ORIGINAL ARICLE

Gold nanorods-mediated efficient synergistic immunotherapy for detection and inhibition of postoperative tumor recurrence

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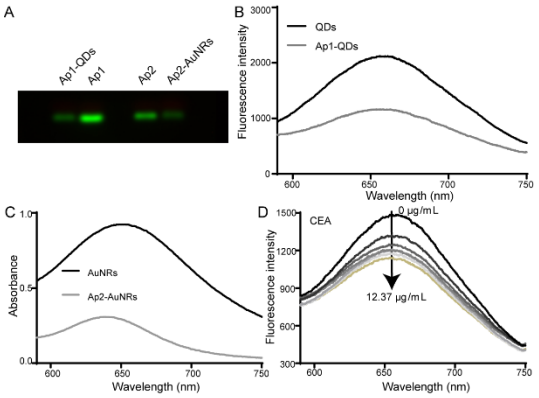

Figure S1 Characterization of CEA aptamer modified CdTe QDs and AuNRs. (A) Polyacrylamide gel electrophoresis to identify the conjugation of CEA Aptamer to CdTe QDs and AuNRs; (B) Fluorescence spectra of CdTe QDs before and after the conjugation of CEA aptamer suspended in ddH_2O at λ_{ex}=570 nm; (C) UV-Vis spectra of AuNRs and Ap2-AuNRs dissolved in ddH_2O; (D) The

Fluorescence spectra of Hydrogel-IFN-γ-QA incubated with different concentrations of CEA standard (from 0 to 12.37 μg/mL).

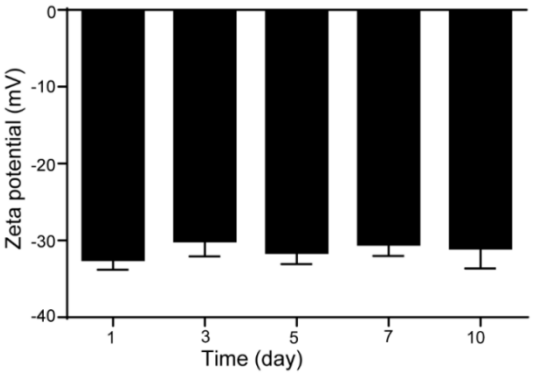

Figure S2 Stability of Hydrogel+IFN-γ+QA. Changes of zeta potential were measured by DLS. Data are presented as mean ±SD (*n*=3).

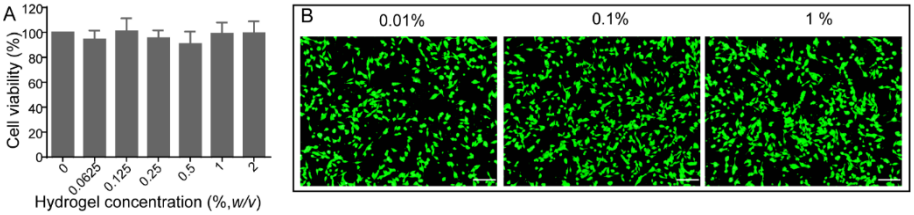

Figure S3 Cytotoxicity of thermal responsive hydrogels to tumor cells. a) Cell viability of LLC cells treated with Hydrogel for 24 h; Data are expressed as mean ±SD (*n*=5). b) AM/PI staining of LLC cells incubated with different concentrations of Hydrogel for 24 h.

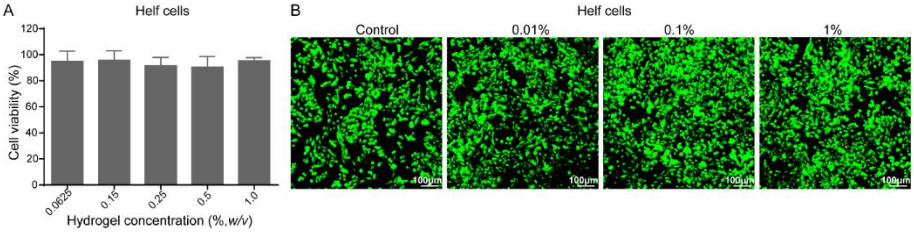

Figure S4 Cytotoxicity of thermal responsive hydrogels to normal cells. a) Cell viability of Helf cells treated with Hydrogel for 24 h; Data are expressed as mean ±SD (*n*=5). b) AM/PI staining of Helf cells incubated with different concentrations of Hydrogel for 24 h.

Table S1 IC_{50} values of IFN-γ and Hydrogel+IFN-γ+QA against LLC cells for 24 h (*n*=5).

Sample	IC_{50} (μg/mL)
IFN-γ	6.41
Hydrogel+IFN-γ+QA	6.66

IFN-γ, interferon-γ; Hydrogel+IFN-γ+QA, thermal responsive hydrogels co-loaded with AP1-QDs, AP2-AuNRs, and interferon-γ.

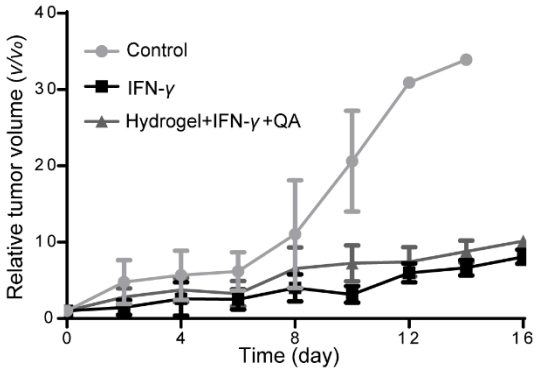

Figure S5 The relative tumor volume changes of *in situ* recurrence mice with different treatments based on Fig. 3E. Data are presented as mean ±SD (*n*=5).

Table S2 The median survival of three groups for inhibition of postoperative tumor recurrence *in situ*.

Group	Median survival (day)
Control	11
IFN-*γ*	13
Hydrogel+IFN-*γ*	16

IFN-*γ*, interferon-*γ*; Hydrogel+IFN-*γ*, thermal responsive hydrogels loaded with interferon-*γ*.

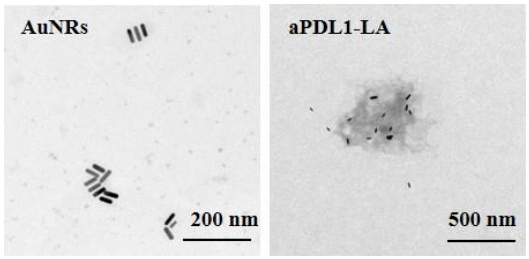

Figure S6 TEM images of AuNRs and aPDL1-LA.

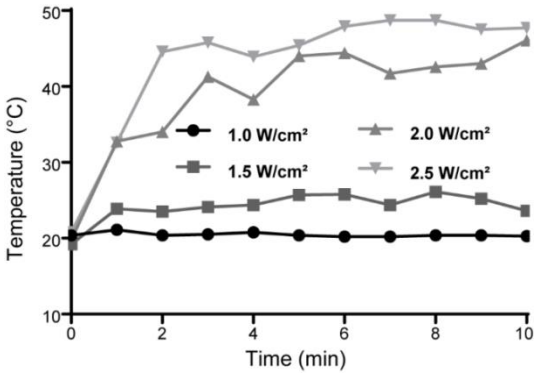

Figure S7 The temperature changes of AuNRs (20 μg/mL) irradiated with 808 nm laser at different power intensities.

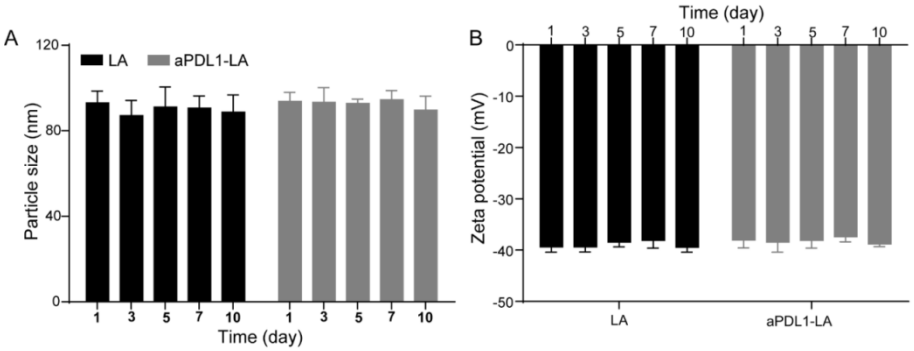

Figure S8 Stabilities of LA and aPDL1-LA. (A) Changes of particle size of LA and aPDL1-LA; (B) Changes of zeta potential of LA and aPDL1-LA. Data are presented as mean ±SD (*n*=3).

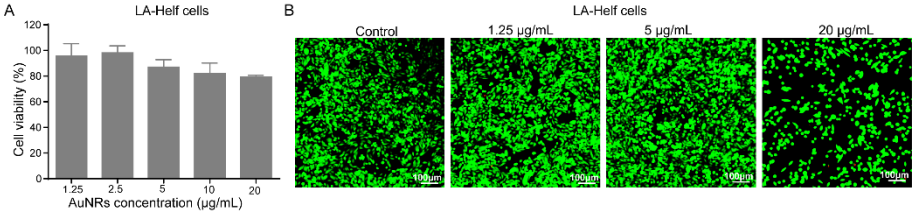

Figure S9 Cytotoxicity of LA to normal cells. (A) Cell viability of Helf cells treated with LA for 24 h; Data are expressed as mean ±SD (*n*=5); (B) AM/PI staining of Helf cells incubated with different concentrations of LA for 24 h.

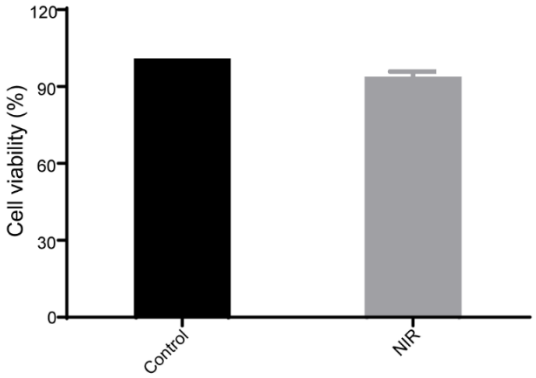

Figure S10 Cytotoxicity of NIR laser. Cell viability of LLC cells treated with 808 nm laser (2.0 W/cm^2, 5 min). Data of NIR group are presented as mean ±SD (*n*=5).

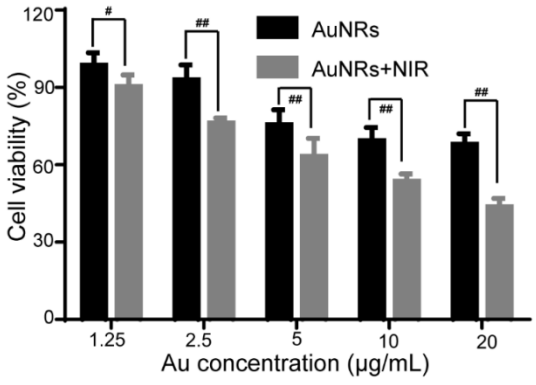

Figure S11 Cell viability of LLC cells treated with different concentrations of AuNRs with or without irradiation for 24 h. Data are presented as mean ±SD (n=5). $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ compared with AuNRs group by Student's t-test.

Table S3 IC50 values of LA, LA+NIR, aPDL1-LA, and aPDL1-LA+NIR against LLC cells (n=5).

Sample	IC_{50} (μg/mL)
LA	30.11
LA+NIR	12.13
aPDL1-LA	10.37
aPDL1-LA+NIR	5.65

LA, liposomes encapsulated AuNRs; LA+NIR, liposomes-encapsulated AuNRs with near-infrared irradiation; aPDL1-LA, anti-PDL1-modified liposomes-encapsulated AuNRs; aPDL1-LA+NIR, anti-PDL1-modified liposomes-encapsulated AuNRs with near-infrared irradiation.

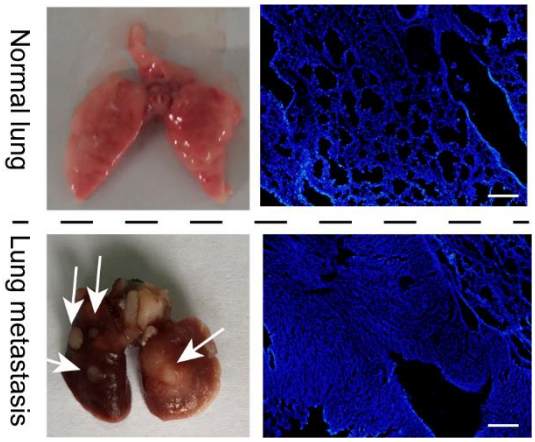

Figure S12 The appearance of tumor nodules in lung tissue. Lung tissues were collected from LLC Lung metastases-bearing mice and frozen sections were stained with Hoechst 33258 observed by confocal laser scanning microscope.

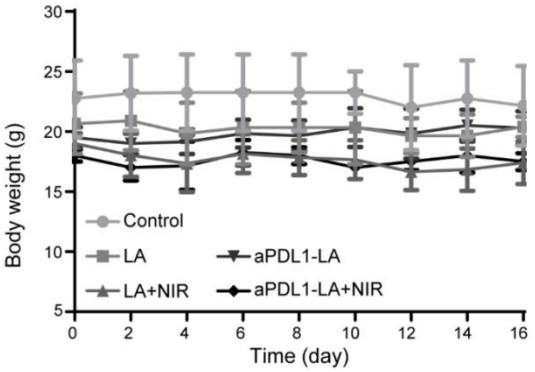

Figure S13 Body weight changes of LLC lung metastasis cancer-bearing mice. Data are presented as mean ±SD (n=5).

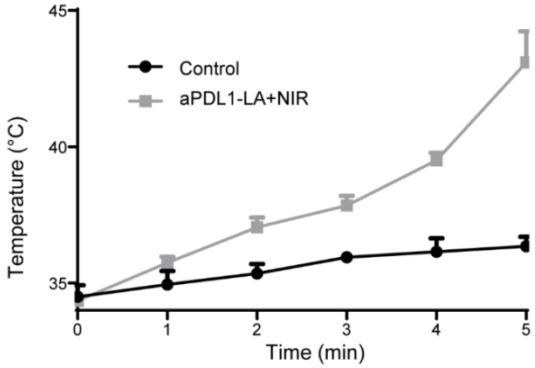

Figure S14 The temperature changes of the lung tissue based on Fig. 5B. Data are presented as mean ±SD (*n*=3).

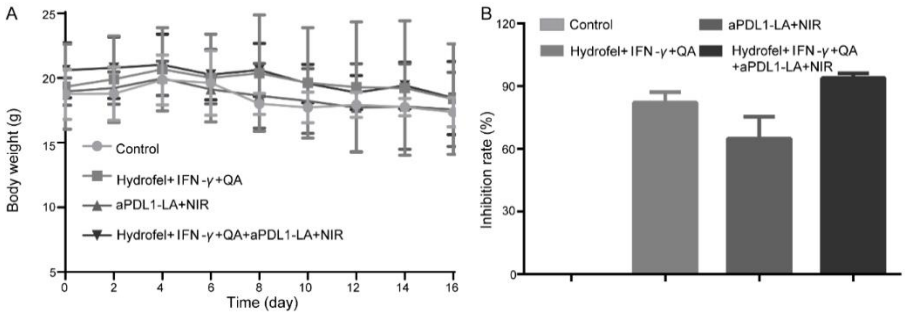

Figure S15 Body weight changes and tumor inhibition rate. (A) Body weight change of subcutaneous LLC and LLC Lung metastases-bearing mice; (B) The tumor inhibition rate of LLC subcutaneous tumor**.** Control group: no treatment group; Hydrogel+IFN-*γ*+QA group: local injection of Hydrogel+IFN-*γ*+QA; aPDL1-LA+NIR group: intravenous injection of aPDL1-LA with 808 nm laser irradiation; Hydrogel+IFN-*γ*+QA+aPDL1-LA+NIR group: local injection of Hydrogel+IFN-*γ*+QA on Day 0 and intravenous injection of aPDL1-LA on Days 1, 3 and 5 with 808 nm laser irradiation. Data are presented as mean ±SD (*n*=5).

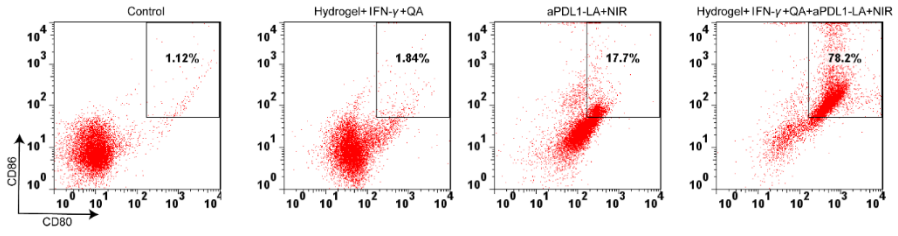

Figure S16 DC maturation induced by synergistic immunotherapy on subcutaneous LLC and LLC Lung metastases-bearing mice (gated on $CD11c^+$ DC cells). Lymph nodes were collected after the treatment, disrupted, stained with anti-CD80-APC and anti-CD86-FITC and tested by flow cytometry (*n*=3, 1×10^4 cells measured). Control group: no treatment group; Hydrogel+IFN-*γ*+QA group: local injection of Hydrogel+IFN-*γ*+QA; aPDL1-LA+NIR group: intravenous injection of aPDL1-LA with 808 nm laser irradiation; Hydrogel+IFN-*γ*+QA+aPDL1-LA+NIR group: local

injection of Hydrogel+IFN-γ+QA on Day 0 and intravenous injection of aPDL1-LA on Days 1, 3 and 5 with 808 nm laser irradiation.

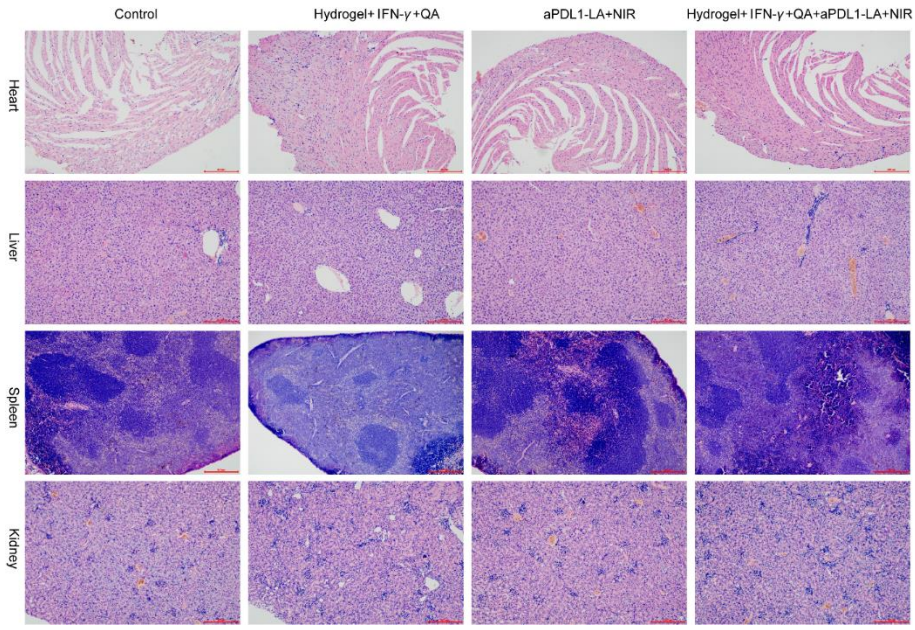

Figure S17 Histopathological images of tissue sections. Main organs were harvested from subcutaneous LLC and LLC Lung metastases-bearing mice (scale bar=200 μm) based on Fig. 6. Control group: no treatment group; Hydrogel+IFN-γ+QA group: local injection of Hydrogel+IFN-γ+QA; aPDL1-LA+NIR group: intravenous injection of aPDL1-LA with 808 nm laser irradiation; Hydrogel+IFN-γ+QA+aPDL1-LA+NIR group: local injection of Hydrogel+IFN-γ+QA on Day 0 and intravenous injection of aPDL1-LA on Days 1, 3 and 5 with 808 nm laser irradiation.

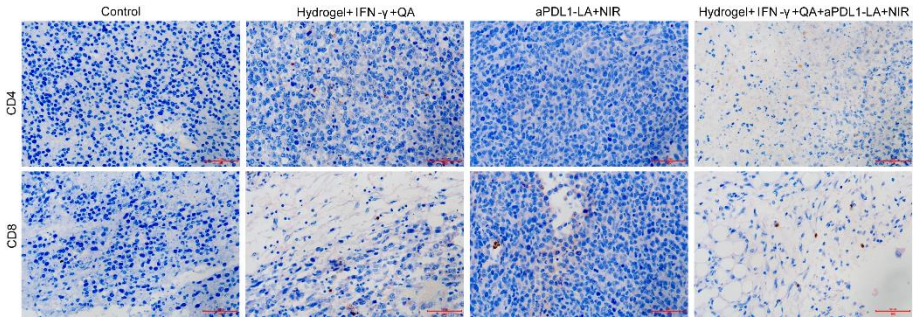

Figure S18 The immunohistochemistry staining images of $CD4^+$ and $CD8^+$ T-lymphocytes in *in situ* recurrence tumors after different treatments. (Scale bar=50 μm). Control group: no treatment group; Hydrogel+IFN-γ+QA group: local injection of Hydrogel+IFN-γ+QA; aPDL1-LA+NIR group: intravenous injection of aPDL1-LA with 808 nm laser irradiation; Hydrogel+IFN-γ+QA+aPDL1-LA+NIR group: local injection of Hydrogel+IFN-γ+QA on Day 0 and intravenous injection of aPDL1-LA on Days 1, 3 and 5 with 808 nm laser irradiation.

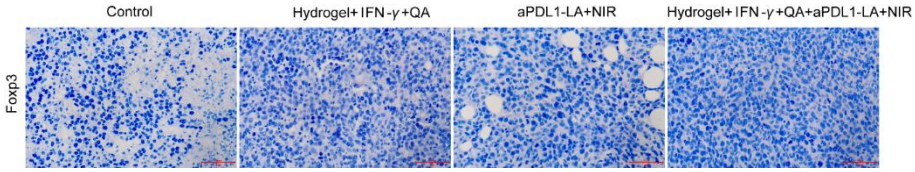

Figure S19 The immunohistochemistry staining images of Treg cells in *in situ* recurrence tumors after different treatments based on Figure 6. (Scale bar=50 μm). Control group: no treatment group; Hydrogel+IFN-*γ*+QA group: local injection of Hydrogel+IFN-*γ*+QA; aPDL1-LA+NIR group: intravenous injection of aPDL1-LA with 808 nm laser irradiation; Hydrogel+IFN-*γ*+QA+aPDL1-LA+NIR group: local injection of Hydrogel+IFN-*γ*+QA on Day 0 and intravenous injection of aPDL1-LA on Days 1, 3 and 5 with 808 nm laser irradiation.

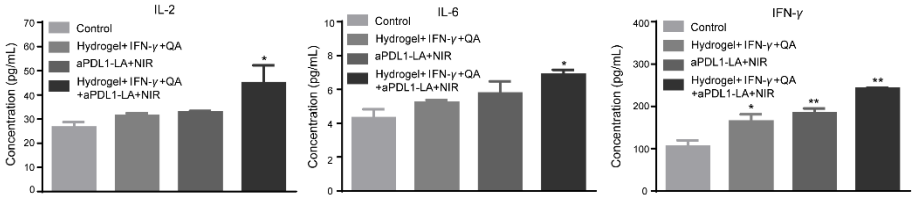

Figure S20 Immune cytokine levels in sera isolated from mice on Day 9. Control group: no treatment group; Hydrogel+IFN-*γ*+QA group: local injection of Hydrogel+IFN-*γ*+QA; aPDL1-LA+NIR group: intravenous injection of aPDL1-LA with 808 nm laser irradiation; Hydrogel+IFN-*γ*+QA+aPDL1-LA+NIR group: local injection of Hydrogel+IFN-*γ*+QA on Day 0 and intravenous injection of aPDL1-LA on Days 1, 3 and 5 with 808 nm laser irradiation. Data are presented as mean±SD (n=3). *P<0.05, **P<0.01 compared with control group by ANOVA analysis.