Supplementary Data

1. Supplementary Methods

Synthesis of peptide (DPA and RGD)

All peptides were synthesized on appropriate resins on an CS bio 336X automated peptide synthesizer using the optimized HBTU activation/DIEA *in situ* neutralization protocol developed by an HBTU/HOBT protocol for Fmoc-chemistry SPPS. After cleavage and deprotection in a reagent cocktail containing 88% TFA, 5% phenol, 5% H2O and 2%TIPS, crude products were precipitated with cold ether and purified to homogeneity by preparative C18 reversed-phase HPLC. The molecular masses were ascertained by electrospray ionization mass spectrometry (ESI-MS).

HPLC.

Analytical HPLC was carried out in a Waters 2535 equipped with a Dynamax Absorbance Rainin Detector. Analytical injections were monitored at 214 nm. Separations were performed using a Waters Xbridge C-4 column (4 μm, 300 Å) at a flow rate of 1 mL/min with a gradient from 5 to 65% of B in 15 min (eluent A: 0.1% TFA/H2O, eluent B: 0.1% TFA in CH3CN).

Preparative HPLC was performed in a Waters Delta Prep 4000 equipped with a Gilson UV detector model 116 and a Waters Xbridge C-4 column at a flow rate of 15 mL/min employing the following gradient: 0->60 min, 20->50% B. Preparative injections were monitored at 214 nm.

Mass spectrometry.

Peptides were characterized using electrospray ionization MS on a LC/MS Waters 2695+ SQD2. Peptides masses were calculated from the experimental mass to charge (m/z) ratios from all

of the observed protonation states of a peptide by using the Waters software.

Analysis of intracellular distribution of AuNp-DPA

To characterize the intracellular distribution of AuNp-DPA, antibodies directed against established marker of subcellular organelles were used: mouse monoclonal antibodies against early endosomes antigen 1 (EEA1) and antibodies against the GTPase RAB7 protein to identify late endosomes. Cells were grown on glass coverslips in McCoy's 5A supplemented with 10% FBS at 37°C until 60% of confluence was achieved. They were incubated with 200 μg/mL of AuNp-DPA, and fixed with paraformaldehyde 4%. Cells were then washed three times with Phosphate Buffered Saline (PBS), and permeabilized with a gelatin dilution buffer GDB2X containing 0,01% (v/v) digitonin, for 30 min at RT. Subsequently, cells were incubated with primary antibody diluted in GDB2X for 2h at 37°C, then washed three times with HS and incubated with rhodamine conjugated secondary antibodies (dilution 1:100) for 1 hr at 37°C. Finally, cells were washed with PBS three times. Coverslips were mounted onto slides with glycerol.

For Lysotracker, firstly, make a dilution of the 1-mM probe stock solution to a final working concentration of 50-75 nM in PBS at 37°C. And then, aspirate the culture medium from cells grown on coverslips, and wash the cells in the culture dish three times with PBS. After that, cover the cells with prewarmed (37°C) probe-containing PBS. Incubate for 30 min to 2 h under the desired growth conditions for the cells under study. Finally, replace the labeling solution with fresh medium. Observe the cells using a LSCM.

Cell culture

Human colon cancer cell lines HCT116 $p53^{+/+}$ carrying wild-type p53 and the isogenic HCT116 $p53^{-/-}$ (p53 deletion) were kindly provided by Prof. Bert Vogelstein (Ludwig Center at John Hopkins, Baltimore, USA), and maintained in McCoy's 5A medium with 10% FBS. All cells were maintained at 37°C in an atmosphere of 5% CO_2. For MTT test, cells were seeded at 3,000 cells/well in 96-well plates and allowed to grow for 24 h before treatment. Spectroscopic readings were taken 1 d and 2 d after treatment, and percent cell viability was calculated on the basis of optical density values of sample wells versus reference wells. The normal cells used as control is the Peripheral Blood Mononuclear Cell (PBMC) separated from c57 mice.

Apoptosis and cell cycle analysis

Necrosis/apoptosis was evaluated by flow cytometry using the FITC Annexin V Apoptosis Detection Kit (BD Biosciences). Briefly, cells were treated with 2 μM AuNp-DPA, 4 μg/ml AuNp, 2 μM DPA and 2 μM Nutlin-3 for 72 h. Cells were then harvested, washed twice with cold PBS, and resuspended in 1×binding buffer at a concentration of 1×10^6 cells/ml. One hundred microliters of the solution (1×10^5 cells) was transferred to a 5-ml culture tube, followed by addition of 5 μl of FITC Annexin V and 5 μl of PI. After gentle vortexing and a 15-min incubation in the dark at room temperature, 400 μl of 1× binding buffer was added to the tube, and cells were analyzed by FACS.

Cells were first serum starved for 12 h, and then treated with 1 μM AuNp-DPA, 2 μg/ml AuNp, 1 μM DPA and 1 μM Nutlin-3 for 24 h. Next, cells were harvested, washed twice in PBS, and fixed in 70% ethanol on ice for at least 30 min. After that, cells were stained with propidium iodide (PI) solution (50 μg/ml PI, 50 μg/ml RNase A, 0.1% Triton-X, 0.1mM EDTA). Cell cycle distributions were then analyzed based on DNA contents by a flow cytometer (BD Biosciences, NJ).

Western blot analysis. Cells were treated with AuNp-DPA (1μM), AuNp (1μM), DPA (1μM) and Nutlin3 (1μM) for 24 h, respectively. Cells were then lysed in prechilled RIPA buffer containing protease inhibitors, and equal amounts of protein lysates were separated by 10% SDS-PAGE and transferred onto PVDF membranes (Roche Diagnostics, Mannheim, Germany). The membranes were subsequently incubated with the indicated primary antibodies at 4°C overnight. Antibodies against p53 and p21 were purchased from Abcam. Antibody against β-actin was purchased from Sigma-Aldrich. This was followed by incubation with their respective HRP-conjugated secondary antibodies from Calbiochem, and immunoblotting signals were visualized using the Western Bright ECL detection system (Advansta, CA).

Immunohistochemical (IHC) staining

Sections were cut at 5 μm thickness, deparaffinized and rehydrated. Endogenous peroxidase activity was blocked with hydrogen peroxide/methanol, and antigen retrieval was performed in a pH 9.0 TE (Tris-EDTA) buffer by autoclave for 10 min. The resultant tissue sections were then incubated with primary antibodies against p53, p21 and Ki67 at 4°C overnight. After incubation with labeled streptavidin-biotin (LSAB) complex for 15 min, the slides were stained and visualized by using the iView DAB detection system (ZSGB-BIO, P.R. China). Each stained section was evaluated by a minimum of 10 randomly selected ×20 high-power fields for further statistical analysis.

To evaluate immunostaining intensity (I), we used a numeric score ranging from 0 to 3, reflecting the intensity as follows: 0, no staining; 1, weak staining; 2, moderate staining; and 3,

intense staining. To evaluate immunostaining area (A), we used a numeric score ranging from 1 to 4, reflecting the intensity as follows: 1, positive area <10%; 2, 10%<positive area <50%; 3, 50%<positive area <90%; and 4, positive area>90%. Using an Excel spreadsheet, the mean score was obtained by multiplying the intensity score (I) by the percentage of positive area and the results were added together (total score: I×A).

2. Supplementary Figures

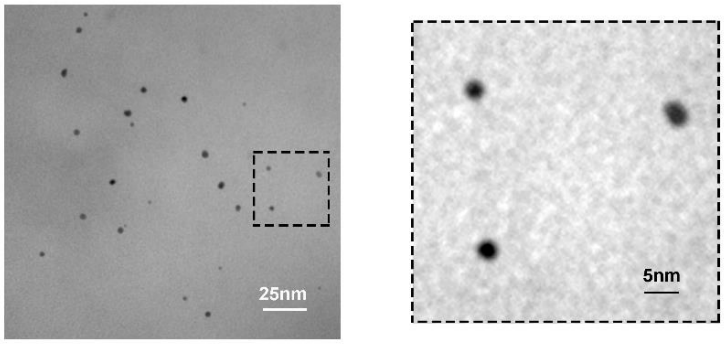

Figure S1. TEM image of Au core. The right picture is the partial enlarged detail of the left one.

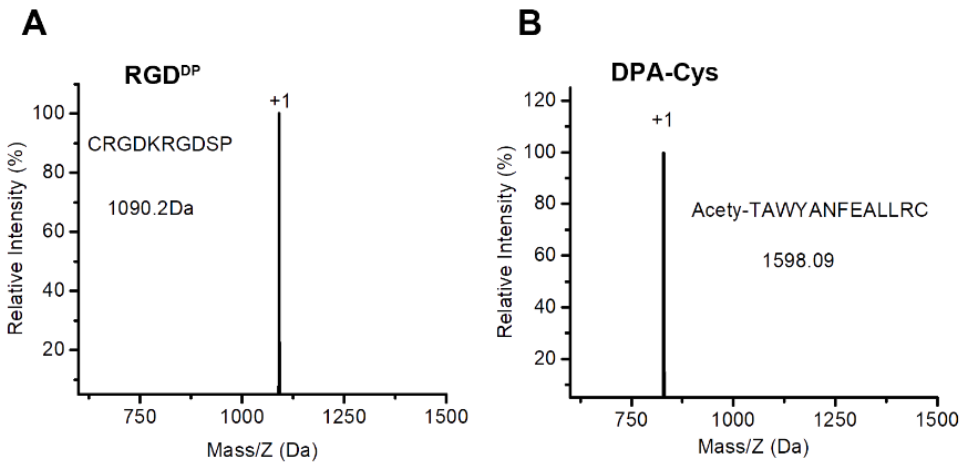

Figure S2. ESI-MS result of the peptide RGD^{DP} (**A**) and DPA-Cys (**B**).

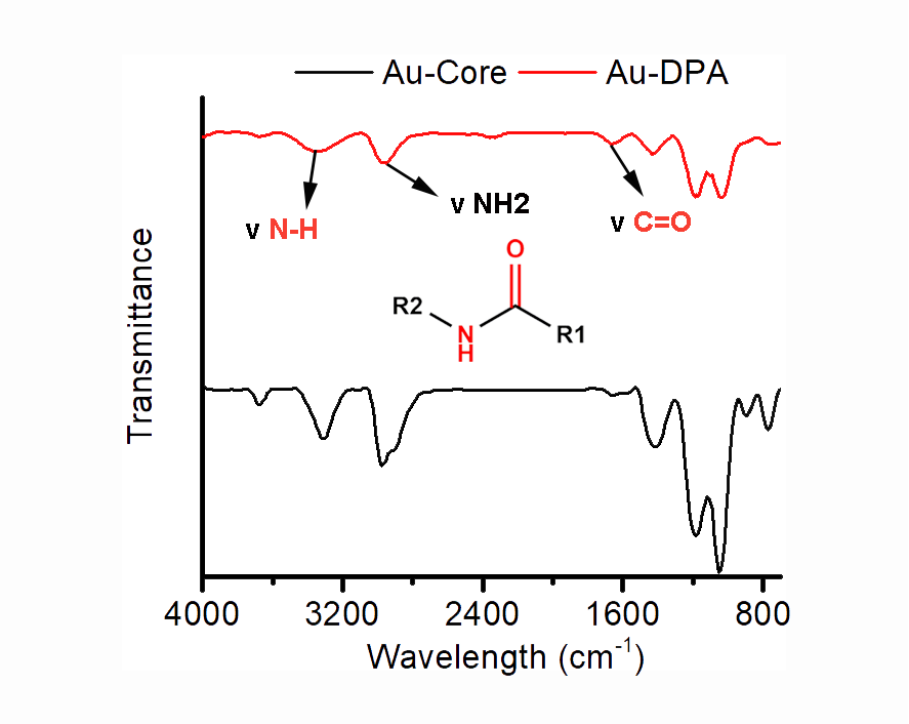

Figure S3. The FTIR of Au-DPA, and Au-core, demonstrating their surface chemical structures before and after DPA conjugation.

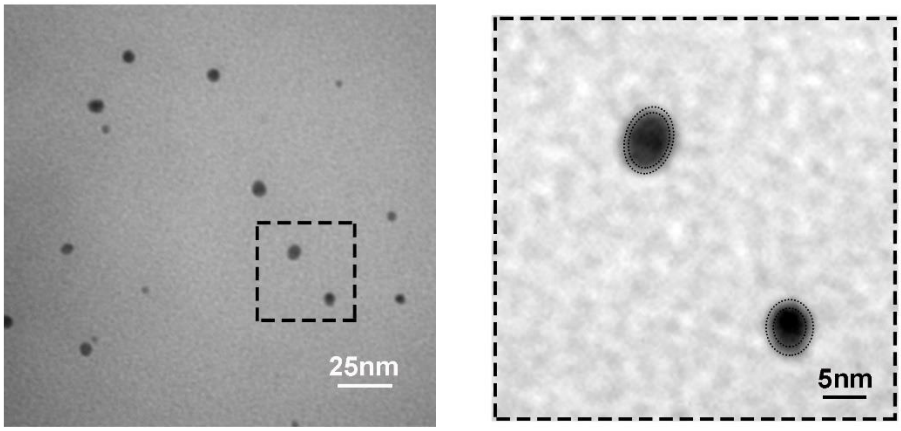

Figure S4. TEM image of Au-DPA-PLL. The right picture is the partial enlarged detail of the left one. The dotted ellipse marked the outline of the PLL outer layer.

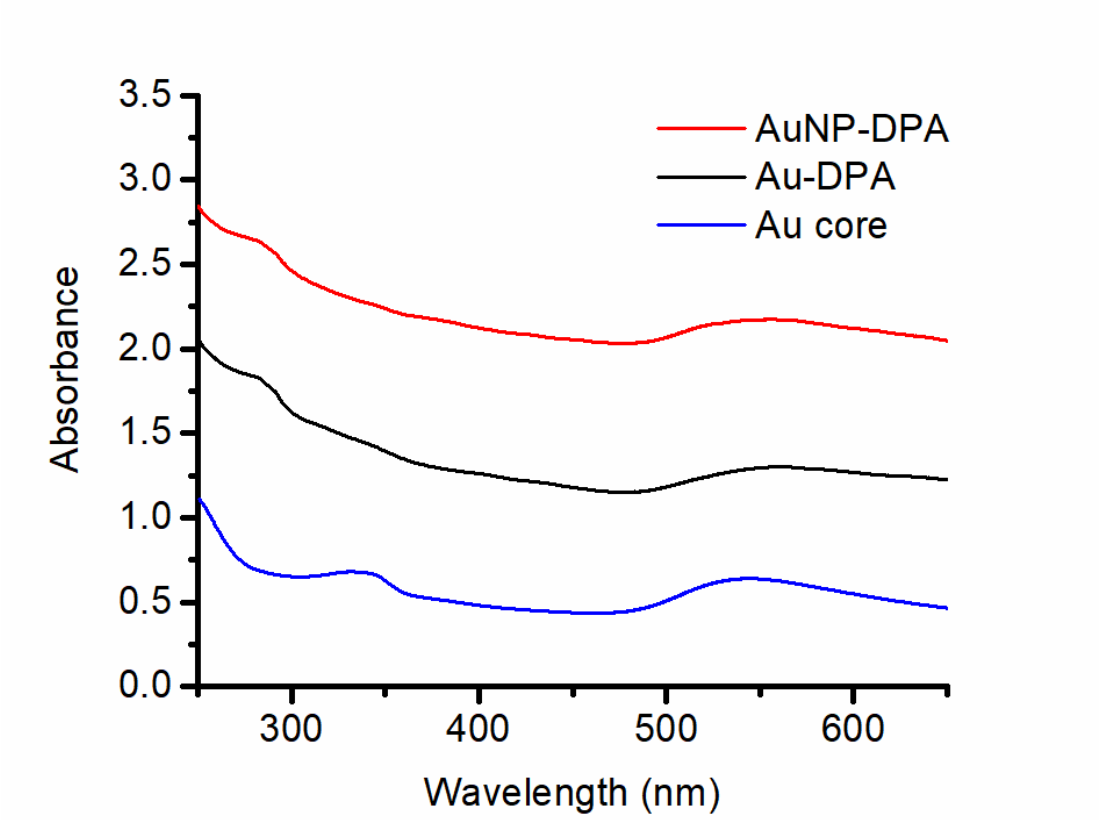

Figure S5. Ultraviolet–visible spectra of Au-DPA, Au-core and AuNp-DPA measured in HEPES buffer (pH 7.4) at the concentration of 0.2 mg/ml. The peaks of three curves at ~540nm were the plasma resonance peak of gold nanoparticles. The peaks in the Au-DPA and AuNp-DPA were the characteristic absorption peak of peptide bond, indicating the successful conjugation of therapeutic D-peptide DPA.

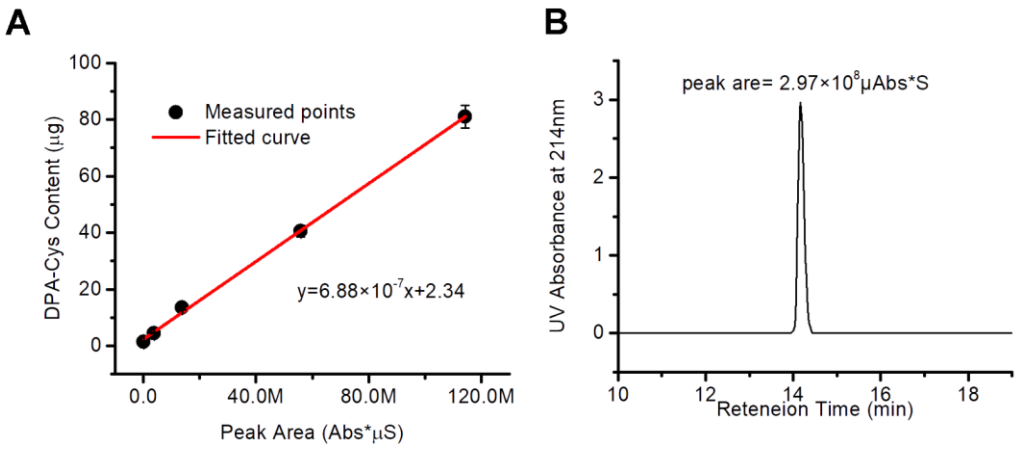

Figure S6. (A) the standard curve of the content of DPA-Cys measured by HPLC. (B) the quantitative curve of AuNp-DPA. AuNp-DPA was firstly dissolved in PBS buffer containing 6 M guanidine hydrochloride (to eliminate the interaction between DPA and PLL) and 1 M dithiothreitol (to break the conjunction between peptide and Au nanoparticle). After 10000g centrifugation, supernate was measured by HPLC.

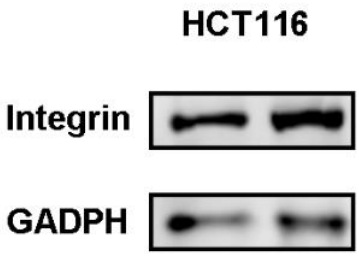

Figure S7. The expression of integrin in HCT116 cells by western blot analysis. GADPH was used as control.

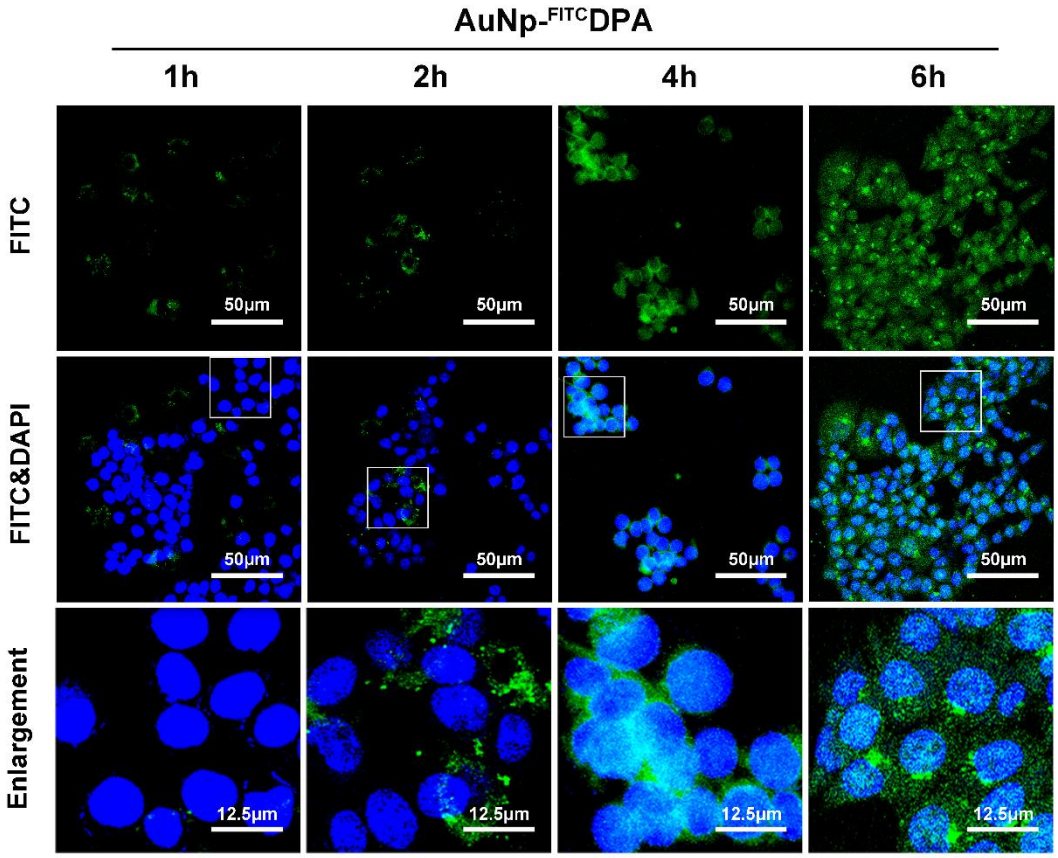

Figure S8. Confocal laser scanning microscope (CLSM) images of HCT116 cells incubating with FITC-labeled AuNp-DPA at the concentration of 200 μg/mL for 1h, 2h, 4h and 6 h. All images were taken under the same exciting light and detector gain. The pictures at last column are the partial enlarged detail of the corresponding dotted boxes at second column.

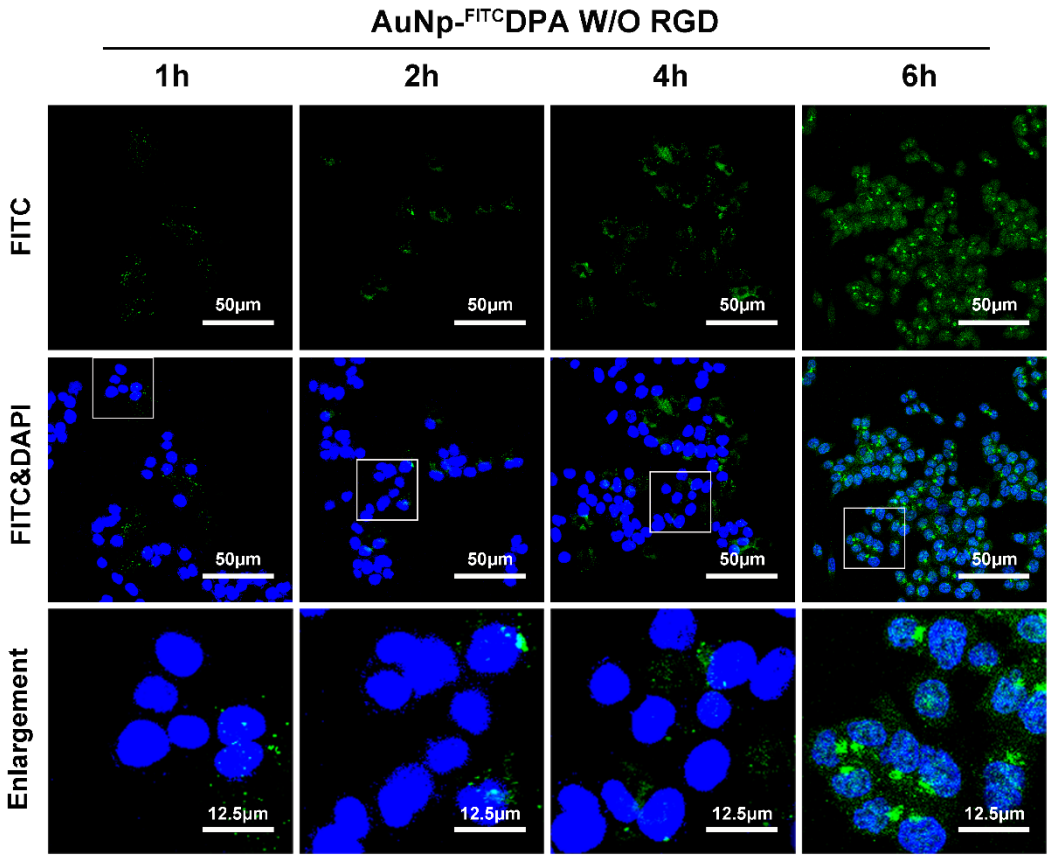

Figure S9. Confocal laser scanning microscope (CLSM) images of HCT116 cells incubating with FITC-labeled AuNp-DPA W/O RGD at the concentration of 200 μg/mL for 1h, 2h, 4h and 6 h. All images were taken under the same exciting light and detector gain. The pictures at last column are the partial enlarged detail of the corresponding dotted boxes at second column.

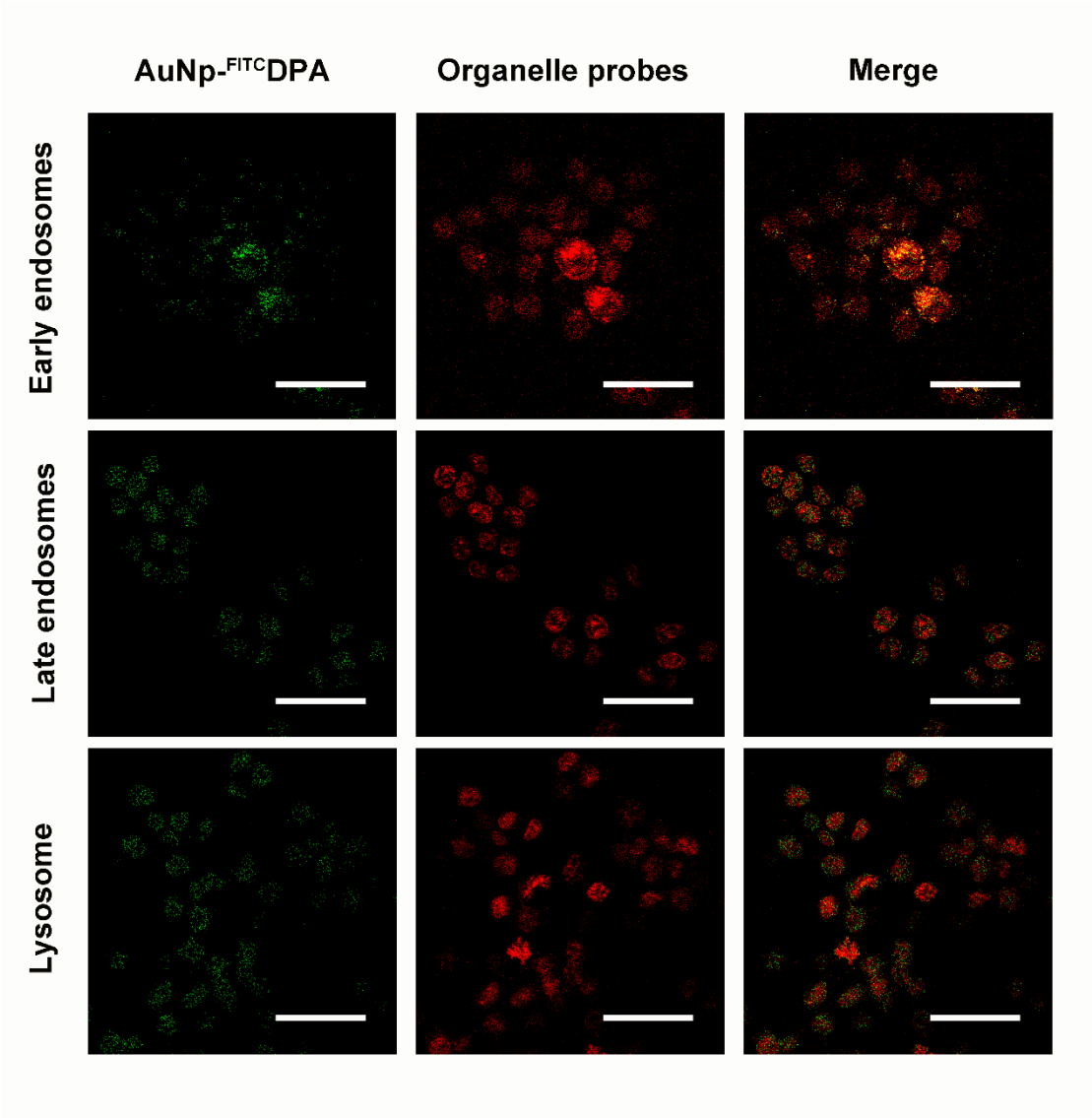

Figure S10. Evaluation of co-localization of FITC-labeled AuNp-DPA with lysosome, early endosomes and late endosomes. The three subcellular organelles were marked in red, and the scale bar stands for 50 μm.

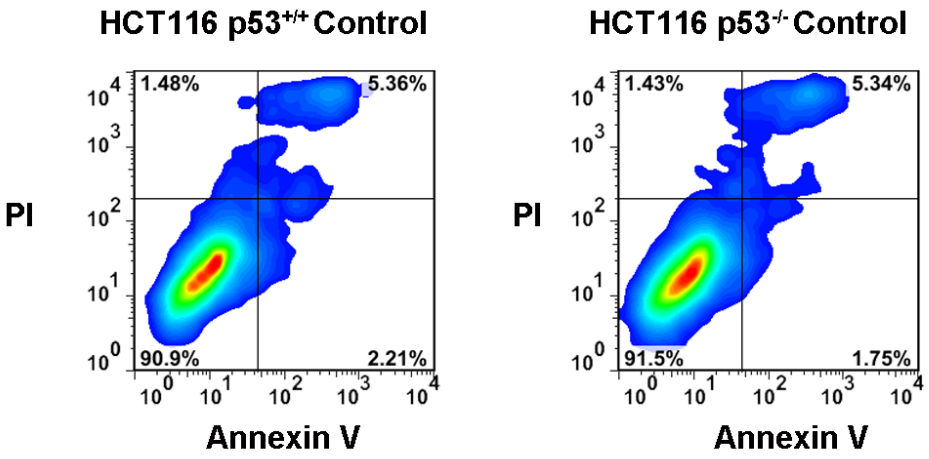

Figure S11. HCT116 $p53^{+/+}$ and HCT116 $p53^{-/-}$ cells were treated with PBS for 72 h, and cell apoptosis was evaluated by Annexin V-PI staining (abscissa: Annexin V; ordinate: PI) and flow cytometry.

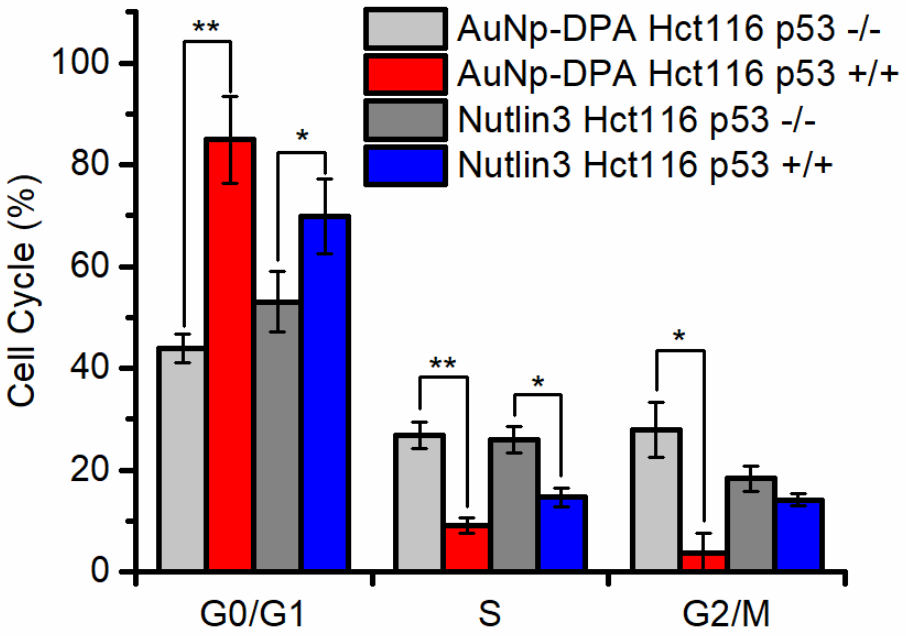

Figure S12. HCT116 $p53^{+/+}$ and HCT116 $p53^{-/-}$ cells were treated with 1 μM nutlin3 or AuNp-DPA for 24 h, and cell cycle distributions were analyzed by flow cytometry. The data were presented as mean ± s.d. of values from three independent experiments. Statistically significant differences were indicated: *, p <0.05; **, p <0.01.

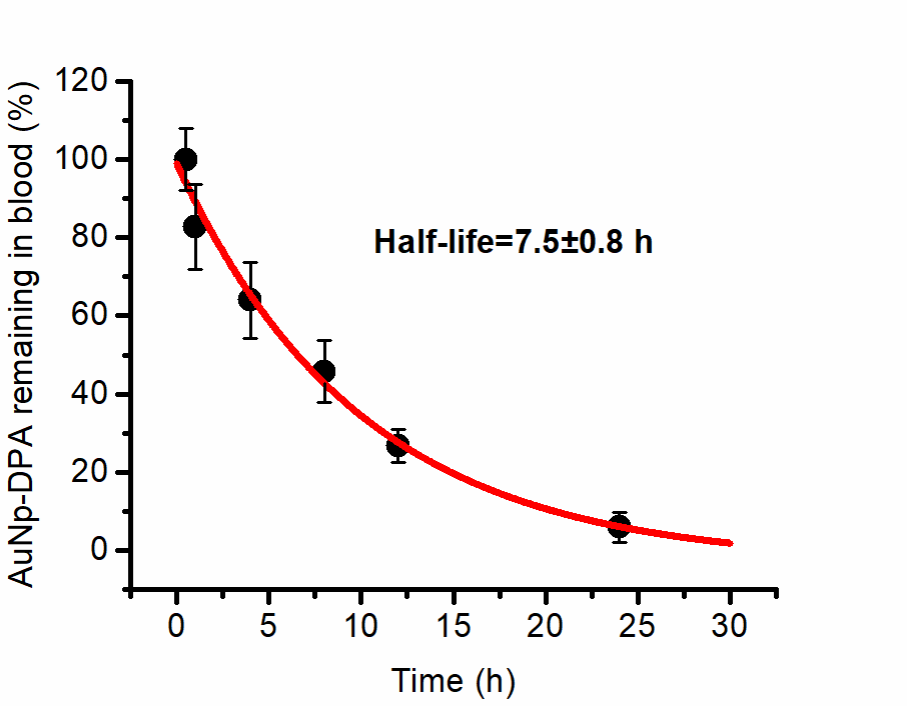

Figure S13. Percentage of FITC-labeled AuNp-DPA remaining in circulation as a function of time with an injection dose of 2 mg/kg (iv). The solid line represents an exponential fit.

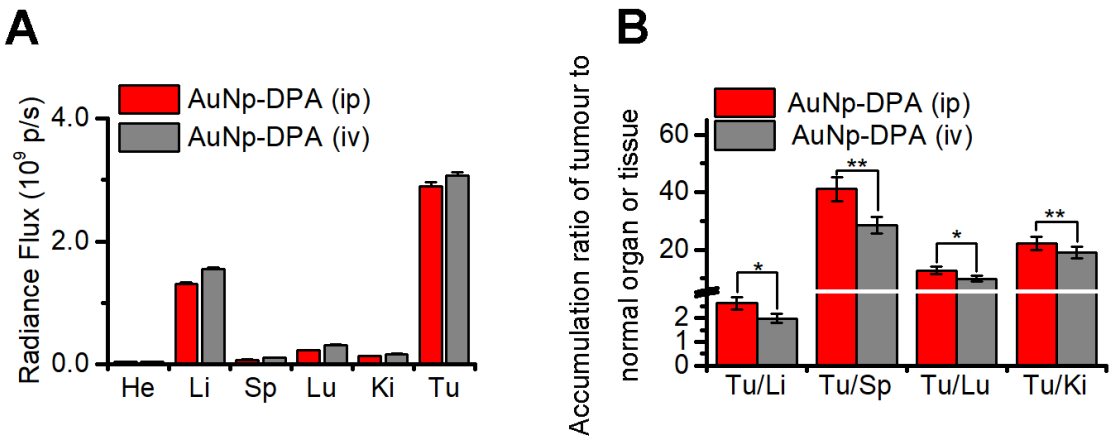

Figure S14. (**A**) *In vivo* quantitative distribution in the ischemic region of the iv and ip results. Fluorescence intensity in the ischemic region was determined using Living Image 3.0. Fluorescence unit determined by IVIS was expressed as radiance efficiency [(photons s^{-1} cm^{-2} sr^{-1})/(μW cm^{-2})]. (**B**) Tumor-to-background (normal organ or tissue) ratios of AuNp-DPA at 6 h post-injection by iv or ip. (n =3, mean ± s.d.).

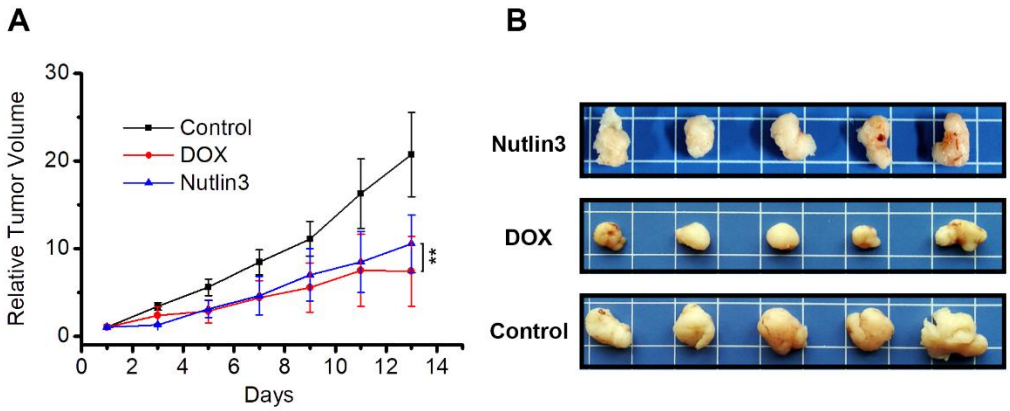

Figure S15. (**A**) Tumor growth curves in nude mice subcutaneously inoculated with 1×10^6 HCT116 $p53^{+/+}$ cells into the right flank. The indicated treatments were administered every other day. Data are presented as mean ± s.e. (n =5). (**B**) Photographs of xenograft tumors collected from mice with the indicated treatments.

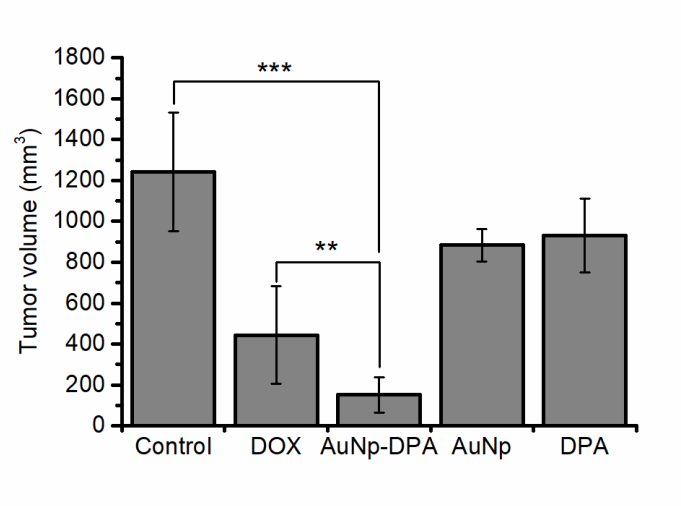

Figure S16. The average volumes of the tumors excised at the end of the experiment. Statistically significant differences were indicated: **, $p < 0.01$; ***, $p < 0.001$

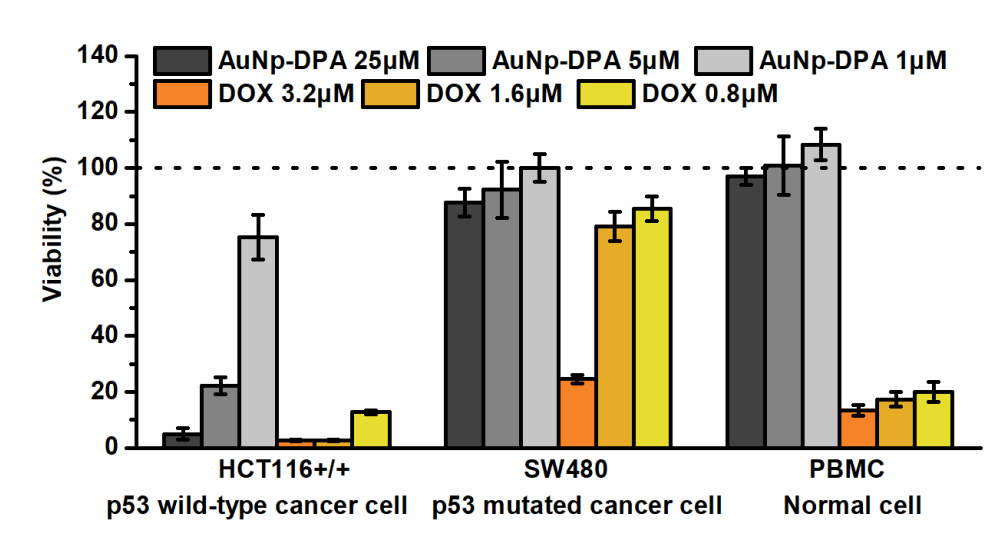

Figure S17. Selective cytotoxicity of AuNp-DPA against HCT116 $p53^{+/+}$, SW480, and PBMC, using DOX as positive control. All cells were incubated with the indicated samples for 48 hours, and then the viability of the treated cells was measured by MTT test.

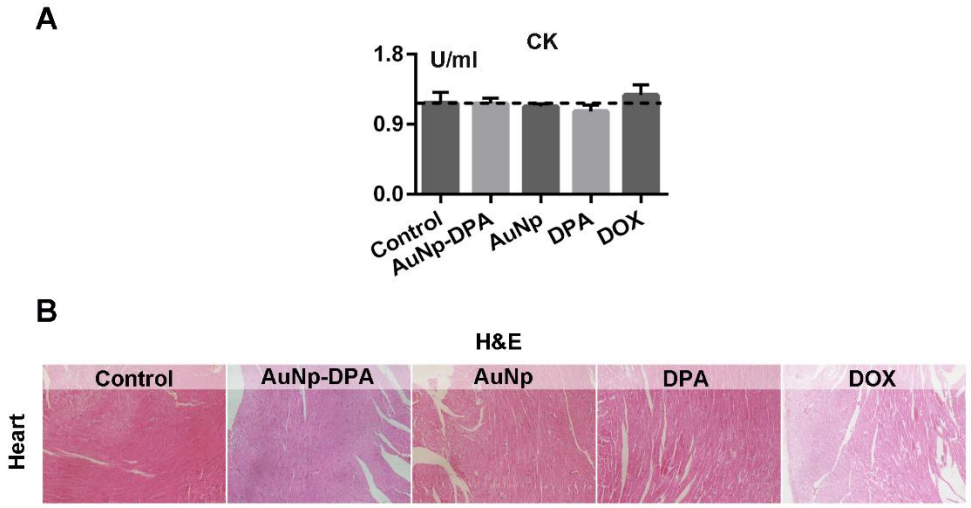

Figure S18. Toxicity evaluation of the Heart in mice with different treatments. (**A**) The activity of serum creatine kinase (CK) in mice with the indicated treatments. Clinically, CK is assayed in blood tests as a marker of damage of CK-rich tissues such as in myocardial infarction (heart attack). *p* values were calculated by *t*-test (unmarked $p > 0.05$). (**B**) The representative H&E staining of heart sections in mice with the indicated treatments (×200).

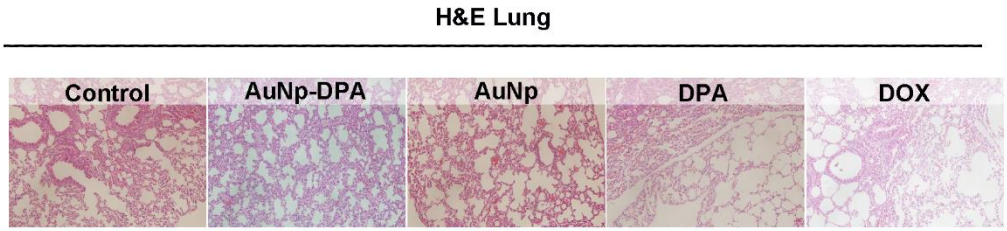

Figure S19. Toxicity evaluation of the lung in mice with different treatments. The representative H&E staining of lung tissues in mice with the indicated treatments (×200).

3. Supplementary Tables

Table S1: particle sizes of the product and intermediates

Sample	Particle size (nm)[*1]	PDI[*2]
Au core	4.68	0.02
Au-DPA	5.90	0.09
Au-DPA-PLL	7.45	0.15
AuNp-DPA	7.66	0.20

[*1] Partice size is the hydrate particle size measured by dynamic light scattering

[*2] PDI: Polydispersity Index

Table S2: The relationship between particle size and DPA loading

Estimated size	Measured Particle size (nm)[*1]	PDI[*2]	DPA loading (mmol/g) [*3]
5	4.68	0.02	0.50
10	9.50	0.05	0.20
20	21.2	0.06	0.05
40	42.5	0.10	<0.01

[*1] Partice size is the hydrate particle size measured by dynamic light scattering

[*2] PDI: Polydispersity Index. It's the PDI of the gold nanoparticle before DPA conjugation.

[*3] mmol/g = peptide molarity/Au mass