Experimental section

Agarose gel electrophoresis of tGd–GNMs$_{siRNA}$ complex.

To identify the siRNA binding ability of tGd–GNMs, different concentrations of tGd–GNMs and 1 μg of siRNA were mixed in nuclease–free water at the designated N/P ratios (N/P = 0.4 : 1, 2 : 1, 4 : 1, 8 : 1, 16 : 1, 32 : 1) for 20 min at room temperature. To assess the release capacity of siRNA from tGd–GNMs$_{siRNA}$ complex, excess amount heparin (about 5 times weight of siRNA) was co–incubated with tGd–GNMs$_{siRNA}$ complex (1 μg of siRNA equivalent) for another 20 min at room temperature. To investigate the protection of siRNA by tGd–GNMs$_{siRNA}$ complex, 2 μL of RNase was mix with tGd–GNMs$_{siRNA}$ complex (1 μg of siRNA equivalent) at 37 °C for 0 min, 30 min, 60 min and 120 min. Finally, 1% agarose gel electrophoresis assay was used to determine the loading efficiency, release of siRNA, and protection of siRNA by tGd–GNMs. Free siRNA was used as control group.

Detection of ROS produced by tGd–GNMs$_{siRNA}$ complex under X–ray Irradiation.

1,3–diphenylisobenzofuran (DPBF) were used to estimate generation of reactive oxygen species (ROS). 1 mL aqueous solutions containing DPBF (0.5 mM,100 μL) and different amount of tGd–GNMs$_{siRNA}$ complex (25 μg/mL, 50 μg/mL, 100 μg/mL, 200 μg/mL, 400 μg/mL) were radiated by different X–ray doses (0, 2, 4, 6 and 8 Gy). Then the specific absorption at 417 nm was recorded, and the corresponding production yield was calculated: ROS production yield (%) = (1–tGd–GNMs$_{siRNA}$ treated absorbance/control absorbance) × 100%.

In vitro cytotoxicity.

HUVEC cells were maintained in ham's F–12K medium supplemented with 10% FBS, 1% ECGS and 1% penicillin–streptomycin. HepG2 cells were maintained in

DMEM medium supplemented with 10% FBS and 1% penicillin–streptomycin. H22 cells were maintained in 1640 medium supplemented with 10% FBS and 1% penicillin–streptomycin. For the in vitro cytotoxicity test, HUVECs cells, HepG2 cells and H22 cells were cultured at 8000 cells per well in 96 well plates at 37 °C for 24 h. As the cells were 60% confluent, the cells were treated with different amount of tGd–GNMs, tGd–$GNMs_{siRNA}$ complex (siRNA concentrations range from 0 to 3,000 μM) or Lipo2000–siRNA complex (siRNA concentrations range from 0 to 3,000 μM) respectively. Then cell viability was evaluated by Cell Counting Kit (CCK–8) assay.

Cellular uptake and lysosomal escape of siRNA delivered by tGd–$GNMs_{siRNA–Cy5}$ complex

To validate cNGR targeting effect, siRNA was labelled with a near infrared–red fluorescent dye Cy5 on the 5'–end of the sense strand in this section. HUVEC cells were plated at a density of 1×10^5 cells per well in confocal dish for 24 h, then incubated with free siRNA–Cy5, $GNMs_{siRNA–Cy5}$ complex, tGd–$GNMs_{siRNA–Cy5}$ complex (100 nM of siRNA–Cy5 equivalent) in culture medium at 37 °C for 4 h. After that, the cells were rinsed thrice with PBS, and counterstained with DAPI. Photographs of cells were obtained by the confocal microscopy.

To confirm whether the siRNA can successfully escape from lysosomes, HUVEC cells were pre–seeded on confocal dish for 24 h and stained by LysoTracker green (Invitrogen, 75 nM) for 1 h. After rinsing with PBS, the cells were incubated with tGd–$GNMs_{siRNA}$ complex (100 nM of siRNA–Cy5 equivalent). At 1 h, 2 h and 4 h post–treatment, cells were rinsed thrice with PBS, photographs of cells were obtained by the confocal microscopy. Excitation wavelengths were set as 488 nm for LysoTracker green and 633 nm for siRNA–Cy5.

Real time–PCR and western blot.

For real time–PCR analysis, total RNA was extracted from transfected cells using TRIzol reagent (TianGen). Then the RNA was converted to cDNA to determine VEGF mRNA expression. VEGF gene–specific primers were showed in Supplementary Table S2. For western blot analysis, total proteins for each treatment were extracted from transfected cells. Proteins were separated on 10% SDS gel and transferred to a polyvinylidene difluoride membrane, which probed with rabbit monoclonal antibody against VEGF (1:1,000) at 4 °C overnight, followed by incubation with horseradish peroxidase–linked goat anti–rabbit secondary antibody (Invitrogen, 1:3,000) for 1 h at 37 °C. Beta–tubulin was used as an internal control (Invitrogen, 1:1,000). The western blot signals were detected using a ChemiDoc XRS System (Bio–Rad). The band intensity of proteins was quantified using ImageJ software.

Blood circulation time and biodistribution of tGd–GNMs$_{siRNA–Cy5}$ complex.

Healthy male ICR mice (4–6 weeks old, 20 g) were obtained from Beijing Vital River Laboratory Animal Technology Co., Ltd. and were subcutaneously inoculated with the second generation H22–related ascites. After 10 days, as the tumour volume reached ~100 mm^3, tumour xenograft models were successfully set up. To evaluate the blood circulation half–life of tGd–GNMs$_{siRNA–Cy5}$ complex, H22 tumor–bearing ICR mice were intravenously injected with free siRNA–Cy5 and tGd–GNMs$_{siRNA–Cy5}$ complex at a dose of 10 ug siRNA–Cy5 per mouse equivalent. At different time points post–injection (5 min, 30 min, 1 h, 6 h, and 24 h), 50 μL of blood samples were collected from the eye socket and photographed by fluorescence imaging system. Then the blood samples were dissoluted by digestive chloroazotic acid (HCL/HNO_3 = 1:3) to quantify the amount of Au^{3+} by ICP–MS. A two–compartment pharmacokinetic model was utilized to calculate the pharmacokinetics parameters of tGd–GNMs$_{siRNA}$ complex.

To investigate the biodistribution of tGd–GNMs$_{siRNA}$ complex, H22 tumor–

bearing ICR mice were injected with tGd–$GNMs_{siRNA}$ complex (10 ug siRNA per mouse equivalent) by tail vein. At 6, 16 and 24 h post injection, the mice were killed to collect tumours and major organs (heart, liver, spleen, lung, kidney). The tissues were executed ex vivo fluorescent imaging and dissoluted by digestive chloroazotic acid (HCL/HNO_3 = 1:3) to quantify the amount of Au^{3+} by ICP–MS.

Multimodal fluorescent/ CT/MR images for diagnosis.

For in vivo fluorescent/CT/MR imaging, H22 tumor–bearing ICR mice were administered tGd–$GNMs_{siRNA}$ complex (10 ug siRNA per mouse equivalent) via the tail vein prior to imaging. At 0, 6, 16 and 24 h post–injection, the image of the mice was performed. Fluorescent imaging was performed by fluorescence imaging system (excitation: 430 nm and emission: 600 nm). CT imaging was acquired using a GE Light Speed VCT clinical imaging system with the parameters were as follows: slice thickness, 2.5 mm; pitch, 1:1; the tube voltage of 120 kV, the tube current of 200 mA; field of view, 512 × 512, gantry rotation time, 1 s. MR imaging was performed on 3.0 T clinical MRI scanner equipped with a small–animal coil. The parameters were as follows: Freq. FOV = 4 mm^2, phase FOV =1.00 mm^2, TR = 609 ms, TE =10.4 ms, slices = 15, slice thickness = 1.0 mm, spacing = 0.2 mm.

In vivo GT/RT synergistic treatment

As the tumours reached approximately ~100 mm^3, mice were randomly divided into five groups: (i) control; (ii) free siRNA; (iii) X–ray radiation; (iv) tGd–$GNMs_{nsRNA}$ complex+RT; (v) tGd–$GNMs_{siRNA}$ complex+RT. For group (ii) the mice were intratumorally injected VEGF–siRNA every 2 days for 7 times. For group (iii), mice were received X–ray radiation (6 Gy) for one time. For group (iv) and (v), the mice were injected with 200 μL tGd–$GNMs_{nsRNA}$ complex and tGd–$GNMs_{siRNA}$ complex (10 ug siRNA per mouse equivalent) every 2 days for 7 times, the RT (6 Gy) was executed

after 16 h of first injection. Subsequently, tumour growth was recorded by measuring the tumor size ($V = ab^2/2$, where a is length and b is width) every other day until the end of the experiment. Meanwhile, body weight was also recorded.

In vivo biocompatibility evaluation of tGd–GNMs$_{siRNA}$ complex.

To evaluate the biocompatibility of tGd–GNMs$_{siRNA}$ complex in vivo, healthy male ICR mice (4–6 weeks old, 20 g) were intravenously administrated with tGd–GNMs$_{siRNA}$ complex (10 ug siRNA per mouse equivalent). The mice injected with PBS were set as the control group. the blood samples were collected at 48 h post–injection and 30 days post–injection for complete routine blood analysis and blood biochemistry. For hematoxylin and eosin (H&E) staining, the mice were sacrificed to harvest the major organs (heart, liver, spleen, lung, and kidney) at 48 h post–injection and 30 days post–injection.

Histological examinations

After the therapy experiments, the tumor tissues in different groups were harvested and immediately fixed with 4% paraformaldehyde. The tumor tissues sectioned into slices for H&E, caspase 3, γ–H2AX, VEGF and HIF–1α staining for histological analysis according to standard protocols. For ROS level and angiogenesis estimate, the tumour tissues in different groups were harvested and immediately preserved in liquid nitrogen. After embedding in optimum cutting temperature (O.C.T.) specimen matrix (Sakura, Leiden, The Netherlands) for cryostat sectioning at −20 °C. The tumour tissue was sectioned into slices for DHE probe, CD 31 and NG2 staining and then imaged under a fluorescence microscope.

Data analysis

Data were presented as the mean ± standard deviation (SD). Two–tailed t–test (* $P < 0.05$, ** $P < 0.01$; *** $P < 0.001$, ****$P < 0.0001$) was applied to analyze significant

differences between groups. * $P < 0.05$ was considered significant.

Supplementary Table 1. The SER of $tGNMs_{nsRNA}$ and $tGNMs_{siRNA}$ in H22 cells

Group	Sensitizer enhancement ratio (SER)
Control	1
$tGNMs_{nsRNA}$	1.17
$tGNMs_{siRNA}$	1.28

Supplementary Table S2. The used primer sequences of real–time PCR

Name	Sequences
vegf	For: 5'AAGCTACTGCCGTCCGATT 3' Rev: 5'GCTTCATCGTTACAGCAG3'
GAPDH	For: 5'GCAAATTCCATGGCACCGTC 3' Rev:5'AGCATCGCCCCACTTGATTT 3'

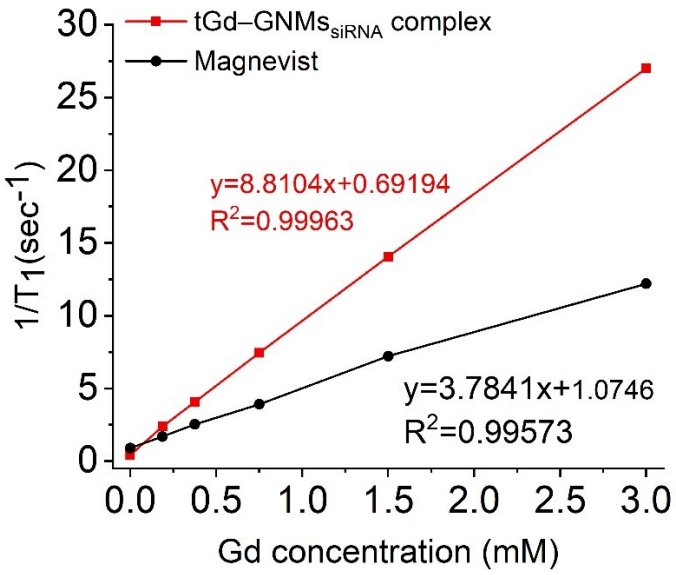

Figure S1. Plots of 1/T1 versus Gd^{3+} concentration.

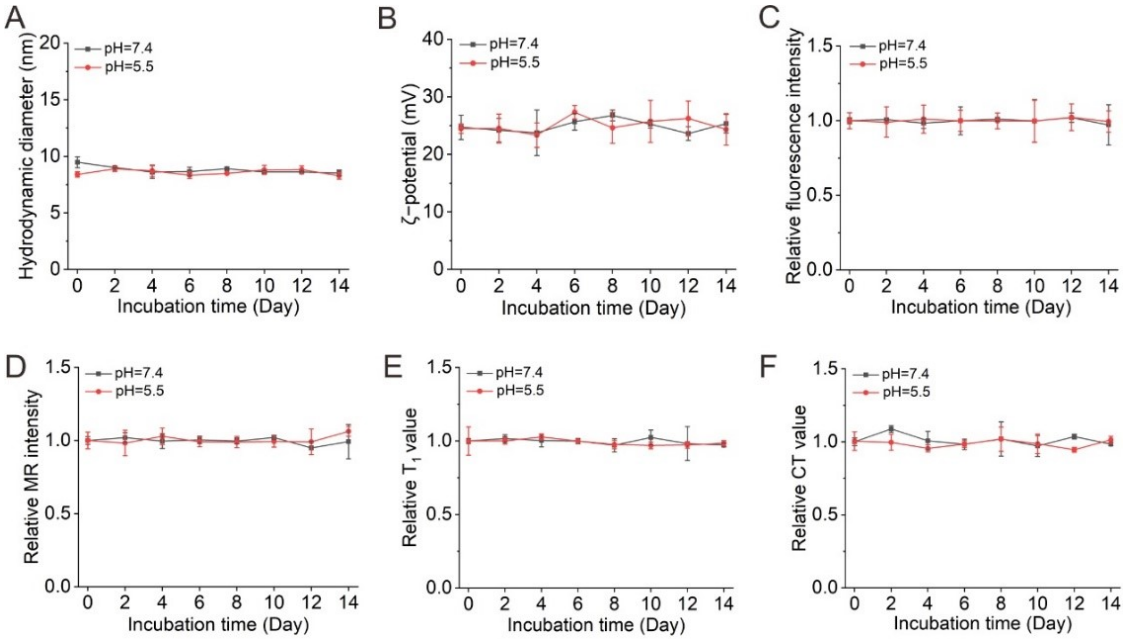

Figure S2. Colloidal and chemical stability study. (A) Particle size, (B) ζ– potential, (C) relative fluorescence intensity, (D) relative MR intensity, (E) relative T_1 value and (F) relative CT value of the tGd–GNMs after different storage time still remained

stable.

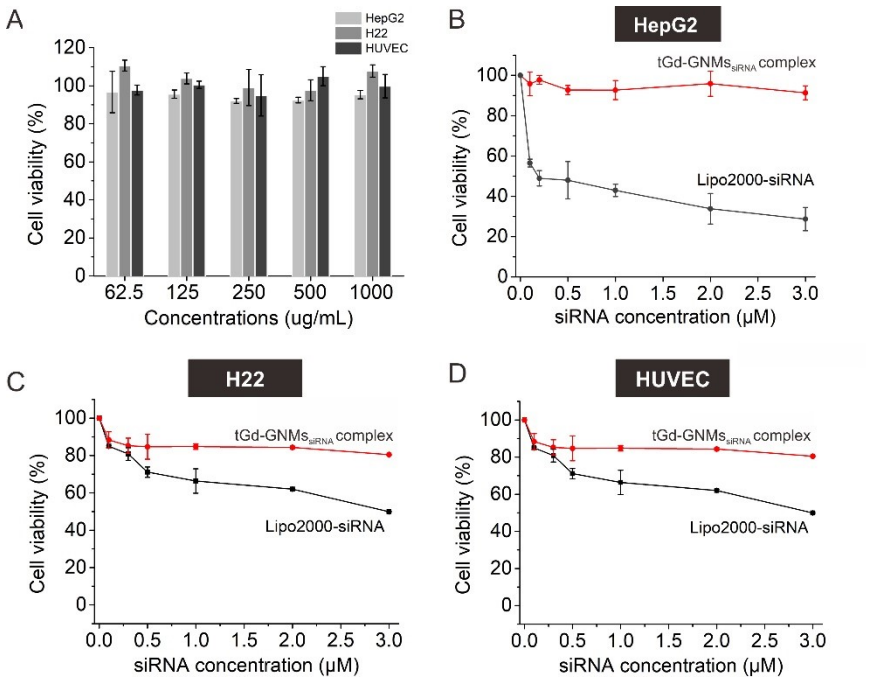

Figure S3. Cytotoxicity of tGd–GNMs$_{siRNA}$ complex. (A) Cytotoxicity of tGd–GNMs for HepG2 cells, H22 cells and HUVEC cells. Viability of (B) HepG2 cells, (C) H22 cells and (D) HUVEC cells after 48 h incubation with tGd–GNMs$_{siRNA}$ complex versus the identical siRNA concentration coupled with Lipo2000–siRNA complex. The quantitative results were represented as the mean ± SD for at least three replicates.

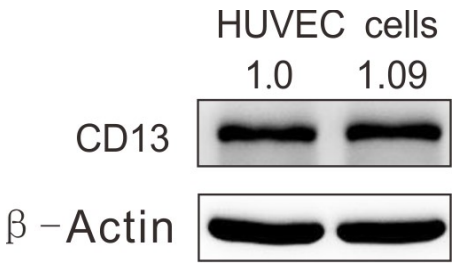

Figure S4 Western blot analysis showed ANP/CD13 over–expression in HUVEC cells.

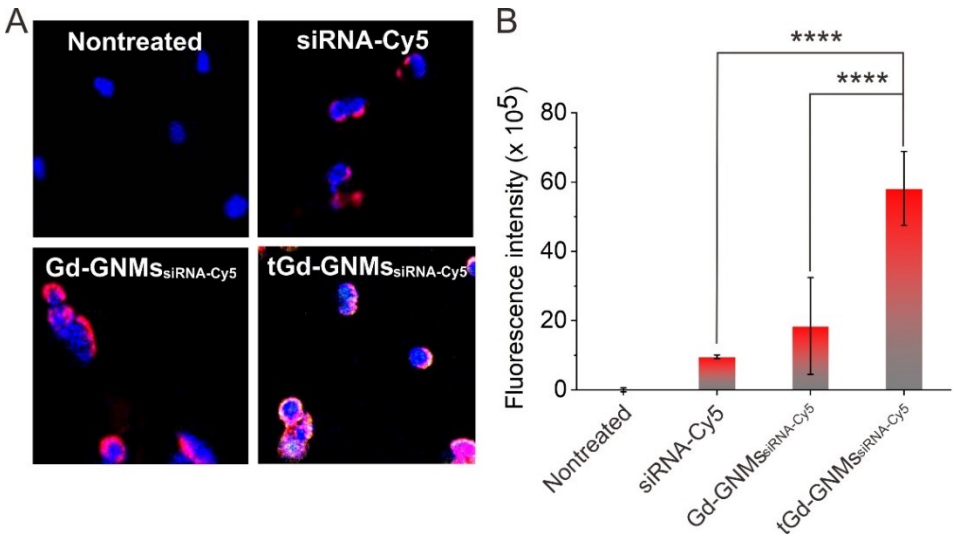

Figure S5. Cellular uptake of free siRNA–Cy5, $GNMs_{siRNA–cy5}$ complex and $tGd–GNMs_{siRNA–cy5}$ complex, 100 nM siRNA equivalent in HUVEC cells. Scale bars, 10 μm. The quantitative results were represented as the mean ± SD for at least three replicates. P values were based on the Student's test: *P < 0.05, **P < 0.01, ***P< 0.001, ****P< 0.0001.

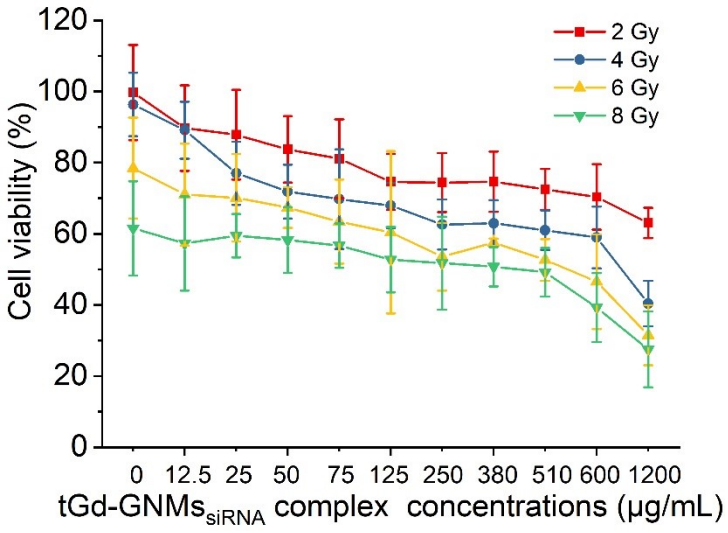

Figure S6. Cell viability of H22 cells incubated with various concentrations of $tGd–GNMs_{siRNA}$ complex for 24 h after irradiation with X–ray (0 Gy, 2 Gy, 4 Gy, 6 Gy and 8 Gy). The quantitative results were represented as the mean ± SD for at least three replicates.

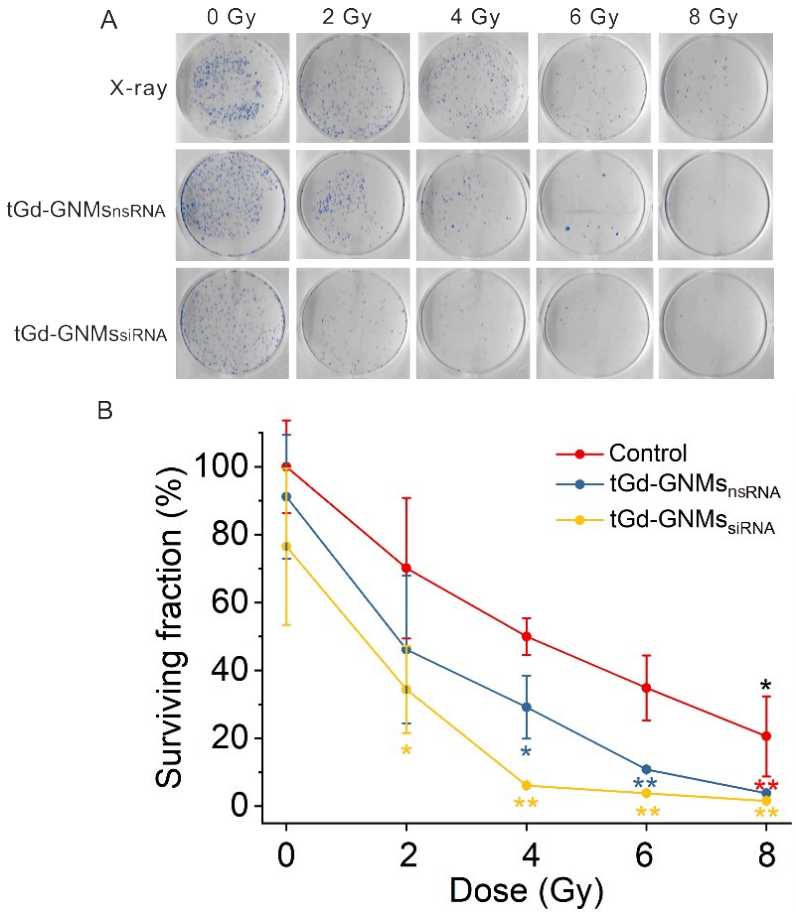

Figure S7. Colony formation assay of H22 cells with tGd–$GNMs_{nsRNA}$ complex and tGd–$GNMs_{siRNA}$ complex treatment under 0, 2, 4, 6, 8 Gy radiation. The quantitative results were represented as the mean ± SD for at least three replicates. P values were based on the Student's test: *P < 0.05, **P < 0.01.

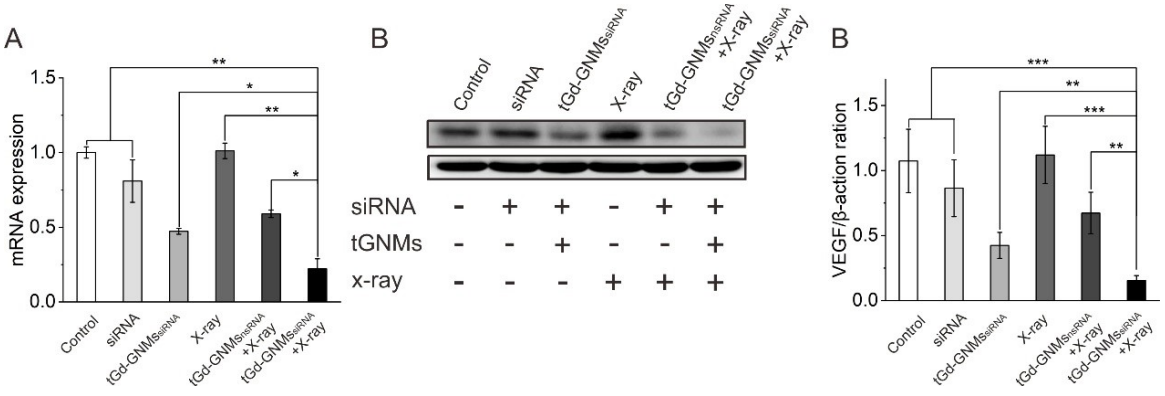

Figure S8. RT-PCR and western blot analysis of VEGF down-regulation in H22 cells after different treatments. (A) RT–PCR and (B, C) Western blot assay. The

quantitative results were represented as the mean ± SD for at least three replicates. P values were based on the Student's test: *P < 0.05, **P < 0.01, ***P< 0.001.

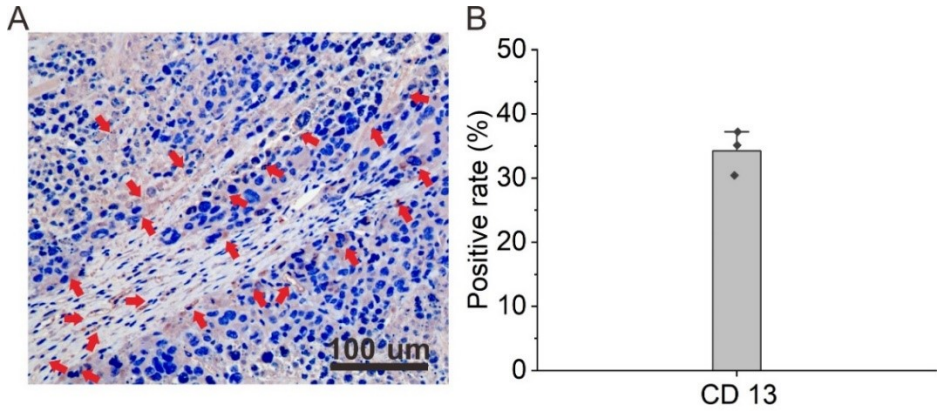

Figure S9. (A) IHC staining analysis (400×) and (B) the quantitative positive rate of CD13 of the tumour tissue.

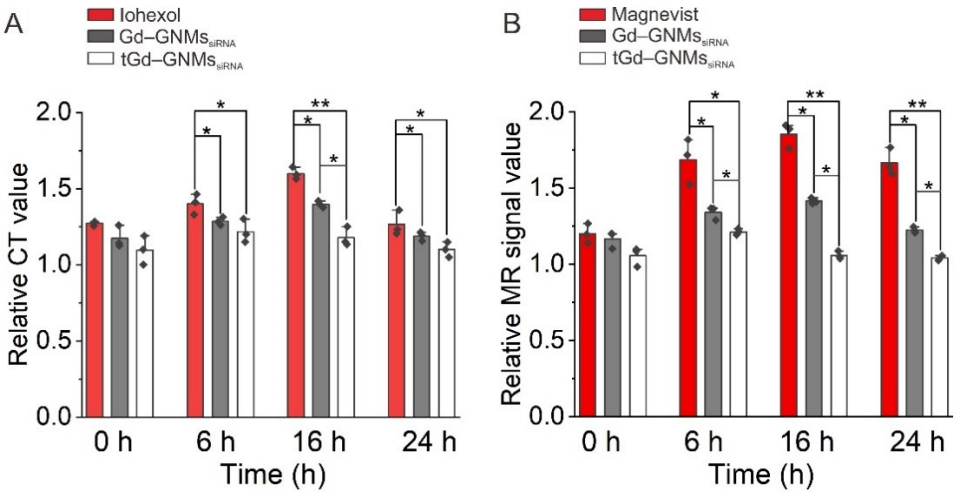

Figure S10. Statistical analysis of (A) relative CT value and (B) relative MR signal intensity after vein intravenous injection of Iohexol, Gd–GNMs$_{siRNA}$ complex and tGd–GNMs$_{siRNA}$ complex. The quantitative results were represented as the mean ± SD for at least three replicates. P values were based on the Student's test: *P < 0.05, **P < 0.01.

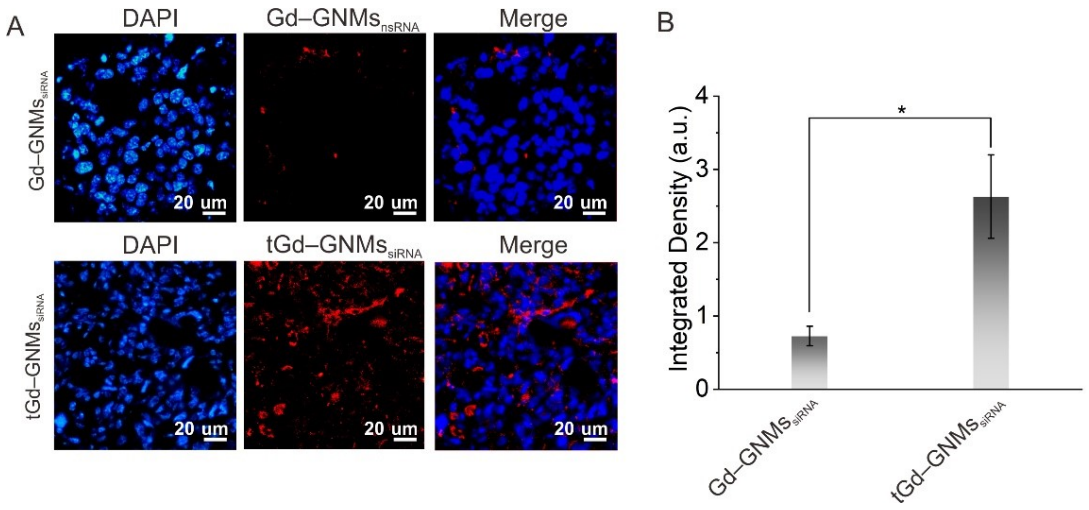

Figure S11. Immunofluorescence of tumour slices from mice at 16 h post–injection of $Gd–GNMs_{siRNA}$ complex and $tGd–GNMs_{siRNA}$ complex. (A) fluorescence imaging, scale bars: 20 μm, (B) quantitative analysis. P values were based on the Student's test: $*P < 0.05$, $**P < 0.01$.

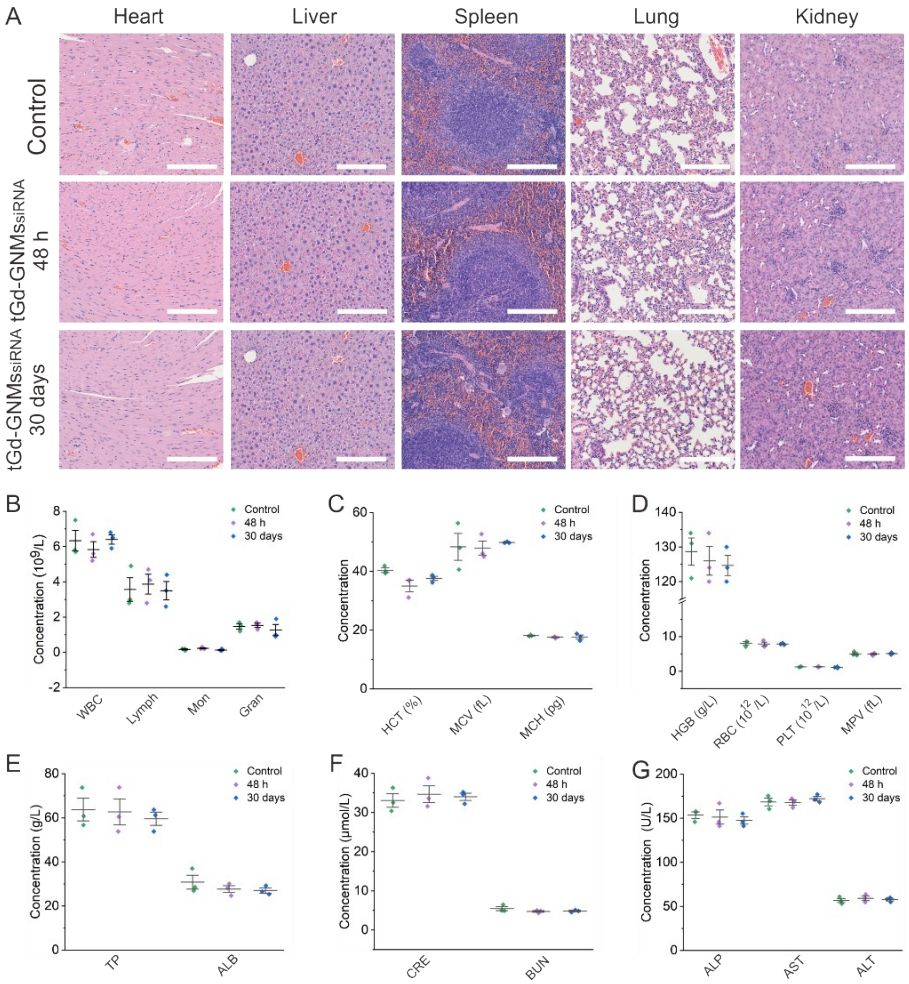

Figure S12. In vivo biocompatibility evaluation of tGd–$GNMs_{siRNA}$ complex. (A) H&Estained images of tissues (heart, liver, spleen, lung, kidney, bar: 100 μm), (B–D) Routine blood analysis and (E–F) Blood biochemistry in normal mice, and mice vein intravenously injected with tGd–$GNMs_{siRNA}$ complex, 48 h and 30 days after injection. The quantitative results were represented as the mean ± SD for at least three replicates.