Supplementary Information for:

# Fabrication of Near-Infrared Light Responsive Photothermal Tea Leaf–Derived Particles with Thermotolerance-Inhibitory and Anticancer Activities

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Contents

**Method of phospholipid analysis by LC–MS/MS.**

Phospholipid species present in TLNPs were characterized by LC–MS/MS using a dedicated phospholipid profiling method package (LC-MS/MS Method Package for Phospholipid Profiling, Shimadzu Corporation, Kyoto, Japan), following the manufacturer's protocol. In brief, TLNPs dispersions were diluted 10-fold with methanol containing 0.1% formic acid and subjected to mass spectrometric analysis. An aliquot of each sample (5 μL) was injected onto a Kinetex C8 column (2.1 mm I.D. × 150 mm, 2.6 μm; Phenomenex, Torrance, CA, USA) and chromatographed at a flow rate of 0.5 mL/min. Separation was achieved using a binary gradient system consisting of mobile phase A (20 mM ammonium formate) and mobile phase B (2-propanol:acetonitrile, 1:1 v/v). The proportion of mobile phase B was programmed as follows: 20% (0–1 min), 40% (2 min), 92.5% (25–26 min), 100% (35 min), and then returned to 20% at 38 min. The column oven temperature was maintained at 45 °C throughout the analysis.

Data acquisition, peak integration, and lipid identification/quantification were carried out using LabSolutions software (version 5.99 SP2, Shimadzu Corporation). Multiple reaction monitoring (MRM) transitions were employed for targeted analysis, and peak area ratios were obtained by normalizing the peak area of each analyte to that of the internal standard (IS). For this purpose, 17:0–20:4 phosphatidylinositol (PI; Avanti Polar Lipids, Alabaster, AL, USA) was added to each sample at a final concentration of 0.38 μmol/L. Only phospholipid species with peak areas exceeding 5000 were included in the quantitative evaluation.

**Calculation of photothermal conversion efficiency (η)**

The photothermal conversion efficiency (η) of TLNPs-ICG was calculated from the photothermal heating/cooling profiles under 800 nm NIR irradiation. The efficiency was determined using the following equation:

$$\eta = \frac{hS(T_{max} - T_{sur}) - hS(T_{max,H_2O} - T_{sur})}{P(1 - 10^{-A_{800}})} \quad (1)$$

where $T_{max}$ is the maximum temperature of the sample solution, $T_{max,H_2O}$ is the maximum temperature of the solvent (water) under identical irradiation conditions, $T_{sur}$ is the ambient temperature, $P$ is the laser power, $A_{800}$ is the absorbance of the sample at 800 nm, $h$ is the heat transfer coefficient, and $S$ is the laser irradiation spot size.

**Determination of $hS$**

The parameter $hS$ was calculated as:

$$hS = \frac{m_i C_{p,i}}{\tau_s} \quad (2)$$

where $m_i$ and $C_{p,i}$ are the mass and specific heat capacity of the solvent (water), respectively, and $\tau_s$ is the system heat transfer time constant.

The time constant $\tau_s$ was obtained from the cooling stage using:

$$\tau_s = -\frac{dt}{d(ln\theta)} \quad (3)$$

where $\theta$ is the dimensionless driving-force temperature and $t$ is time. At each time point, $\theta_t$ was calculated by:

$$\theta_t = \frac{T_t - T_{sur}}{T_{max} - T_{sur}} \quad (4)$$

Thus, a plot of $\ln(\theta)$ versus time $t$ was constructed from the cooling data, and $\tau_s$ was derived from the slope of the linear regression.

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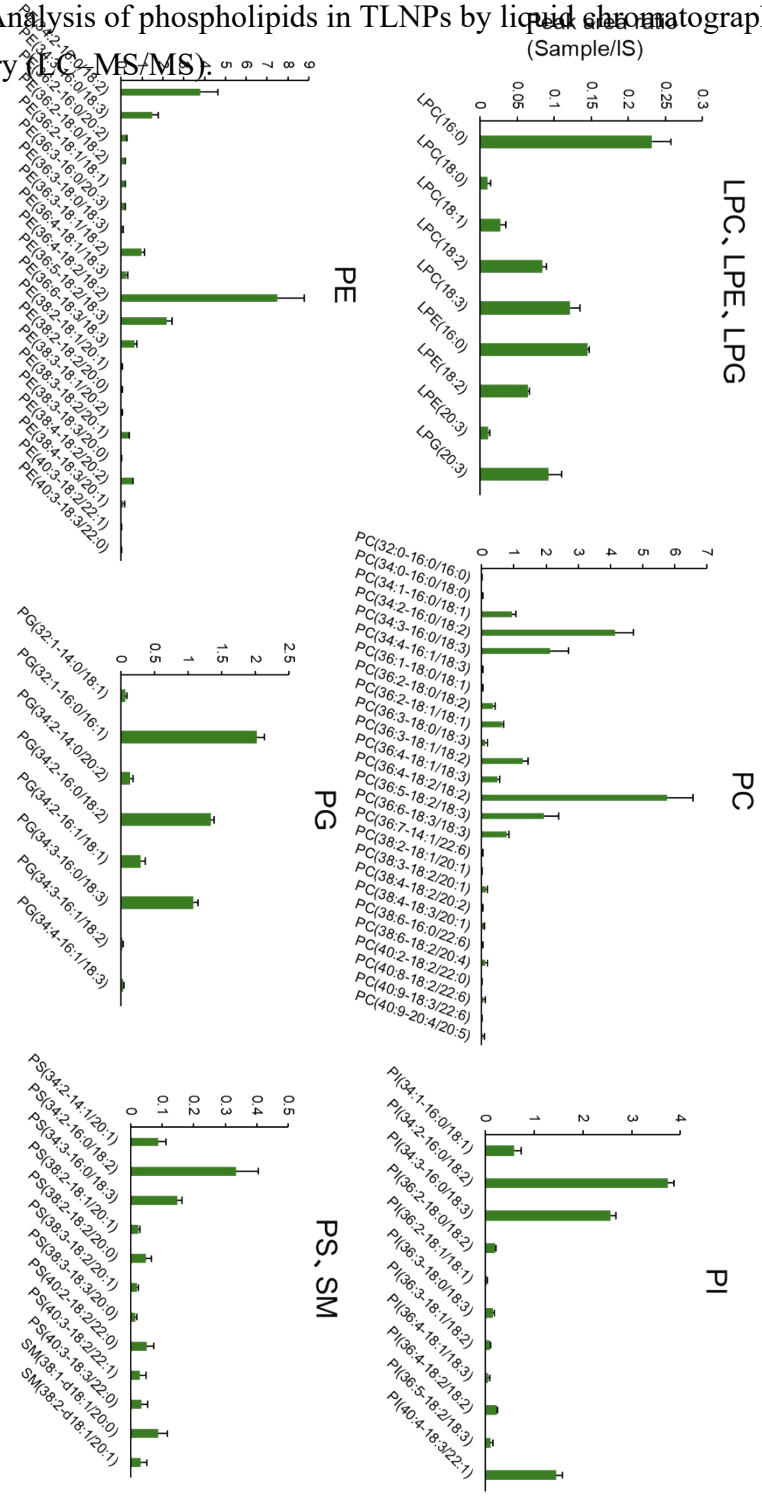
Table S1 Sequences of various primers used for HSP measurement

| | | | |
|---|---|---|---|
| A375 | GAPDH | Forward | 5'-GGGTGATGCTGGTGCTGAGTATGT-3' |
| | | Reverse | 5'-AAGAATGGGTGTTGCTGTTGAAGTC-3' |
| | HSP70 | Forward | 5'-TTTTGGTCCTAAGAATCGTTCA-3' |
| | | Reverse | 5'-ACACTTTCGGCTGTCTCCTTCA-3' |
| | HSP90 | Forward | 5'-TTTCAAACCGCAAACCGGATA-3' |
| | | Reverse | 5'-GACCTTAAGAATCTTTCATGG-3' |
| B16 melanoma | GAPDH | Forward | 5'-AACTTTGGCATTGTGGAAGG-3' |
| | | Reverse | 5'-ACACATTGGGGGTAGGAACA-3' |
| | HSP70 | Forward | 5'-TGGTGCTGACGAAGATGAAG-3' |
| | | Reverse | 5'-AGGTCGAAGATGAGCACGTT-3' |
| | HSP90 | Forward | 5'-AAAGGCAGAGGCTGACAAGA-3' |
| | | Reverse | 5'-AGGGGAGGCATTTCTTCAGT-3' |

Figure S1 Analysis of phospholipids in TLNPs by liquid chromatography-tandem mass spectrometry (LC-MS/MS).



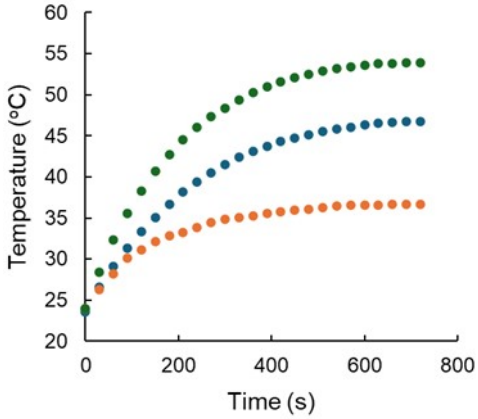



Figure S2 Photothermal effect of TLNPs-ICG at different irradiation intensities (Protein concentration: 50 μg $mL^{-1}$, wavelength 800 nm, irradiation time 12 min). Green plot: 2.0 W $cm^{-2}$. Blue plot: 1.0 W $cm^{-2}$. Orange plot: 0.5 W $cm^{-2}$.

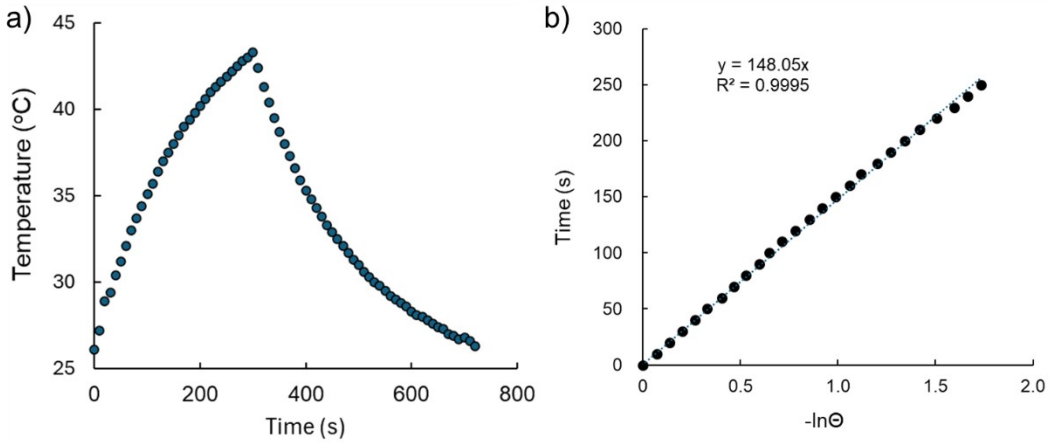



Figure S3 Calculation of photothermal conversion efficiency. a) Plot of the heating and cooling process due to the photothermal effect of TINPs-ICG (protein conc.: 50 μg $mL^{-1}$). b) Linear fitting curves were calculated for the system heat transfer time constant $\tau_s$.

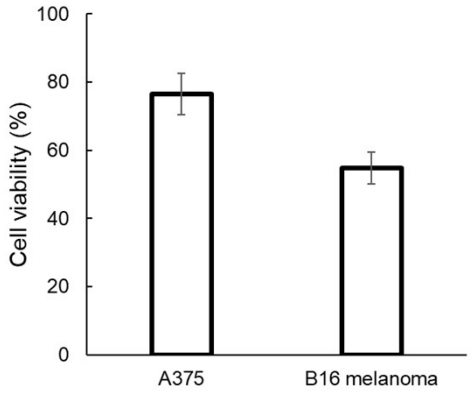



Figure S4 Cell viability in each cancer cell line after treatment with free EGCG at a dose equivalent to the EGCG content in TLNPs-ICG.

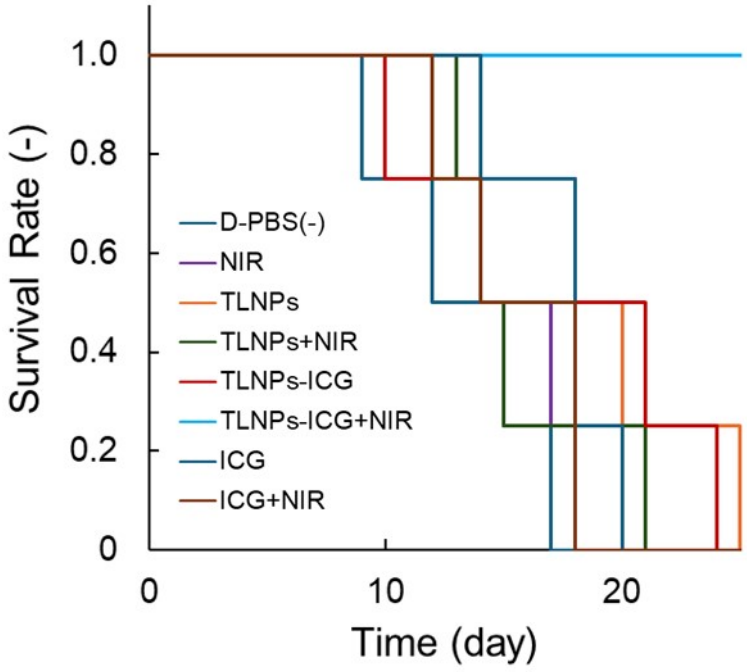



Figure S5 Survival curves of tumor bearing mice treated with D-PBS (−), NIR only, TLNPs, TLNPs + NIR, TLNPs–ICG, TLNPs–ICG + NIR, free ICG, or free ICG + NIR. Survival was monitored over time (days).