (m, 6H, CH_3).

NHS-type cleavable monomer for conjugated template (**6**)

Compound **5** (0.433 g, 0.535 mmol) and N,N-diisopropylethylamine (0.095 mL) were dissolved in DMA (100 mL), after which NHS-PEG_5-NHS (0.605 g, 1.2 mmol) was added to the solution. The reaction mixture was stirred for 12 h at room temperature and evaporated in vacuo. The residue was dissolved in $CHCl_3$ and washed with brine, and the organic phase was dried over $MgSO_4$ and evaporated. The resulting yellow solid was dried in vacuo to give **6**. Yield: 0.280 g (33.0%). ^{1}H NMR (400 MHz, $CDCl_3$): δδ 6.28 (s, 1H, C=CHC=O), 5.53 (quint., 1H, CH_2O-CH-CH_2O), 4.53 (d, 6H, O-CH_2CH) 3.62 (t, 2H, C(=O)-NHCH_2), 3.52 (t, 20H, NHC(=O)-CH_2(CH_2OCH_2)$_5$), 3.41 (t, 8H, CH_2NH), 2.95 (s, 1H, C(=O)-CHC), 2.83 (t, 8H, CH_2SSCH_2), 2.55 (t, 8H, C(=O)CH_2CH_2C(=O)), 1.17-1.30 (m, 6H, CH_3).

Synthesis of template molecule iRGD-PEG-ss-aPS (**7**)

Compound **6** (0.159 g, 0.10 mmol), EDC (0.50 mmol) and NHS (0.50 mmol) were dissolved into phosphate buffered solution (PBS) (pH = 6.0) and stirred at room temperature for 4 h to activate the carboxyl group of compound **6**. Then, iRGD peptide (0.284 g, 0.3 mmol) was added to the above solution. The above mixture was stirred at room temperature for 48 h. The solution was subsequently dialyzed with a Float-A-

Lyzer G2 device (MWCO: 2-3 kD) to remove unreacted monomers. The dialyzed solution was ultra-filtered using a Vivaspin 20 (4000 rpm, 10 min) and lyophilized to give **7**.

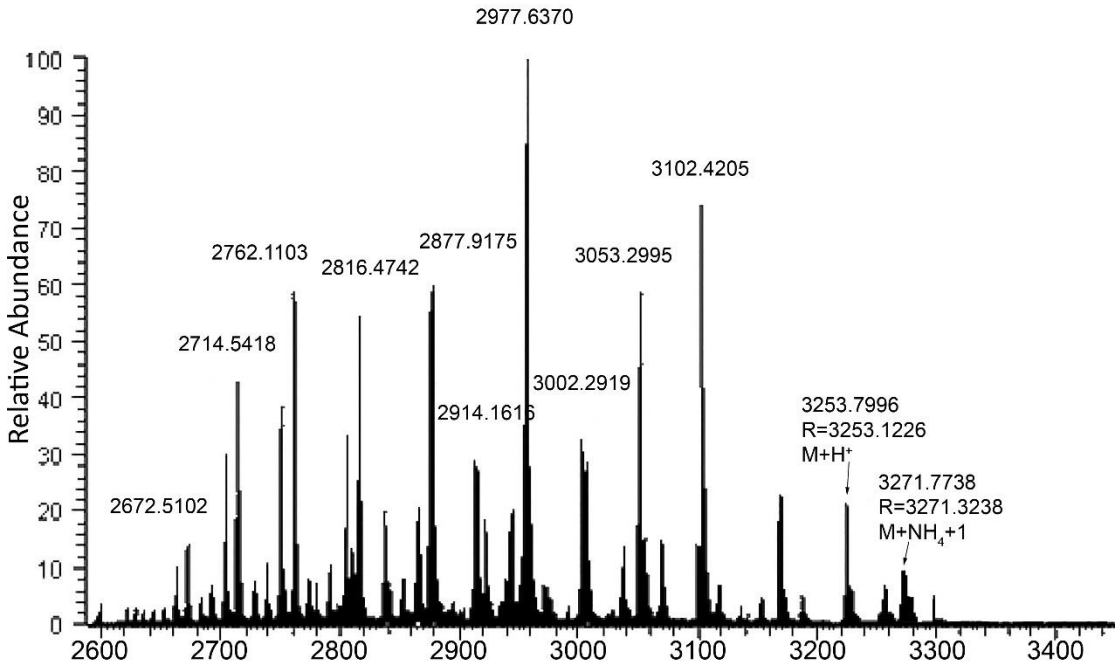


Figure S2. The electrospray ionisation mass spectra (positive mode) for iRGD-PEG-ss-aPS.

Lc-Ms/Ms conditions

Liquid chromatography was performed on a Shimadzu HPLC system (Shimadzu, Tokyo, JPN). Curcumin and I.S. (hesperetin) were separated on a 50 × 2.1 mm BetaBasic C8 column, 5 μm column (Thermo Hypersil-Keystone, Bellefonte, USA). The mobile phase comprising water containing 0.1% formic acid-acetonitrile (94:6, v/v) was delivered isocratically at a flow rate of 0.5 mL/min. HCQ and the I.S. were monitored using a Finnigan TSQ Quantum EMR Triple Quadrupole mass spectrometer (Thermo Fisher Scientific Corp., San Jose, USA) equipped with an electrospray ionization (ESI) source. The mass spectrometer was operated in positive ESI mode with a collision gas (Argon) pressure of 1.5 mTorr, a typical electrospray needle voltage of 3000 V, a sheath nitrogen gas flow of 49 (arbitrary unit) and a heated capillary temperature of 350°C. HCQ was analyzed by the multiple reaction monitor (MRM) mode using ion transitions at a proper collision energy as follows: The precursor to product ion transitions of m/z 336 → 247 and m/z 340 → 251 were used to measure the analyte and IS, respectively. The mass spectrometer was tuned to its optimal sensitivity by direct infusion of HCQ. The assay demonstrated a good linear dynamic range of 1-3200 ng/mL for hydroxychloroquine, with coefficient of determination (r^2) of =0.9999. The chromatographic run time was 3 min. The retention time of HCQ is

4.6 min.

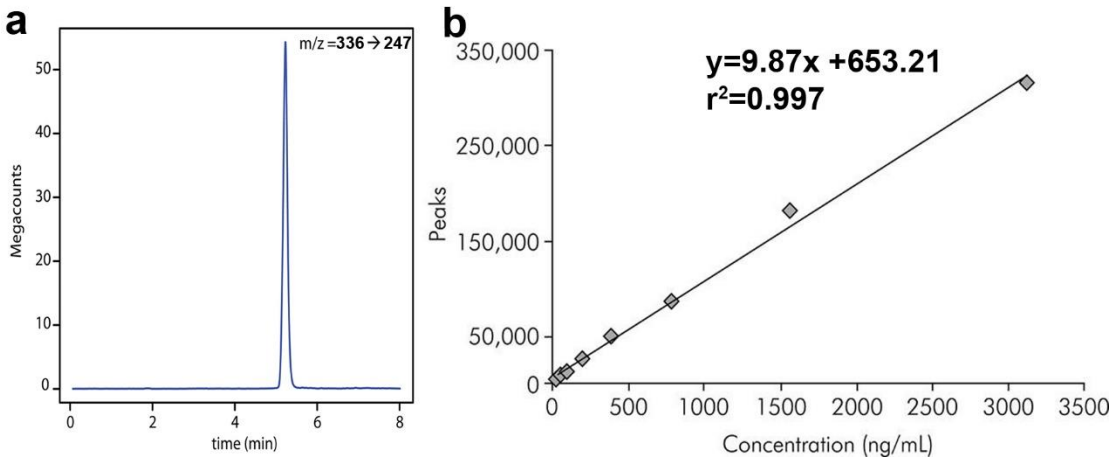


Figure S3. (a) Typical chromatograms based on the multiple reaction monitor (MRM) of HCQ. (b) The standard curve for carboplatin. Calibration standards containing HCQ in the concentrations (ng/mL) of 1, 5, 10, 200, 400, 800, 1600, and 3200 were prepared before injection into LC-MS/MS for analysis. Calibration curves of HCQ exhibited excellent linearity over the concentration ranges of 1-3200 ng/mL.

Effect of HCQ@ZnS@exo@iRGD on cell invasion and migration *in vitro*

In experiments to measure cell invasion, U87 cells (5×10^4) in 300 μL serum-free DMEM were seeded into the upper chambers of 24-well cell culture plates with polycarbonate inserts with pore diameters of 8 μm (Millipore, Billerica, MA, USA). After 24-h incubation, 800 μL of DMEM containing 10 % serum was added to the lower chamber. Then cells were incubated for 24 h with PBS, free HCQ, HCQ@ZnS, HCQ@ZnS@exo or HCQ@ZnS@exo@iRGD, after which supernatants were aspirated, fresh standard medium was added, and cells were cultured for another 48 h. Cells that had not penetrated the filter were removed using cotton swabs, and cells that had migrated to the lower surface of the filter were fixed with 4% paraformaldehyde and stained with 0.1% crystal violet. Cells were counted in five randomly selected fields at 40× magnification (Olympus, Japan). Each assay was performed in triplicate.

In experiments to measure cell migration, U87 cells were cultured to confluence for 24 h in 6-well plates in a 5 % CO_2 incubator. The cell monolayer was scratched with a 200 μL pipette tip, then washed twice in PBS to remove floating cells. Cells were then cultured for 6 h in DMEM containing 0.5 % FBS in the presence of PBS, free HCQ, HCQ@ZnS, HCQ@ZnS@exo or HCQ@ZnS@exo@iRGD. Supernatants were aspirated, fresh standard medium was added, and cells were cultured for another 18 h. Cells migrating from the leading edge were photographed under a light microscope.

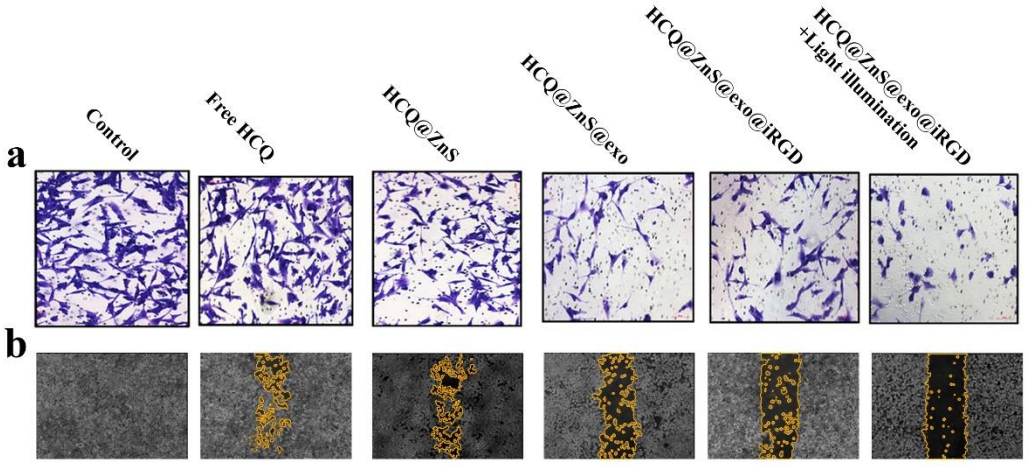


Figure S4. HCQ@ZnS@exo@iRGD effects on invasion, migration, sphere formation activity, as well as androgen receptor and b-catenin levels in U87 cells. (a) Typical images showing inhibition of U87 cell invasion by different formulations (magnification, 100x). (b) Quantitation of U87 cell invasion. (c) Cells were scratched with a pipette tip, then treated with free HCQ, HCQ@ZnS, HCQ@ZnS@exo or HCQ@ZnS@exo@iRGD with/out light illumination. The control group was left untreated. After incubation in fresh culture medium for another 18 h, migration was photographed under phase-contrast microscopy (magnification, 20x).

Using a Boyden transwell chamber assay, we found that treating U87 cells with HCQ@ZnS@exo@iRGD under light illumination significantly attenuated the invasiveness of cells. In the wound healing assay, the gap was little changed compared to the original width in cultures treated with HCQ@ZnS@exo@iRGD under light illumination. The gap was nearly completely covered in control cells left untreated and in cells treated with free HCQ and HCQ@ZnS after 18 h of incubation.

Preliminary evaluation of side effects *in vivo*

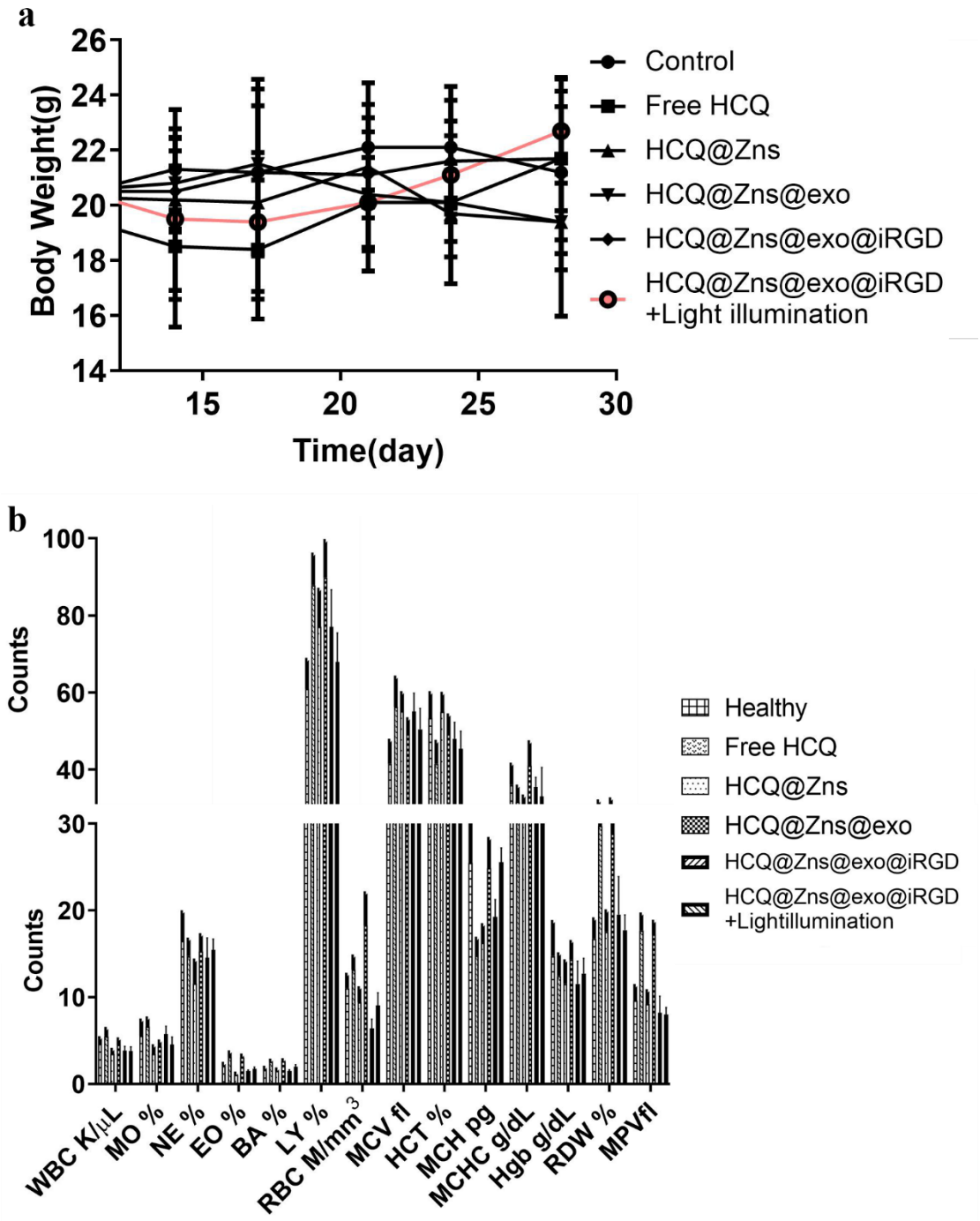


Figure S5. Toxicity of different treatment groups showing no significant systemic damages. (a) Cumulative body weight during treatments. (b) Hematological changes in blood of different treatment groups. WBC, white blood cells; LY, lymphocytes; MO, monocytes; NE, neutrophils; EO, eosinophils; BA, basophils; RBC, red blood cells; MCV, mean corpuscular volume; HCT, hematocrit; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; Hgb, hemoglobin; RDW, red blood cell distribution width; MPV, mean platelet volume. Healthy group was treated with saline.