Supporting Information

Escherichia coli adhesin protein-conjugated thermal responsive hybrid nanoparticles for photothermal and immunotherapy against cancer and its metastasis

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Materials and Methods

Purification of FimH

The purification of recombinant FimH has been described in detail in a previous study. [43] Briefly, competent BL21 cells and the pET28a-FimH plasmid were mixed on ice for 30 min. The cells were incubated for 60–90 s in a 42 °C water bath and then quickly transferred to ice. The transformed BL21 cells were incubated in a Luria-Bertani (LB) medium, for 1 h, in a 37 °C shaking incubator at 0.94 × *g* ($g = r \times 11.18 \times 10^6 \times RPM^2$), and the cells were grown overnight at 37 °C in LB containing kanamycin. After incubation, a single colony was transferred from the culture medium to 1 L of LB, containing kanamycin, and centrifuged at 1.41 × *g* and 37 °C. After harvesting BL21 cells the following day, the cells were lysed using 60% power of ultrasonication for 15 min. The disrupted cells were centrifuged at 10,000 × *g* for 15 min, and the supernatant was collected. Using a low-pressure chromatography system, the Ni-IDA column was equilibrated with equilibration buffer (pH 8.0, 8 M urea, 100 mM Tris, and 100 mM NaH_2PO_4) at 0.5 mL/min flow rate. The supernatant was loaded through an equilibrated column at the same flow rate. When the OD_{280} value reached baseline, the column was washed with an Ni-IDA wash buffer (pH 6.3, 8 M urea, 100 mM Tris, and 100 mM NaH_2PO_4) at 1 mL/min flow rate. After the OD_{280} value reached baseline, a Ni-IDA elution buffer (pH 4.5, 8 M urea, 100 mM Tris, and 100 mM NaH_2PO_4) was loaded at a flow rate of 1 mL/min, and FimH was eluted from the collected effluent.

Measurement of photothermal conversion efficiency

The F-TRH solution (0.2 mg/mL) was irradiated with an 808 nm laser at a power density of 2.0 W/cm^2 for 10 min. The photothermal efficiency of F-TRH was calculated using the following formula:

$$\eta = \frac{hA(T_{max} - T_{surr}) - Q_{dis}}{I(1 - 10^{-A_{808}})} \times 100\%$$

where h is the heat transfer coefficient, A is the surface area of the container, T_{max} is the reached equilibrium temperature, T_{surr} is the ambient temperature, Q_{dis} is the heat associated with the light absorbance, I is the laser power density, and A_{808} is the absorbance of F-TRH at 808 nm.

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Supplemental results

Figure S1

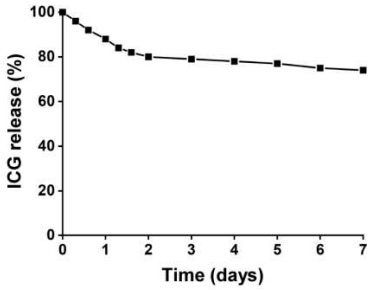

Figure S1. Cumulative release of ICG in F-TRHs through laser irradiation. The percentage of the ICG released was measured in the supernatant after the indicated time.

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Figure S2

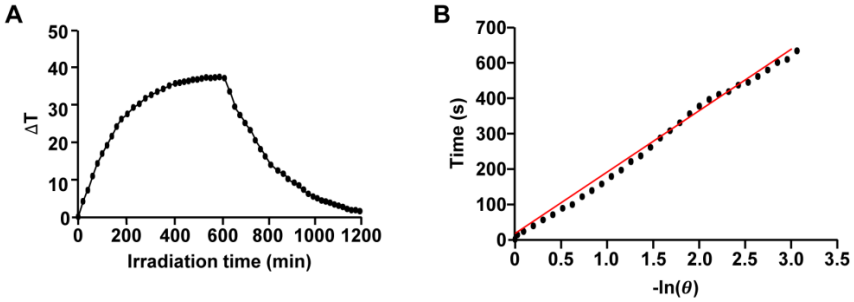

Figure S2. (A) Photothermal effect of F-TRH (0.2 mg/mL) under 808 nm laser irradiation (2 W/cm^{-1}) for 10 min. (B) Linear time data *versus* -ln$\boldsymbol{\theta}$ obtained from the cooling time in (A).

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Figure S3

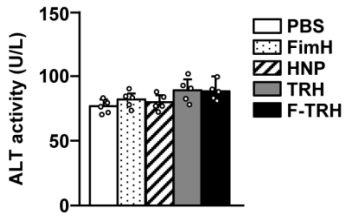

Figure S3. Levels of alanine aminotransferase (ALT) in mice sera. CT-26-bearing mice were intratumorally (*i.t.*) injected with PBS, FimH, HNP, TRH, and F-TRH with NIR laser irradiation (2 W/cm^2, 5 min). ALT levels in the blood serum were determined 24 h after treatment.

Figure S4

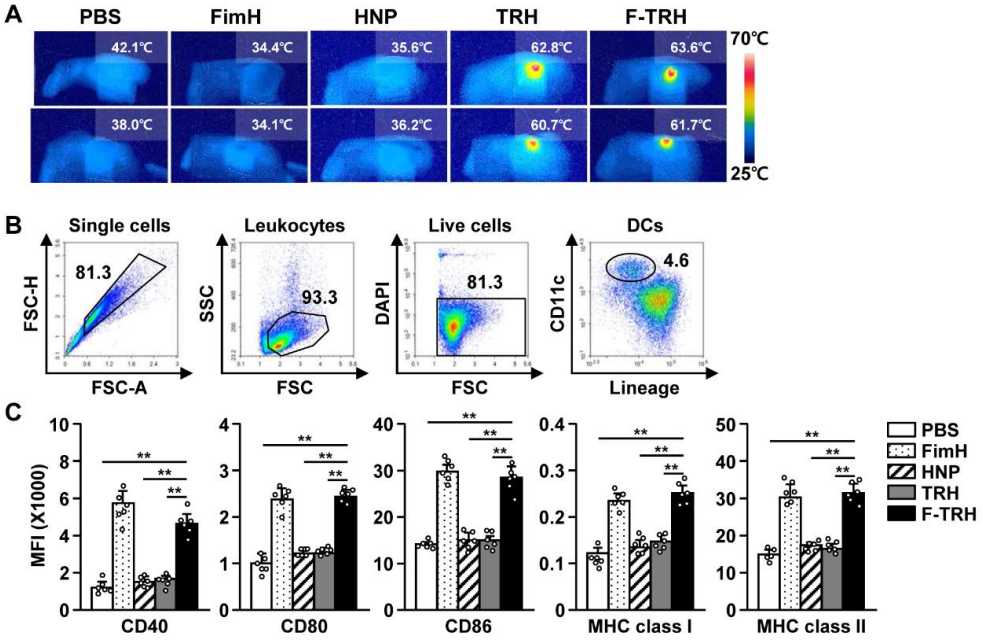

Figure S4. F-TRH treatment with laser irradiation promoted the activation of splenic DCs. CT-26-bearing mice were *i.t.* injected with PBS, FimH, HNP, TRH, and F-TRH followed by NIR laser irradiation (2 W/cm^2, 5 min). (A) The elevated temperature by NIR laser irradiation is shown. (B) Definition of DCs in the spleen. (C) The mean fluorescence intensities of the co-stimulatory molecules and MHC class I and II were analyzed 18 h after treatment. The data are representative of the average of the analyses of six independent samples.

Figure S5

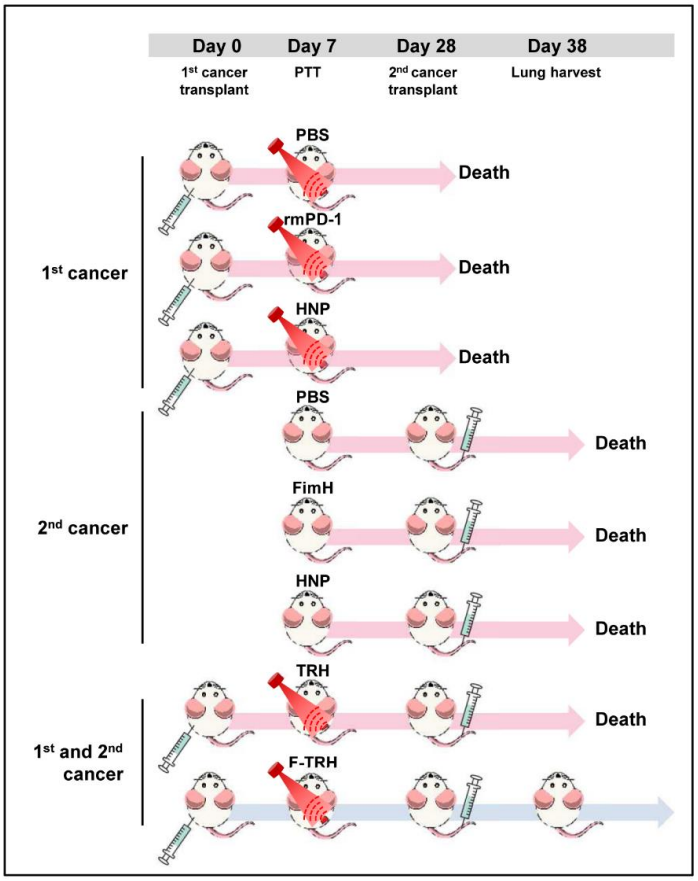

Figure S5. Schematic illustration of the 1st and 2nd tumor challenge models and treatment strategies.

Figure S6

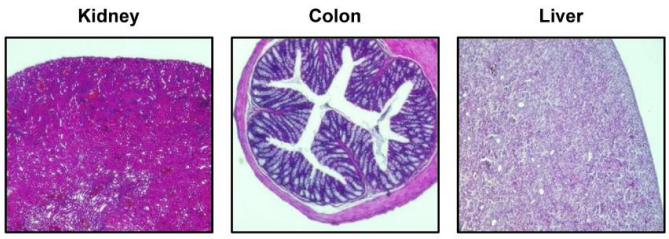

Figure S6. Hematoxylin and eosin (H&E) staining of peripheral tissues. On day 49 following the 1st tumor challenge, the cured mice, via F-TRH with NIR laser irradiation, were sacrificed, and the kidney, colon, and liver were stained with H&E.

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Figure S7

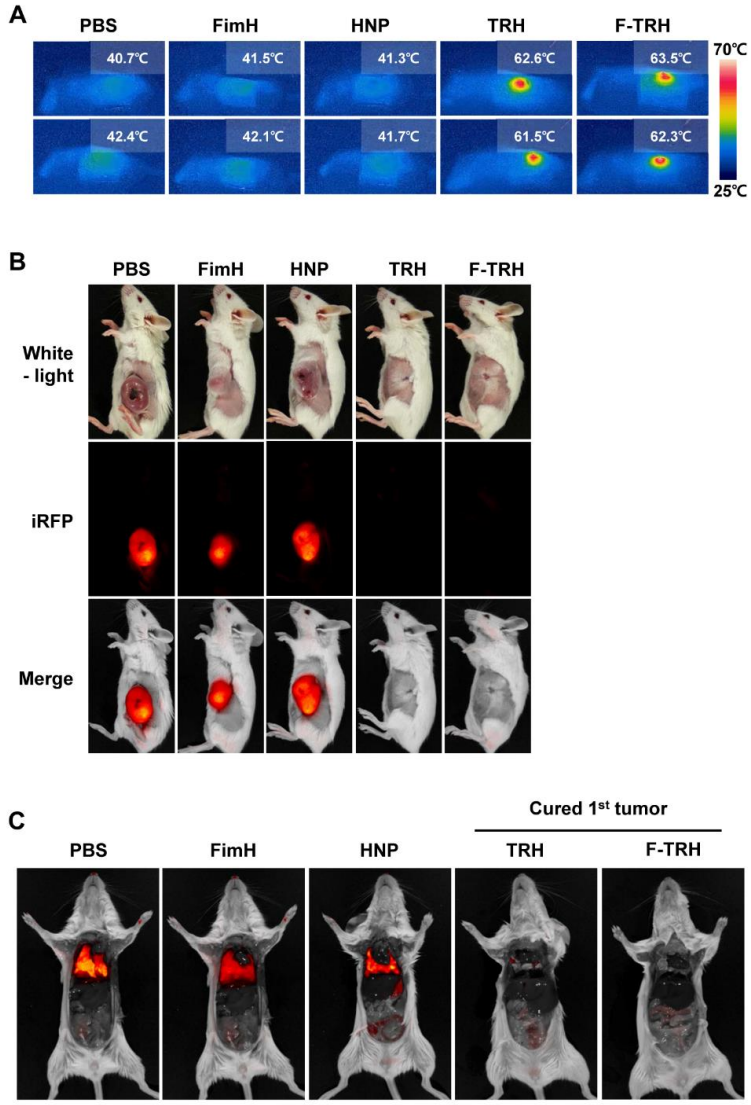

Figure S7. Anticancer and antimetastatic effects of F-TRH against 4T1 breast cancer cells. (A) The thermographic images of 4T1 tumor-bearing BALB/c mice which were treated with PBS, FimH, HNP, TRH, and F-TRH, followed by NIR laser irradiation (2 W/cm^2, 5 min) on day 7 after the tumor challenge, n = 5. (B) Fluorescence images of 4T1-iRFP tumor-bearing mice on day 17, n = 5. (C) Fluorescence images of metastatic 4T1-iRFP cell infiltration in the lung was detected on day 10 after the 2nd challenged 4T1-iRFP cancer, n = 5.

Figure S8

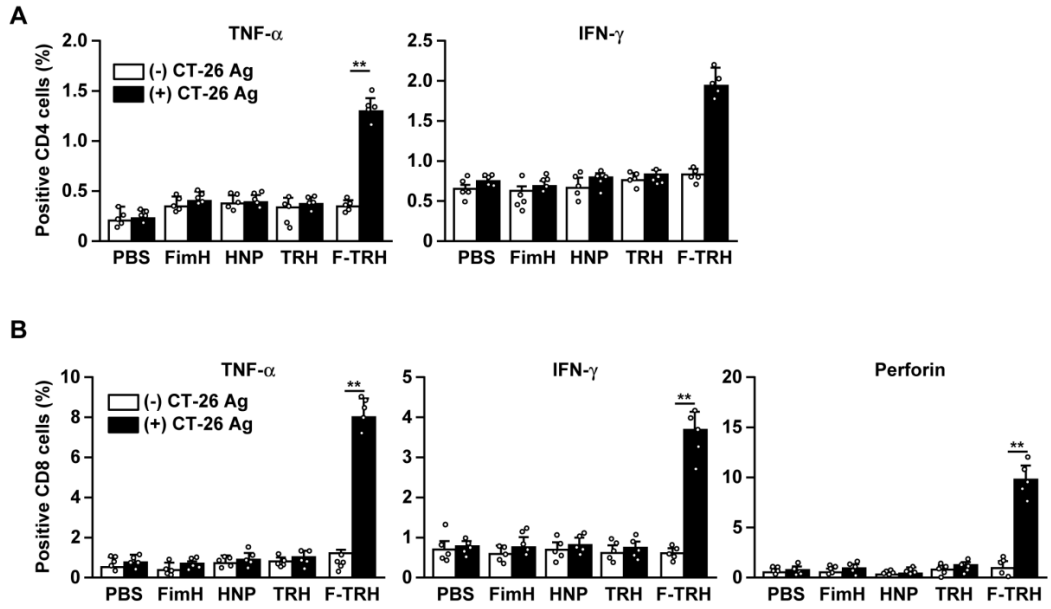

Figure S8. Ag-specific T-cell activation. On day 10 of the 2nd tumor challenge, the splenocytes were incubated with or without CT-26 Ag for 24 h. Intracellular cytokine levels in CD4 (A) and CD8 (B) were measured, n = 5, ***p <0.01*.