

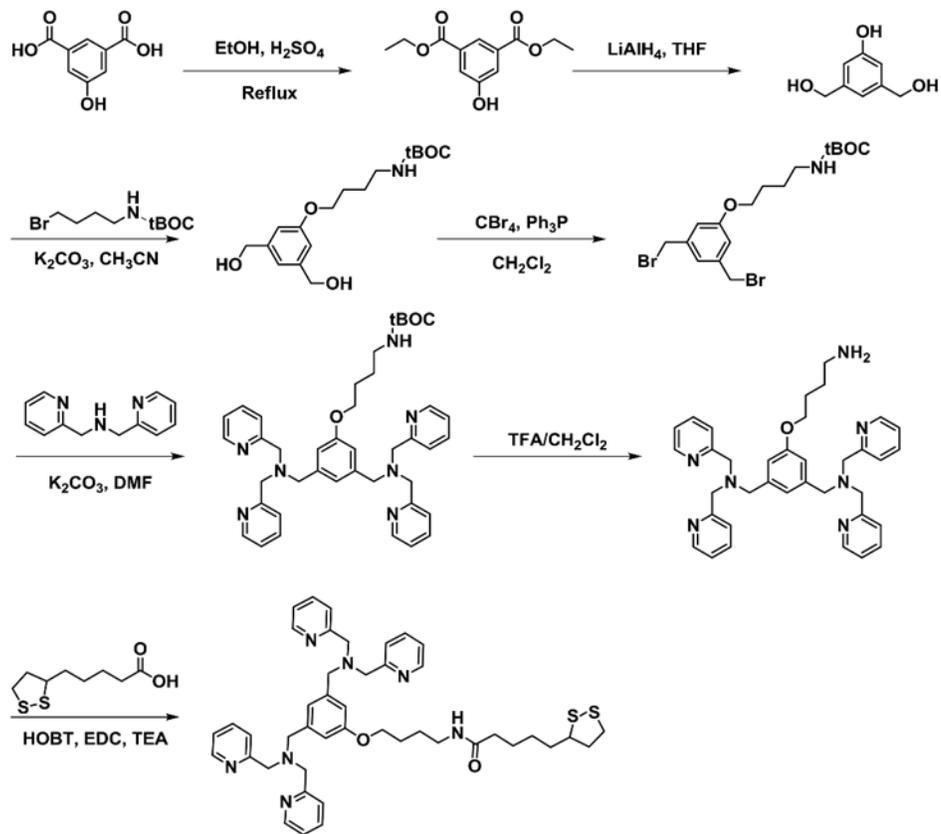
## **SUPPORTING INFORMATION**

### **Engineered Zn(II)-Dipicolylamine-Gold Nanorod Provides Effective Prostate Cancer Treatment by Combining siRNA Delivery and Photothermal Therapy**

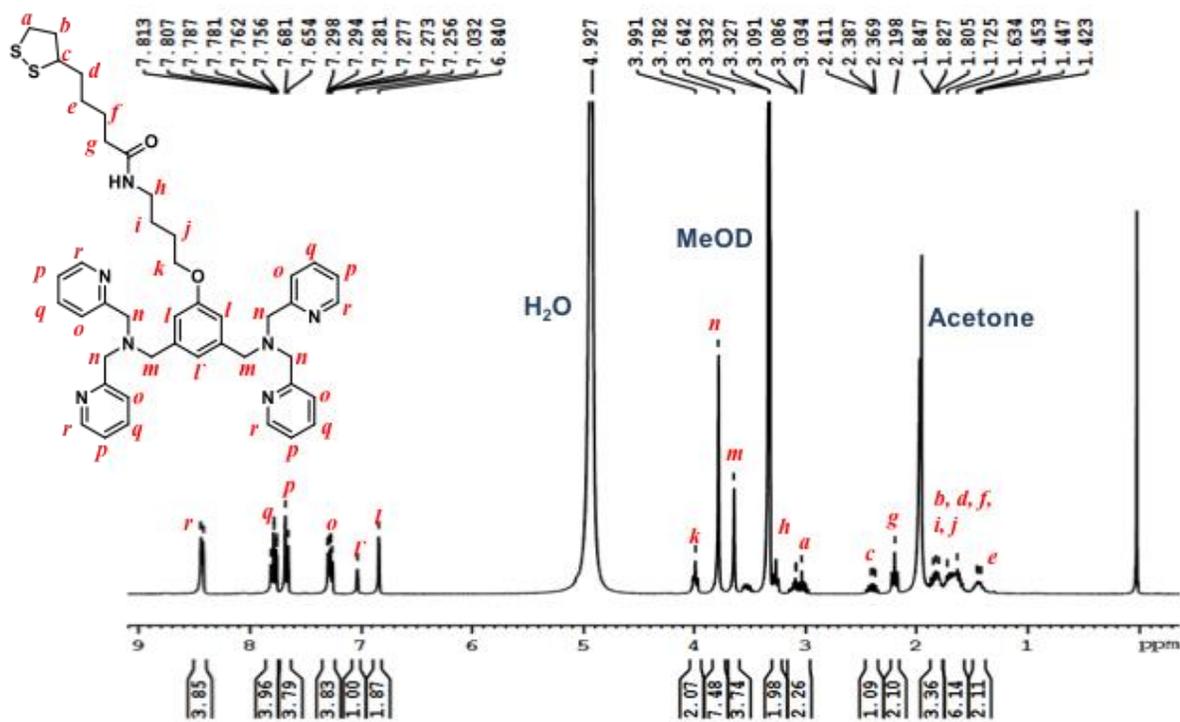
**Kyung Hyun Min,<sup>1,2</sup> Young-Hwa Kim,<sup>1</sup> Zhantong Wang,<sup>1</sup> Jihoon Kim,<sup>1</sup> Jee Seon Kim,<sup>1</sup> Sun Hwa Kim,<sup>2</sup> Kwangmeyung Kim,<sup>2</sup> Ick Chan Kwon,<sup>2</sup> Dale O. Kieseewetter,<sup>1</sup> and Xiaoyuan Chen<sup>1\*</sup>**

<sup>1</sup> Laboratory of Molecular Imaging and Nanomedicine, National Institute of Biomedical Imaging and Bioengineering, National Institutes of Health, Bethesda, Maryland 20892, United States

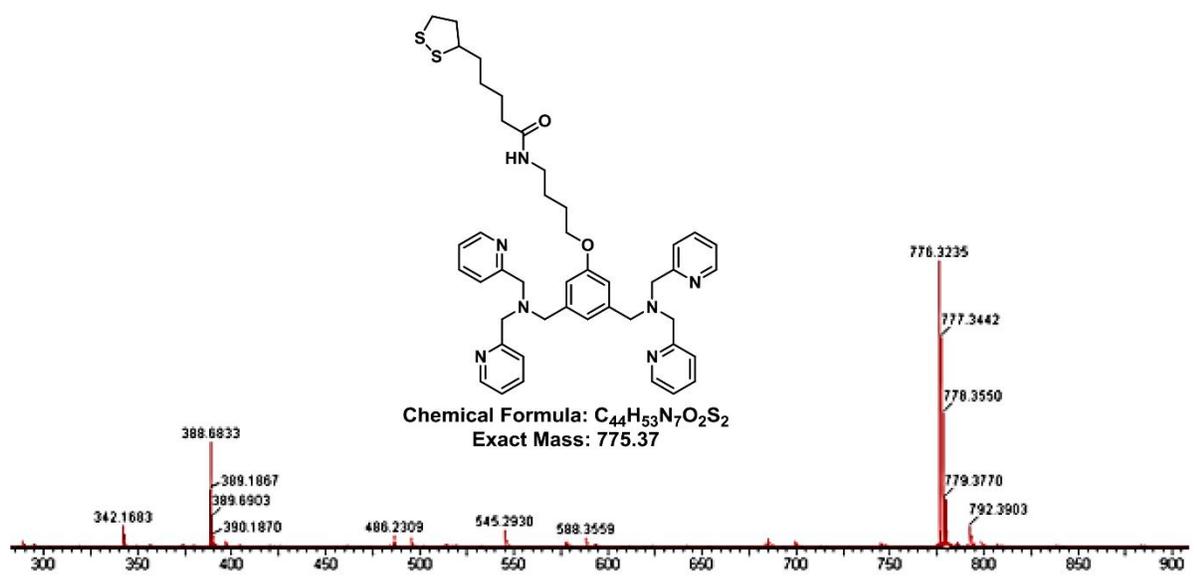
<sup>2</sup> Center for Theragnosis, Biomedical Research Institute, Korea Institute of Science and Technology, Seoul 136-791, Republic of Korea



**Figure S1.** Synthesis scheme of lipoic acid-bis-DPA



**Figure S2.** Confirmation of liponic acid-bis-DPA using  $^1\text{H-NMR}$

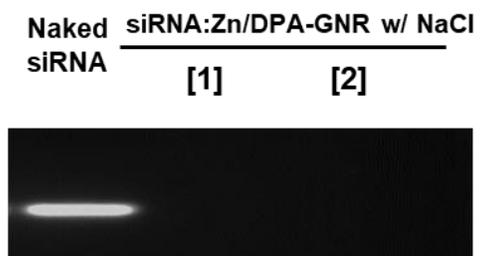


**Figure S3.** Confirmation of lipoic acid-bis-DPA using LCMS.

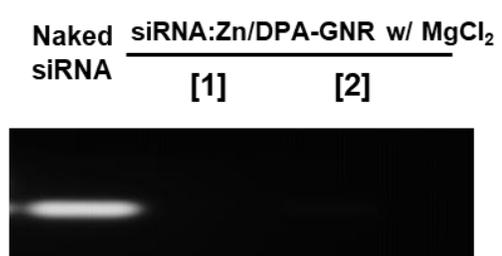
**Table S1.** The feed ratio, composition ratio, and conversion of DPA from various DPA-GNR.

Nanoparticle	Feed ratio ([DPA]/[GNR])	Composition ratio ([DPA]/[GNR])	Conversion of DPA (%)
DPA-GNR1	10	8.3±0.8	83.2±7.6
DPA-GNR2	50	35.3±2.6	70.6±5.1
DPA-GNR3	100	59.3±4.0	59.3±4.0
DPA-GNR4	500	154.1±28.1	30.8±5.6

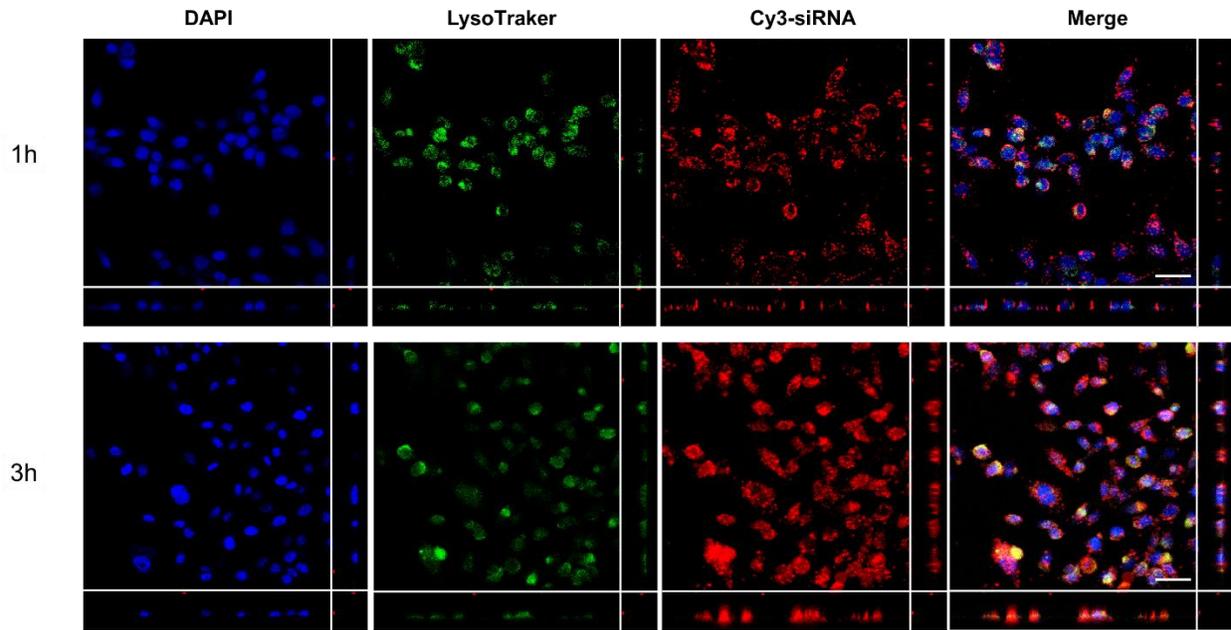
a)



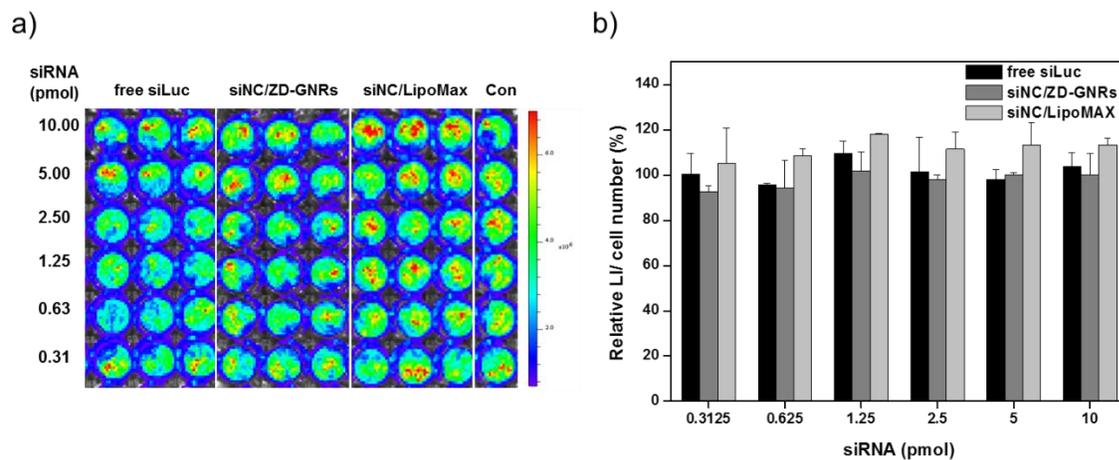
b)



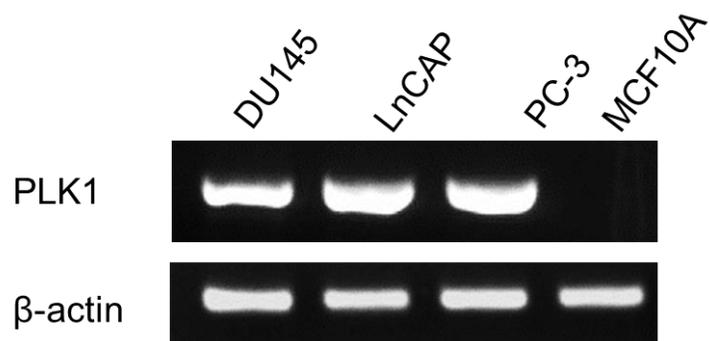
**Figure S4.** (a-b) Electrophoretic retardation analysis of siRNA binding with ZD-GNRs: (a) After addition of NaCl (Lanes 1-2: complexes at siRNA/ZD-GNR molar ratios of 100 and 50); (b) After addition of MgCl<sub>2</sub> (Lanes 1-2: complexes at molar ratio of 100 and 50).



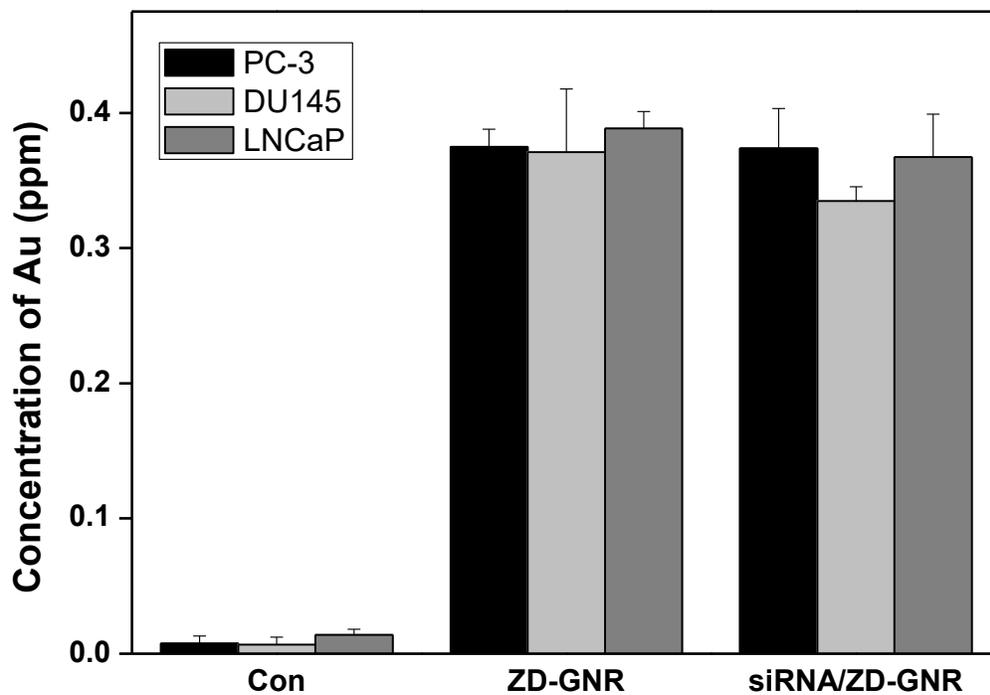
**Figure S5.** Confocal microscopy images of 143B cells treated with Cy3-labeled siRNA/ZD-GNRs after 1 h incubation and 3 h incubation. (Blue fluorescence is associated with DAPI, the green fluorescence is expressed by LysoTracker, and the red fluorescence is released from Cy3-labeled siRNA). Scale bar: 40  $\mu\text{m}$ .



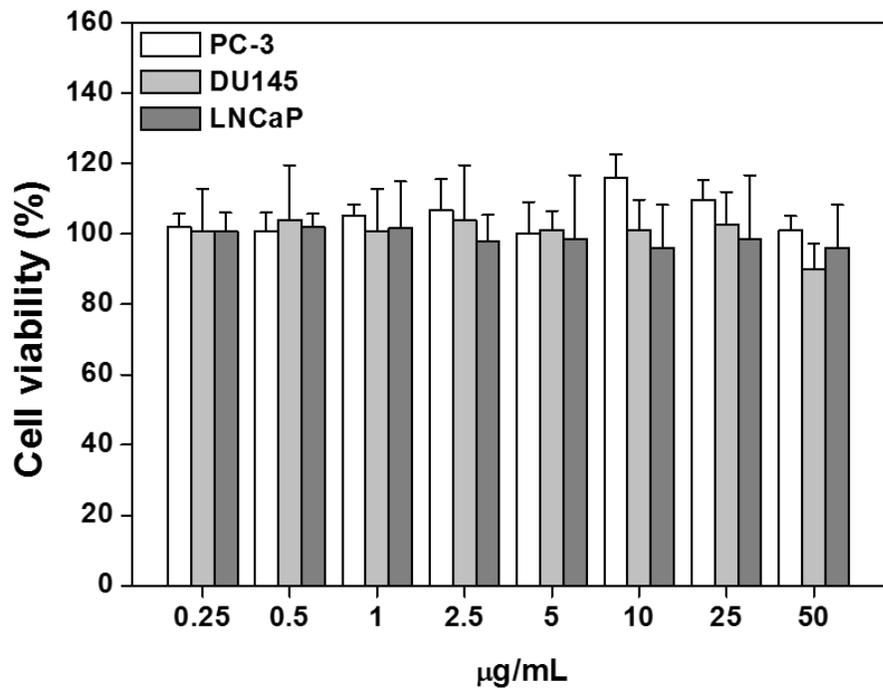
**Figure S6.** a-b) Suppression of fLuc gene expression of 143B-fLuc cells after treatment with free siLuc, siNC/ZD-GNRs or siNC/LipoMax.



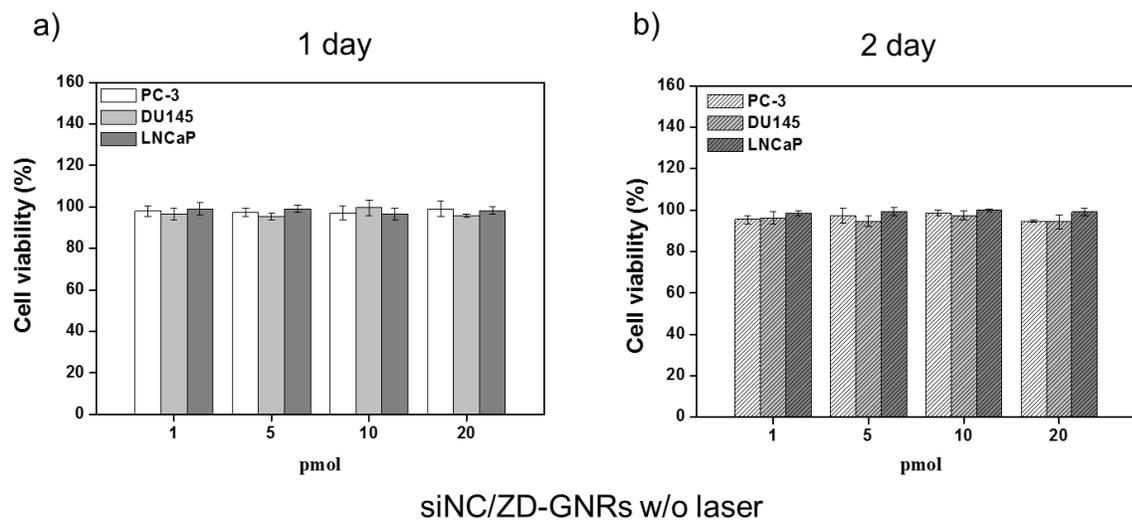
**Figure S7.** mRNA expression of PLK1 in DU145, LNCaP, PC-3 and MCF10A.



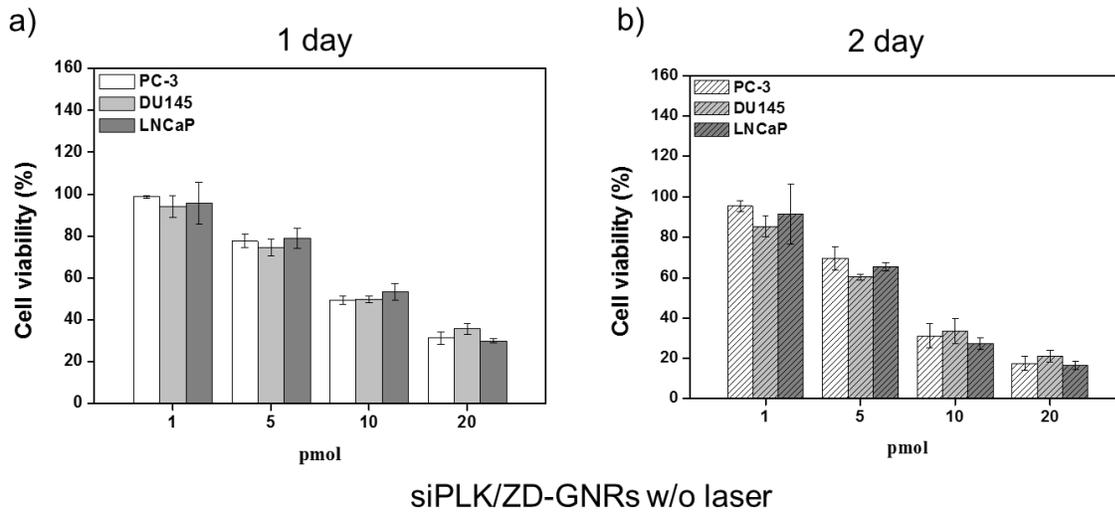
**Figure S8.** ICP-OES analysis of ZD-GNR and siRNA/ZD-GNRs after 3  $\mu\text{g}/\text{mL}$  AuNP treatment for 24 h in prostate cancer cells. Data are means  $\pm$  SD (n=5).



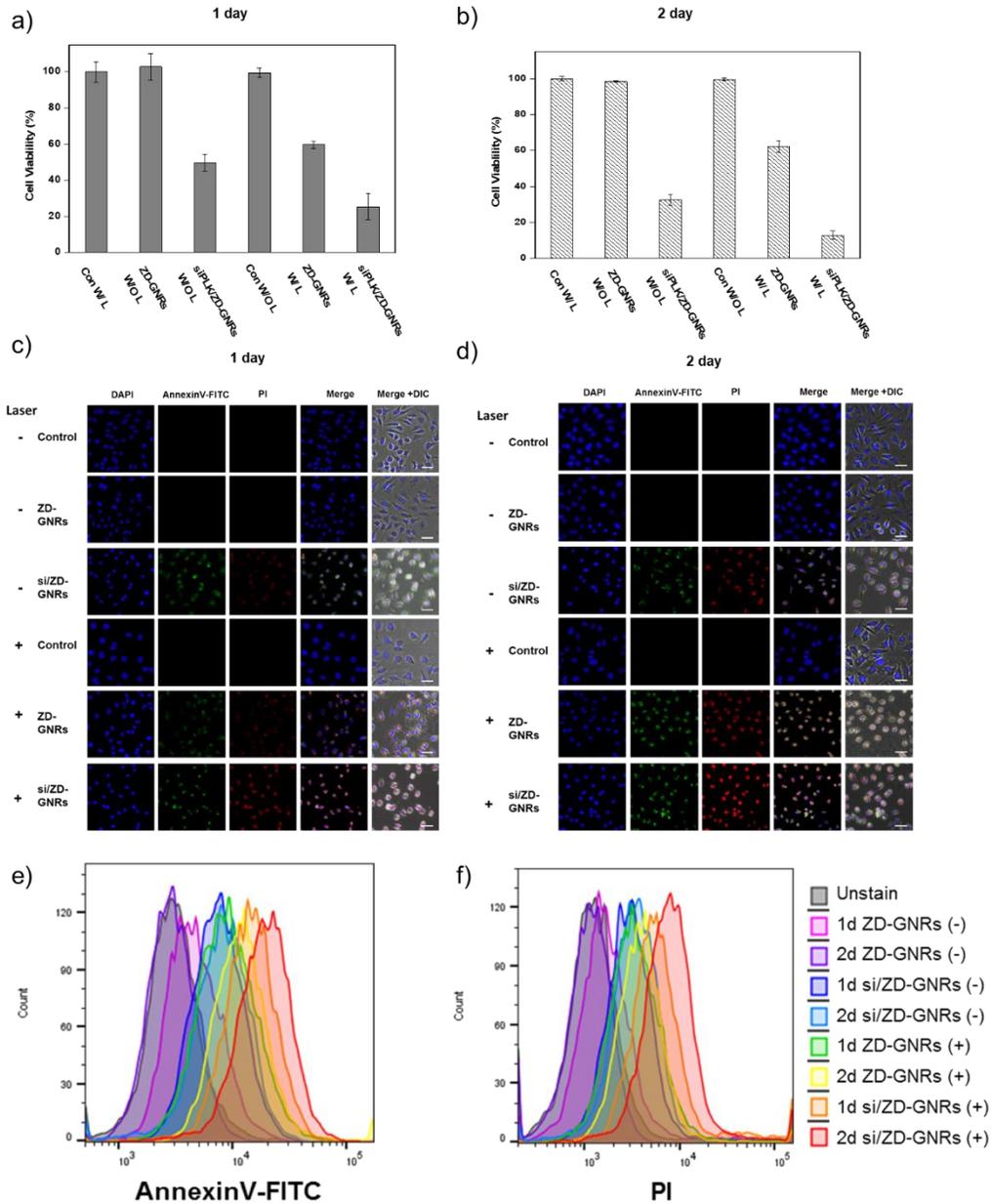
**Figure S9.** *In vitro* cytotoxicity of ZD-GNRs without irradiation after 48 h of incubation. The results represent means  $\pm$  SDs (n = 5).



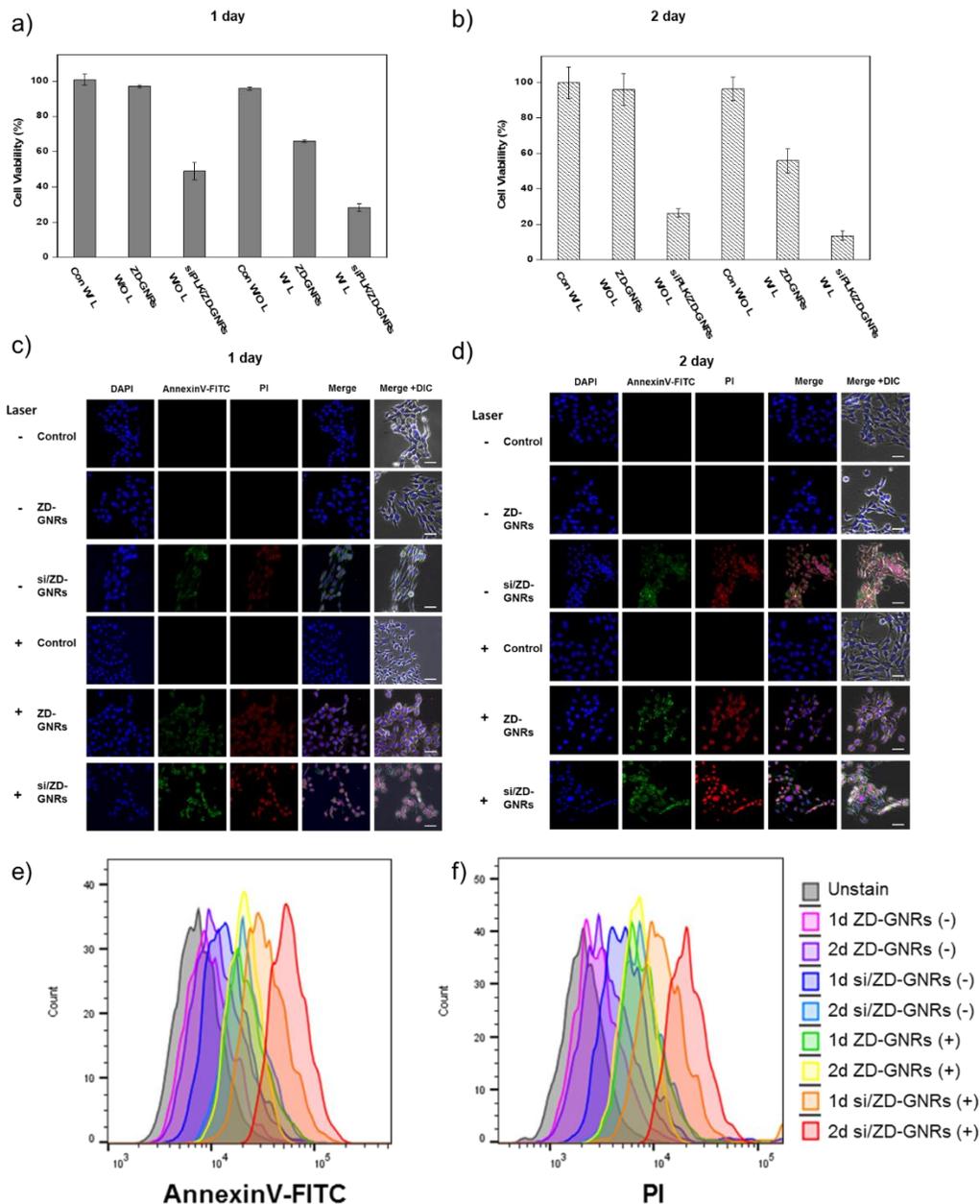
**Figure S10.** (a-b) *In vitro* cytotoxicity of siNC/ZD-GNRs without irradiation after 24 h of incubation (a) and 48 h of incubation (b). The results represent means  $\pm$  SDs (n = 5).



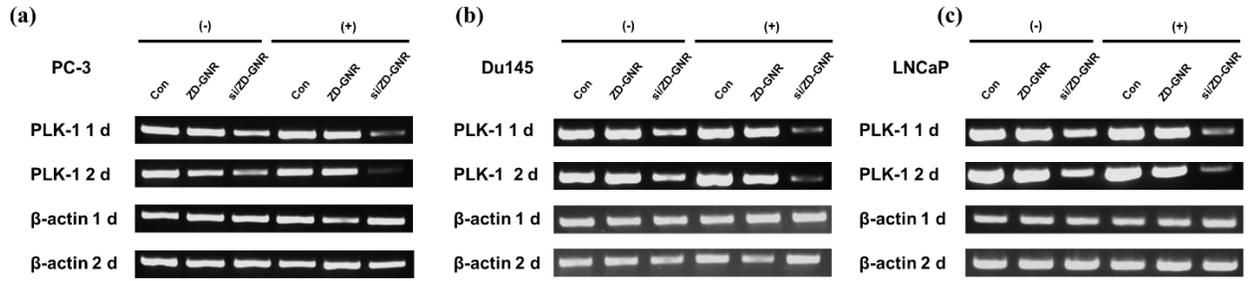
**Figure S11.** *In vitro* cytotoxicity of siPLK/ZD-GNRs without irradiation after 24 h of incubation (a) and 48 h of incubation (b). The results represent means  $\pm$  SDs (n = 5).



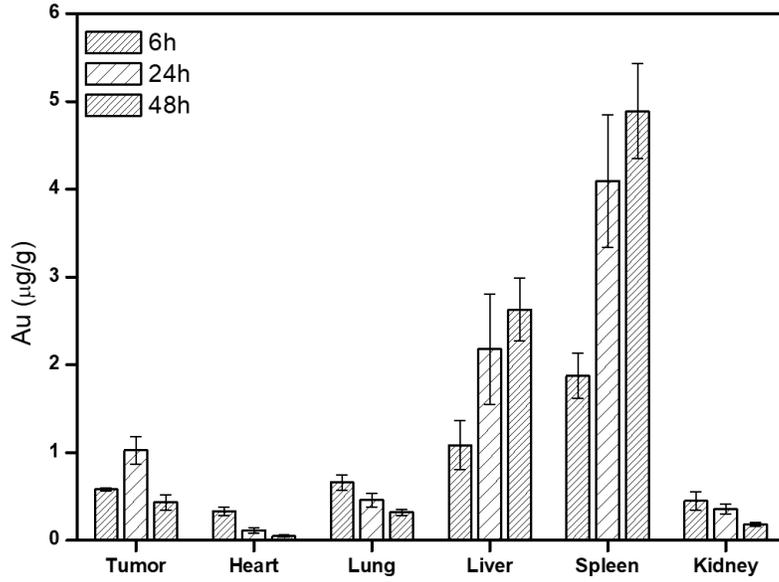
**Figure S12.** *In vitro* combinational gene/photothermal therapy of siPLK/ZD-GNRs on DU145 cell lines. (a-b) *In vitro* cytotoxicity of saline, ZD-GNRs, and siPLK/ZD-GNRs with or without 808 nm laser irradiation at power density of  $0.5 \text{ W/cm}^2$  against prostate cancer cells after incubation for 1 day (a) and 2 days (b). (c-d) Confocal microscopy images of DU145 cells treated with PI and Annexin V (AV) after treatment after incubation for 1 day (c) and 2 days (d) (Blue fluorescence is associated with DAPI; the green fluorescence is expressed by Annexin V-FITC, and the red fluorescence is released from PI). (e-f) Scale bar:  $20 \mu\text{m}$ . Flow cytometry data of DU145 cells treat with PI and Annexin V (AV) after treatment after incubation for 1 day (e) and 2 days (f).



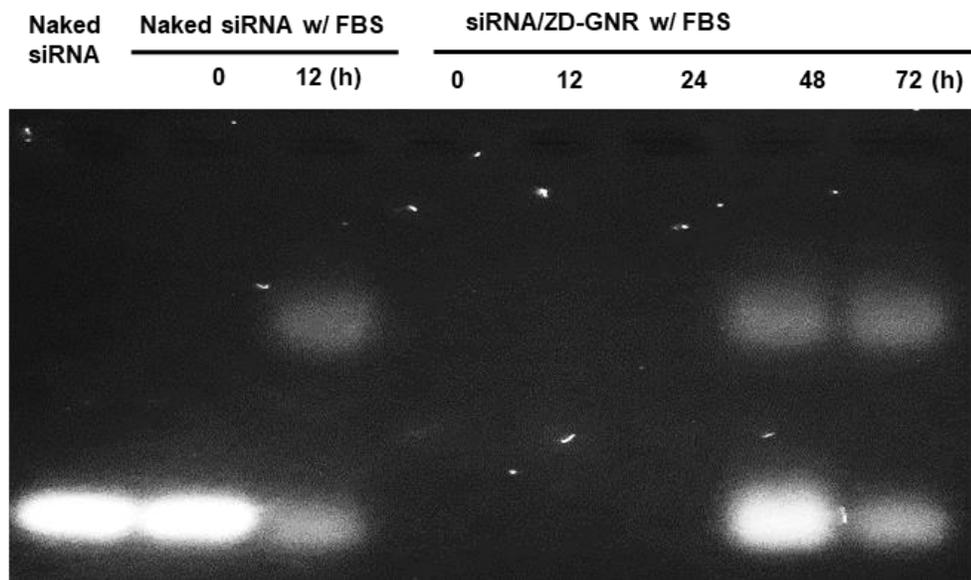
**Figure S13.** *In vitro* combinational gene/photothermal therapy of siPLK/ZD-GNRs on LNCaP cell lines. (a-b) *In vitro* cytotoxicity of saline, ZD-GNRs, and siPLK/ZD-GNRs with or without 808 nm laser irradiation at power density of 0.5 W/cm<sup>2</sup> against prostate cancer cells after incubation for 1 day (a) and 2 days (b). (c-d) Confocal microscopy images of LNCaP cells treated with PI and Annexin V (AV) after treatment after incubation for 1 day (c) and 2 days (d) (Blue fluorescence is associated with DAPI; the green fluorescence is expressed by Annexin V-FITC, and the red fluorescence is released from PI). (e-f) Scale bar: 20  $\mu$ m. Flow cytometry data of LNCaP cells treat with PI and Annexin V (AV) after treatment after incubation for 1 day (e) and 2 days (f).



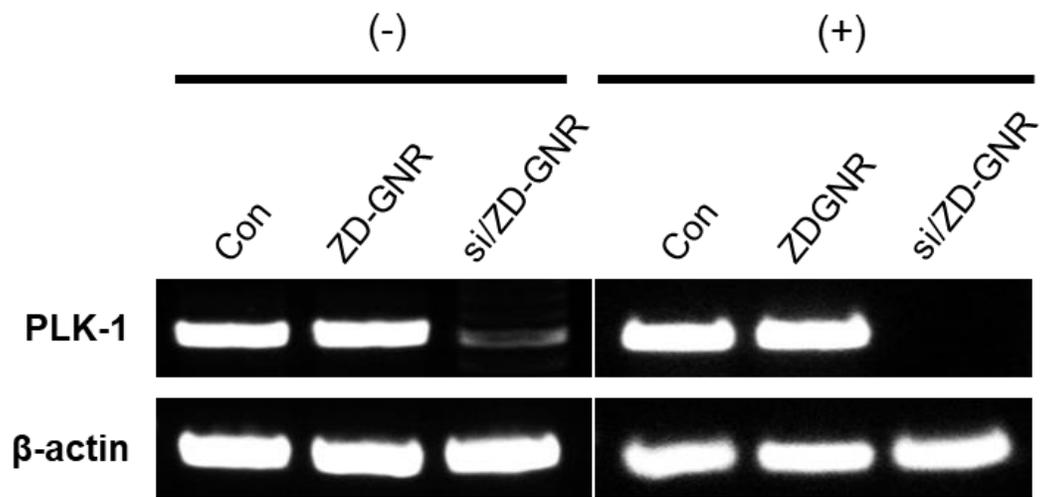
**Figure S14.** (a-c) RT-PCR analysis of the PLK1 gene expression in PC-3 (a), DU145 (b) and LNCaP (c) cells after sample treatment with or without laser irradiation for 1 day and 2 days.



**Figure S15.** Biodistribution study of gold content as measured by ICP-EOS. Biodistribution of siRNA/ZD-GNRs in the tumor, heart, lung, liver, spleen, and kidneys at 6, 24, and 48 h after systemic administration.



**Figure S16.** Stability study under FBS condition. siRNA/ZD-GNR was incubated in 40% FBS solution for 0, 12, 24, 48 and 72 h at 37 °C and then analyzed by agarose gel (2%) under TBE running buffer.



**Figure S17.** RT-PCR analysis of the PLK1 gene expression in PC-3 tumor tissue.