Electronic Supplementary Information

NIR luminogen for low-temperature photothermal therapy by triggering HSP90α down-regulation

Xinzhe Yang,[a,b+] *Keke Ding,*[f+] *Guiquan Zhang,*[a,b] *Pengbo Han,*[a,b] *Rong Hu,*[a,b,e*] *Zhiming Wang,*[a,b] *Anjun Qin,*[a,b*] *and Ben Zhong Tang*[b,c,d*]

[a] State Key Laboratory of Luminescent Materials and Devices, Guangdong Provincial Key Laboratory of Luminescence from Molecular Aggregates, South China. University of Technology, Guangzhou 510640, China.
E-mail: msqinaj@scut.edu.cn, hurong@usc.edu.cn.
[b] Center for Aggregation-Induced Emission, AIE Institute, South China University of Technology, Guangzhou 510640, China.
[c] School of Science and Engineering, Shenzhen Institute of Aggregate Science and Technology, The Chinese University of Hong Kong, Shenzhen, Guangdong 518172, China.
E-mail: tangbenz@cuhk.edu.cn.
[d] Hong Kong Branch of Chinese National Engineering Research Centre for Tissue Restoration and Reconstruction, The Hong Kong University of Science & Technology, Clear Water Bay, Kowloon, Hong Kong, China.
[e] School of Chemistry and Chemical Engineering, University of South China, Hengyang 421001, China.
[f] Department of Urology, The First Affiliated Hospital of Soochow University, NO. 188 Shizi Road, Suzhou 215006, China.
[+] These authors contributed equally to this work.

Experimental Section

Materials

Tetrakis(triphenylphosphine)palladium(0), (4-(bis(4-methoxyphenyl)amino)phenyl) boronic acid, 4,8-bis(5-bromo-4-(2-ethylhexyl)thiophen-2-yl)benzo[1,2-c:4,5-c']-bis[1,2,5]thiadiazole and potassium hexafluorophosphate were purchased from Soochiral Chemical Science & Technology Co., Ltd, Energy Chemical. Ltd. or Bide Pharmatech Ltd. and used as received. The Recombinant Lentivirus (HSP70 promoter-EGFP-mir155(HSP90α)-EF1A-orange, HSP70 promoter-EGFP-mir155(Control)-EF1A-orange) were constructed by Genechem Co., Ltd (Shanghai, China). All other reagents and solvents were purchased from commercial company with analytical grade and used without further purification. DMEM (Dulbecco's modified Eagle medium) and fetal bovine serum (FBS) were purchased from Termo Fisher Scientific (Shanghai, China). MCF-7 cells and NIH 3T3 cells were obtained from cell culture center of Institute of Basic Medical Sciences, Chinese Academy of Medical Science (Beijing, China). A549 cells were purchased from Shanghai Cell Bank of the Chinese Academy of Sciences (Shanghai, China). Female BALB/c Nude mice were purchased from Zhuhai BesTest Bio-Tech Co., Ltd (Zhuhai, China).

Instruments

^{1}H and ^{13}C NMR spectra were tested on a spectrometer (Bruker, AV500). High resolution mass spectra (HRMS) were recorded on MALDI-TOF mode (maXis). The UV-vis spectra and PL spectra were carried out on a UV-vis spectrometer (Shimadzu, UV-2600) and PL spectrofluorometer (Horiba, Fluoromax-4). PL quantum yields (PLQYs) were collected using a spectrometer equipped with a calibrated integrating sphere (Hamamatsu, C13534). The size distribution and zeta potential of nanoparticles were determined by a dynamic light scattering (DLS) instrument (Nano-ZSE, Malvern). The morphology of the nanoparticles was studied using transmission electron microscopy (TEM) (JEOL, JEM-1400, operating at 120 kV). Confocal laser scanning microscope (CLSM) characterization was used with a confocal laser

scanning biological microscope (LSM710, Zeiss, Germany). MTT analysis was recorded on a microplate reader (Thermofisher, USA) at a wavelength of 570 nm. Small animals' fluorescence imaging was carried out by AniView Phoenix (BLT, China). Temperature change of photo-thermal conversion behavior was recorded by a digital thermometer (CEM DT-8891E, CN) with an accuracy of ± 0.1 °C. Infrared (IR) thermal images and temperature changes were recorded using a thermal imaging camera (Ti480, Fluke). Fluorescence imaging of the mice tumors were using ChemiDoc MP imaging system (BioRad, USA).

Synthesis of compound TST

4,8-Bis(5-bromo-4-(2-ethylhexyl)thiophen-2-yl)benzo[1,2-c:4,5-c']bis[1,2,5]thiadiazole (1) (0.200 g, 0.26 mmol), (4-(bis(4-methoxyphenyl)amino)phenyl) boronic acid (2) (0.284 g, 0.81 mmol), potassium carbonate (0.168 g, 1.22 mmol), $Pd(PPh_3)_4$ (0.025 g, 0.022 mmol), tetrahydrofuran (40 mL) and deionized water (0.6 mL) were added in a two-neck flask. And then the mixture was degassed and stirred at 80 °C under nitrogen for 48 h. After cooling to room temperature, the reaction mixture was concentrated, the solid was dissolved in DCM and washed with water and brine. Then the organic layer was dried over anhydrous Na_2SO_4. The crude products were purified by column chromatography using DCM/PE (1:1, v/v) as eluent. Blackish green solid was obtained in 66% yield (0.212 g). ^{1}H NMR (500 MHz, THF-d_8) δ 8.85 (s, 1H), 7.30 (d, 2H), 6.99 (d, 4H), 6.85 (d, 2H), 6.80 – 6.75 (m, 4H), 3.67 (s, 6H), 2.70 (d, 2H), 2.34 (s, 2H), 1.22 – 1.12 (m, 8H), 0.76 (7H). ^{13}C NMR (126 MHz, THF-d_8) δ 156.68, 151.21, 148.70, 145.18, 140.52, 137.72, 135.91, 135.47, 129.56, 126.81, 126.36, 119.41, 114.58, 112.76, 54.72, 40.66, 32.76, 32.53, 29.74, 28.69, 25.79, 23.03, 13.58, 10.25. HRMS (MALDI-TOF) m/z $[M]^+$ calcd. for $C_{70}H_{72}N_6O_4S_4$, 1189.4498; found, 1189.4324.

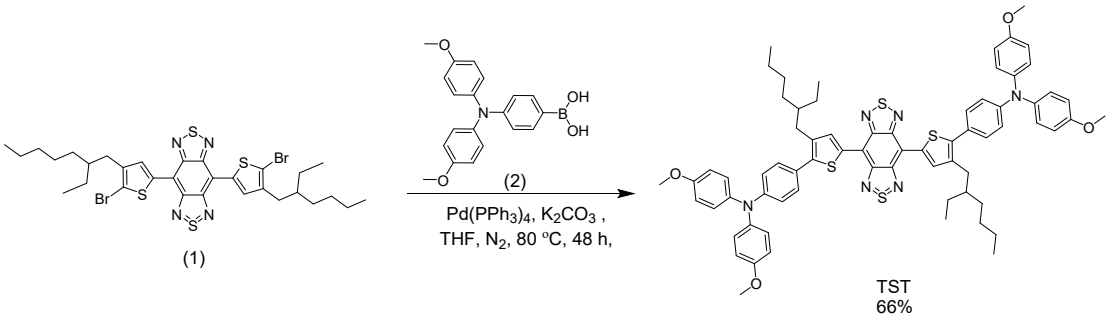


Photo-thermal property observation

The photo-thermal performance of TST NPs (29.7 μM) solutions were investigated by continuously exposing to 808 nm laser (1 W cm^{-2}, 10 min). The photo-thermal performance of TST NPs solution with varied concentration under different power of laser irradiation was measured by a digital thermometer and a thermal imaging camera. The photothermal conversion efficiency (PCE) (η) of TST NPs was calculated by Equations:

$h = (hS(T_{Max} - T_{surr}) - Q_s)/(I(1\text{-}10^{-A}))$

$hS = m_D C_D/\tau_s\tau_s = \tau/(\ln((T_{RT} - T_{surr})/(T_{Max} - T_{surr})))$

η represents the conversion efficiency value, T_{Max} and T_{surr} denote the maximum equilibrium and ambient temperature, respectively. T_{RT} means room temperature. Q_s is the light absorbance of PBS, I is the laser energy (1 W cm^{-2}) for the 808 nm laser, and A is the absorbance value of TST NPs at 808 nm. τ_s is the system time constant of the sample. m_D and C_D index the solution mass (0.1 g) and heat capacity (4.2 J g^{-1}) of PBS, respectively.

Theoretical calculation

The molecular geometries of TST at ground state were optimized using the density functional theory (DFT) method at the B3LYP/6-311g (d, p) level in the Gaussian 16 program.

Cell culture

MCF-7 cells, A549 cells and NIH 3T3 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM). All the cells were finally cultured with 10% FBS and 1%

penicillin streptomycin in a culture flask at 37 °C in a humidified atmosphere containing 5% CO_2.

Cells incubation with recombinant lentivirus

Cells were seeded into cell culture dishes at a density of 1.0×10^6 cells/mL. After the cells adhered to the wall overnight, a solution of recombinant lentivirus at a density of 1.0×10^7 TU mL^{-1} mixed with transfection HitransG A was added. After incubating with the recombinant lentivirus for 24 h, the cells were further incubated with fresh culture medium for 48 h. Then puromycin was added with the final concentration of 2 μg mL^{-1}. The cells were further cultured for 2 days and the growth process was observed.

Thermal-responsive gene activation

Temperature optimization: Cells transfected with recombinant lentivirus (MCF-7-C, MCF-7-H, A549-C and A549-H) were seeded into 6-well chamber slides at a density of 2×10^5 cells mL^{-1}. 24 h later, the cells were collected in tubes, followed with incubation at 42 °C and 45 °C for 5, 10 and 20 min, respectively. Cells incubated at 37 °C were set as control groups. After high temperature treatment, the cells were further incubated in 6-well chamber slides for 48 h under 37 °C in a humidified atmosphere containing 5% CO_2. Finally, these cells were observed under fluorescence microscope to evaluate the thermal-responsive gene activation behavior.

Photo-thermal responsibility evaluation: Cells transfected with recombinant lentivirus (MCF-7-C, MCF-7-H, A549-C and A549-H) were seeded into 6-well chamber slides at a density of 2×10^5 cells mL^{-1}. After 24 h incubation, the cells were incubated with fresh culture medium containing TST NPs (2.9 μM) for 8 h. Then the cells were further irradiated with or without 808 laser (1 W cm^{-2}) for 10 min. And the cells without light irradiation was set as the control group, followed with further incubation for 48 h under 37 °C in a humidified atmosphere containing 5% CO_2. Finally, these cells were observed under fluorescence microscope to evaluate the thermal-responsive gene activation behavior.

Cytotoxicity assay

The cytotoxic potential of TST NPs towards cells was assessed using the MTT assay. MCF-7 cells (MCF-7-C and MCF-7-H), A549 cells (A549-C and A549-H) and NIH 3T3 cells were respectively treated with the different concentrations of TST NPs (0 and 2.9 μM) for 48 h in 96-well plate with 4×10^3 cells/well. After incubation for 8 h, the cells were irradiated with or without 808 laser (1 W cm^{-2}) for 10 min, respectively. Then cells were further treated with the different concentration of TST NPs (0, 0.3, 0.5, 0.7, 1.5, 2.1 and 2.9 μM) in 96-well plate. After incubated with TST NPs for additional 8 h, the cells were also irradiated with or without 808 laser (1 W cm^{-2}) for 10 min, respectively. And the incubated cells were further cultured for 24 h under the standard cell culture conditions. The absorbance of MTT at 570 nm was recorded by the microplate reader.

Western blotting analysis

The related protein expression of cells and tissues were analyzed though western blotting according the literature. MCF-7 cells (MCF-7-C and MCF-7-H), A549 cells (A549-C and A549-H) cells were incubated with TST NPs for 8 h. Then the cells were irradiated with or without 808 laser (1 W cm^{-2}) for 10 min, respectively. The treated cells were further cultured for 48 h under the standard cell culture conditions. Cell total proteins were extracted and collected for western blot assay. Briefly, cells were collected and lysed in RIPA lysis buffer (Servicebio) supplemented with a protease inhibitor cocktail (Servicebio) according to the protocol by the manufacturer. Total proteins were extracted and loaded in the wells of 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). After the separation by gel electrophoresis, the proteins were transferred to PVDF membranes (Servicebio). The membrane was labeled with primary antibodies after blocking with 5% non-fat milk (Servicebio) overnight at 4 °C. Primary antibodies include anti-HSP90 (1:1000, abcam) and anti-GAPDH (1:2000, Servicebio). After washing with TBST, membranes were incubated with corresponding HRP-conjugated secondary antibody for 1 h at room temperature. The membrane was washed 3 times by TBST and

illuminated by an enhanced chemiluminescent (ECL) kit (Servicebio).

BALB/c Nude mouse model and in vivo imaging

Female BALB/c Nude mice (6-8 weeks old) were purchased from Zhuhai BesTest Bio-Tech Co., Ltd. All animal experiments were conducted in strict observance of the guide for the care and use of laboratory animals by the Institutional Animal Care and Use Committee (IACUC) of the South China Agricultural University (2022-D080). MCF-7-H cells (3×10^6) were injected into the right back of mice. The tumor sizes were measured by a caliper every other day, and the tumor volume was calculated as (tumor length) × (tumor width)^2/2. Relative tumor volumes were calculated as V/V_0 (V_0 was the tumor volume when the treatment was initiated. The TST NPs were administered intravenously via intravenous injection into each BALB/c Nude mouse. Mice imaging was carried out using an in vivo imaging instruments spectrum system using the excitation wavelength of 808 nm and fluorescence emission signal wavelength of NIR-Ⅱ within a certain time frame. Mice were sacrificed for western blotting analysis and imaging of tumor tissue.

In vivo biosafety assessment

To estimate the functional biomarkers level of kidney and liver, tumor-bearing mice with different treatments treated with and without TST NPs were sacrificed, and the blood of heart was collected for further analysis. Healthy mice were used as negative controls. At the end of the treatment period (18 days), blood was collected and analyzed for liver function markers (ALT, AST and ALB) and renal function (UREA and CREA). The results were compared with blood from healthy mice (n = 5). In addition, the organs (heart, liver, spleen, lung, and kidney) of mice with different treatments were also collected for the hematoxylin and eosin (H&E) staining. The tumor tissues were analyzed for H&E, Ki67 and TUNEL staining.

Figures and tables

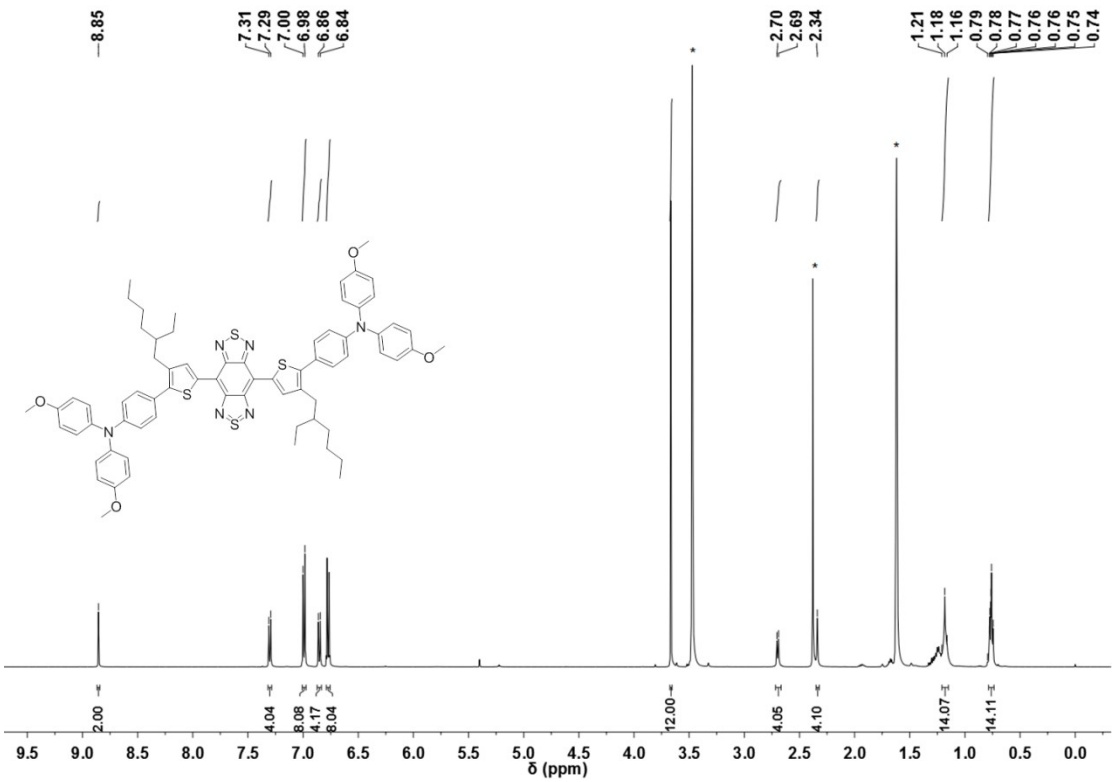


Fig. S1 ^{1}H NMR spectrum of TST in THF-d_8.

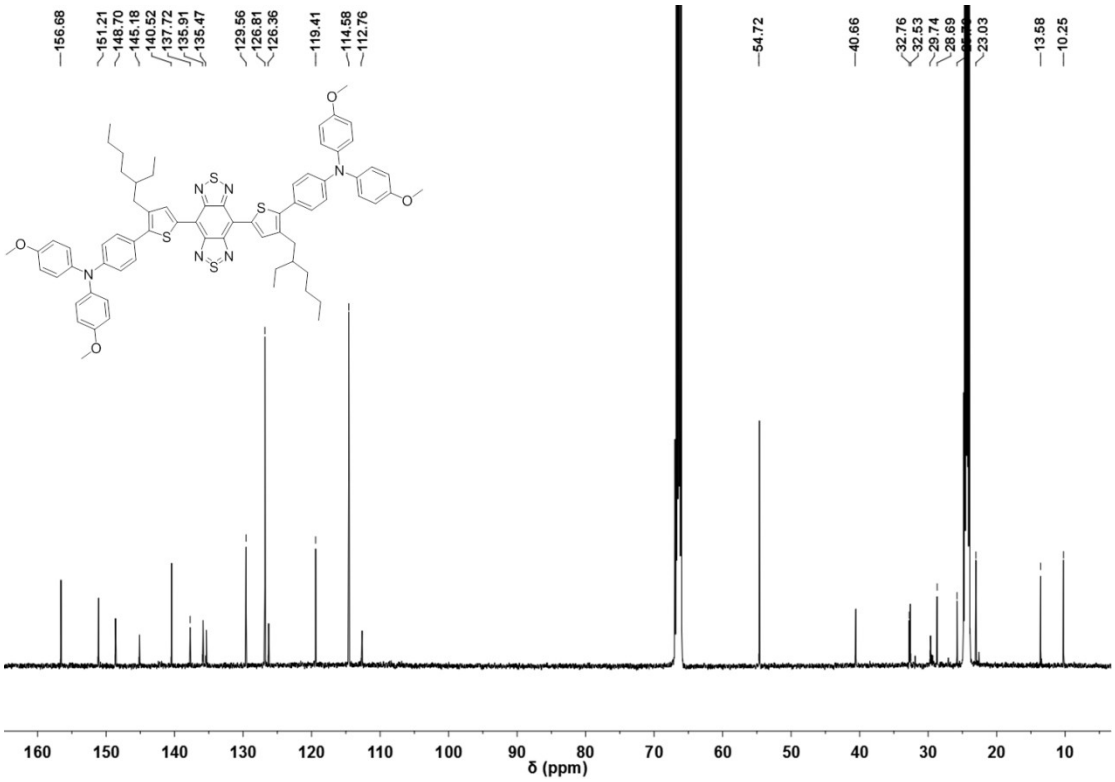


Fig. S2 ^{13}C NMR spectrum of TST in THF-d_8.

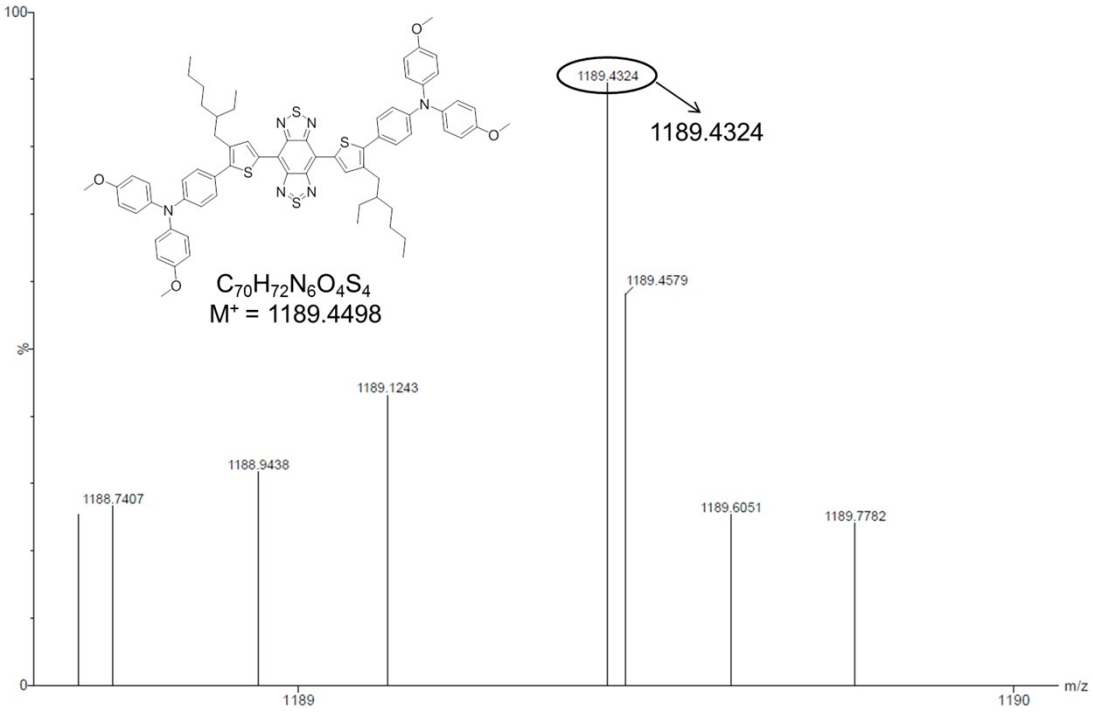


Fig. S3 MALDI-TOF spectrum of TST.

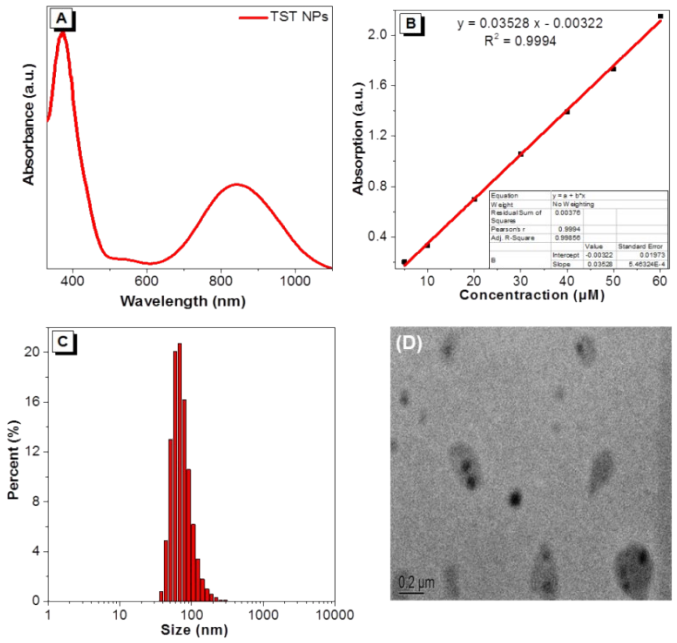


Fig. S4 (A) UV-visible absorption spectrum of TST NPs (10 μM) in water. (B) Absorbance of TST NPs with different concentration at 850 nm. (C) Size distribution of TST NPs in water measured by DLS. (D) TEM images of TST NPs.

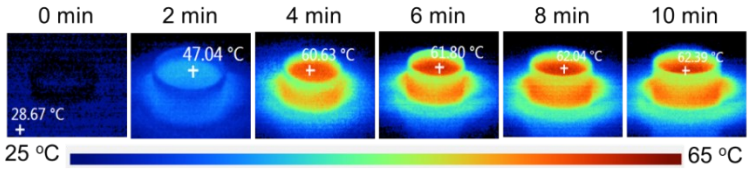


Fig. S5 Near-infrared thermal images of TST NPs (29.7 μM) in water under 808 nm laser irradiation (1 W cm^{-2}) for 10 min.

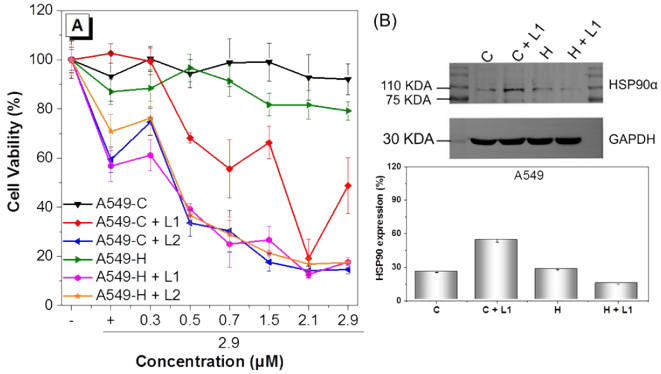


Fig. S6 (A) Cell viability of A549-C and A549-H cells treated with TST NPs with once and twice light irradiation. (B) Western blot analysis of the HSP90α protein

expression in A549-C and A549-H cells treated by 2.9 μM of TST NPs with or without irradiation of 808 nm laser (808 nm laser irradiation, 1 W cm^{-2} for 10 min)

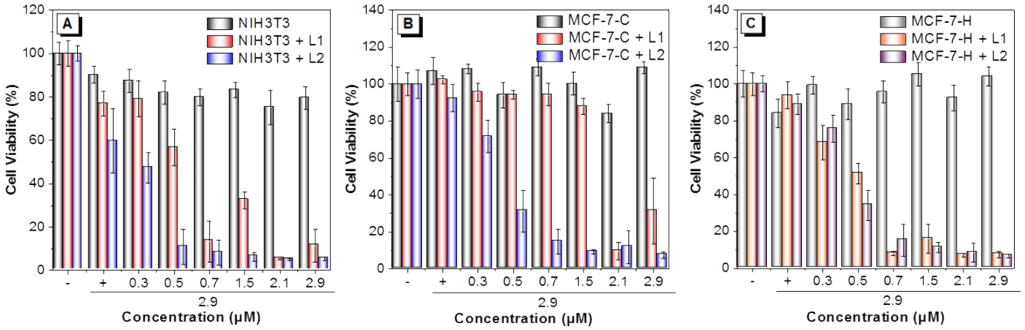


Fig. S7 Cell viability of NIH 3T3 (A), MCF-7-C (B) and MCF-7-H (C) cells treated with TST NPs with once and twice light irradiation (808 nm laser irradiation, 1 W cm^{-2} for 10 min).

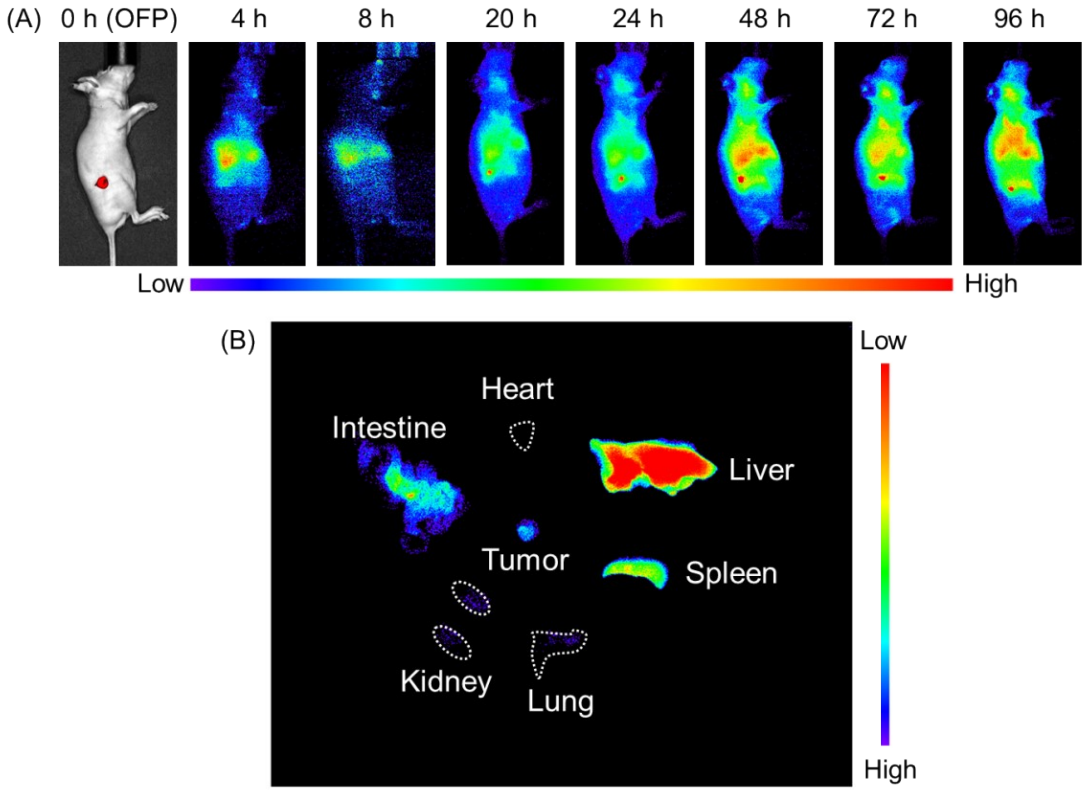


Fig. S8 (A) *In vivo* NIR FLI of MCF-7-H tumor-bearing mice at different time points post i.v. injection of TST NPs. (B) *Ex vivo* NIR FLI of major organs and tumors excised from mice after i.v. injection with for 96 h. (Excitation: 808 nm; [TST

NPs]=232 μM).

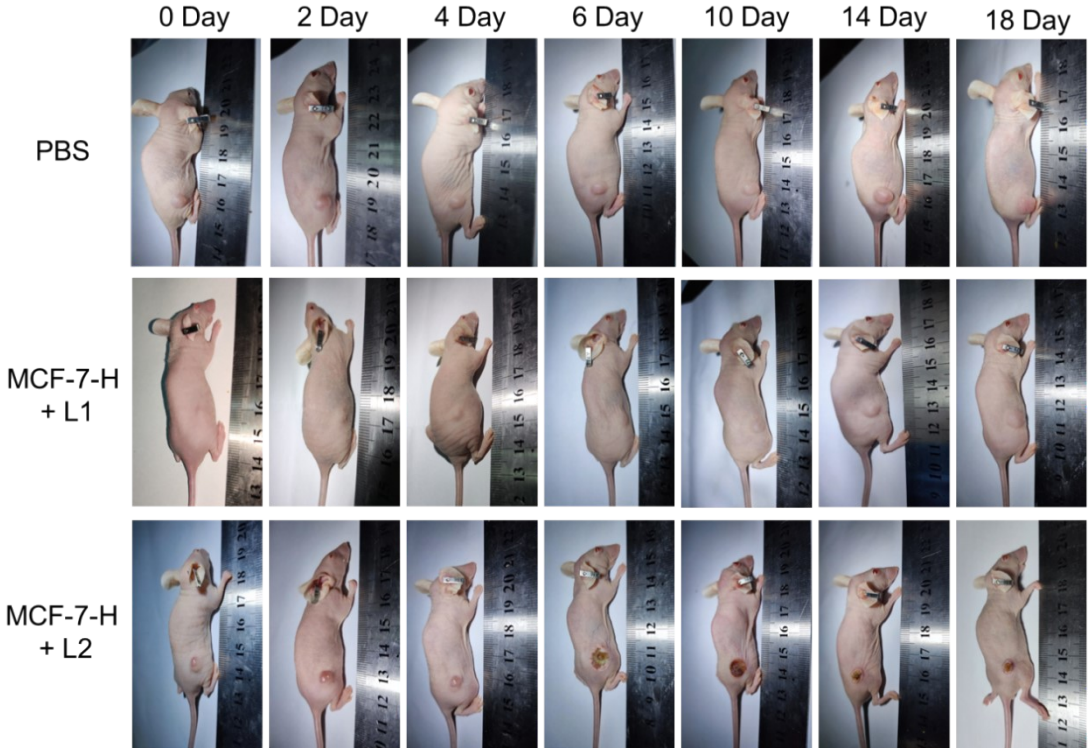


Fig. S9 Representative photos of MCF-7-H tumor-bearing mice with different treatments during 18-days.

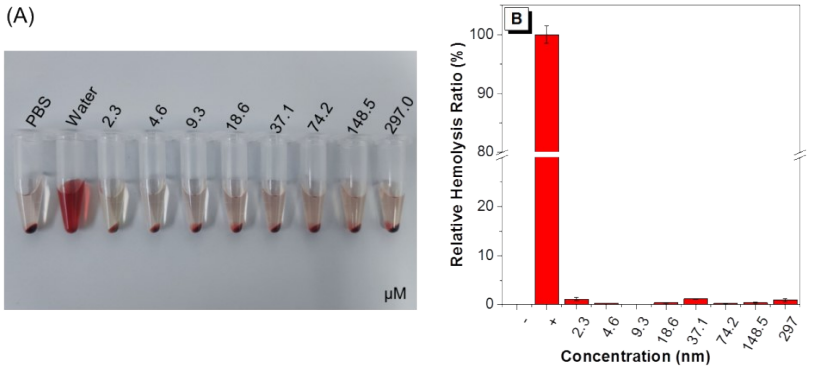


Fig. S10 (A) Photographs of the hemolysis experiment (annotated with the concentration of TST NPs) and (B) hemolysis percentage of mouse red blood cells based on the data in Figure A.

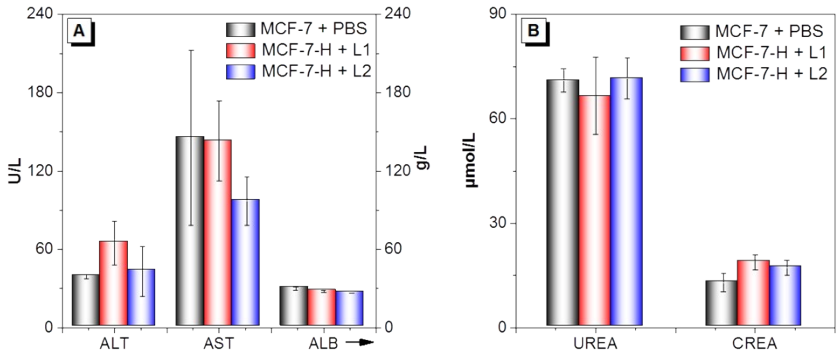


Fig. S11 (A) Levels of ALT, AST and ALB, and (B) UREA and CREA in blood samples from mice with different treatments.

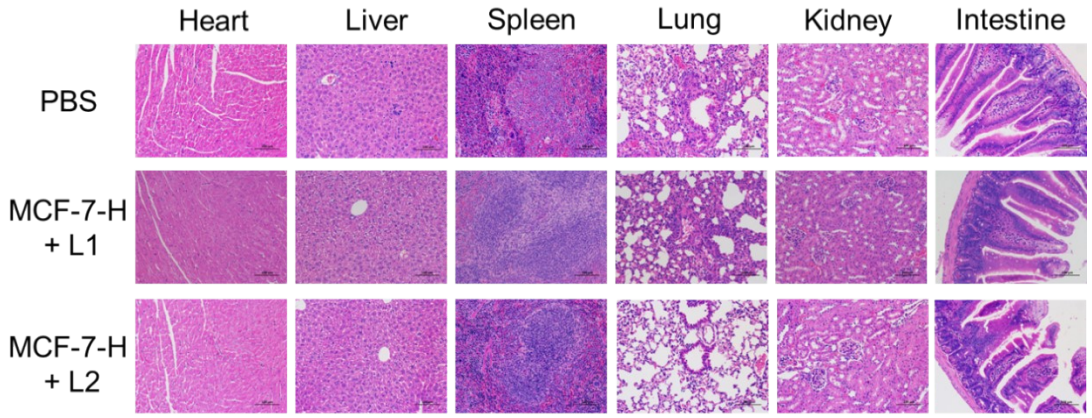


Fig. S12 Histological photomicrographs of organs (intestine, lung, liver, spleen, kidney and heart) after completion of the *in vivo* anti-tumor experiment. Scale bar: 200 µm.

Table S1. CLSM images of different cells after transfection of the designed exogenous gene for 48 h. (scale bar, 50 μm)

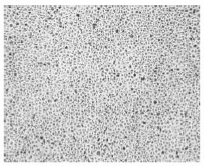
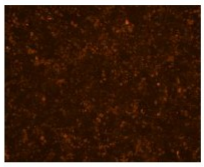
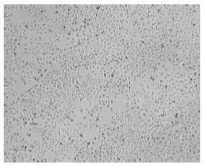
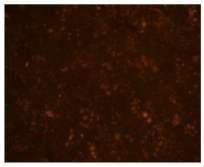
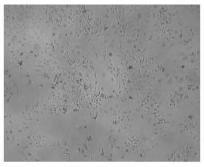
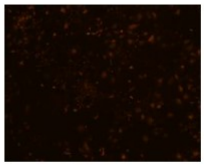
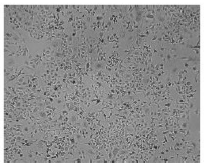
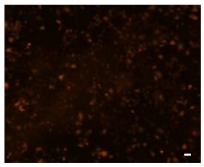
	Bright field	Fluorescent channel
MCF-7-H		
MCF-7-C		
A549-H		
A549-C		

Table S2. CLSM images of two types of MCF-7 cells after incubation with different temperature and incubation time for 48 h. (scale bar, 50 μm)

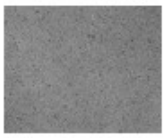
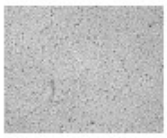
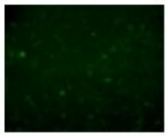
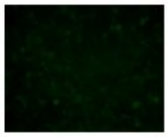
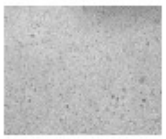
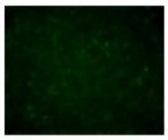
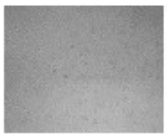
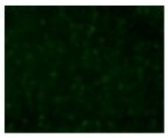
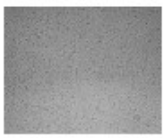
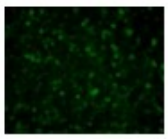
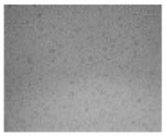
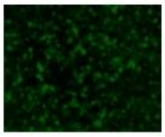
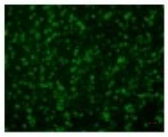
	Heating			
	MCF-7-H		MCF-7-C	
	Bright field	Fluorescent channel	Bright field	Fluorescent channel
37 °C				
42 °C 5 min				
42 °C 10 min				
42 °C 20 min				
45 °C 5 min				
45 °C 10 min				
45 °C 20 min				

Table S3. CLSM images of two types of A549 cells after incubation with different temperature and incubation time for 48 h. (scale bar, 50 μm)

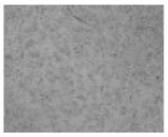
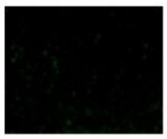
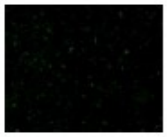
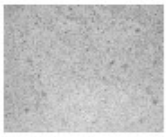
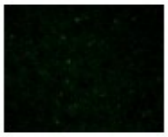
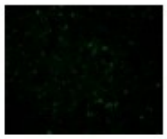
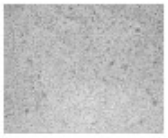
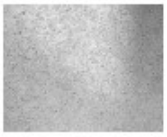
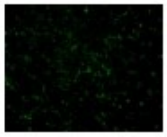
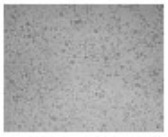
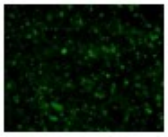
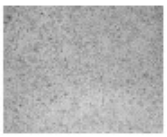
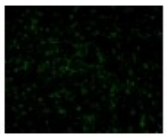
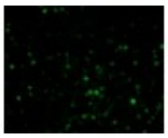
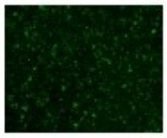
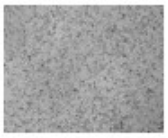
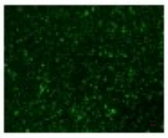
	Heating			
	A549-H		A549-C	
	Bright field	Fluorescent channel	Bright field	Fluorescent channel
37 °C				
42 °C 5 min				
42 °C 10 min				
42 °C 20 min				
45 °C 5 min				
45 °C 10 min				
45 °C 20 min				

Table S4. CLSM images of MCF-7-H and A549-H cells after the incubation with 2.9 μM of TST NPs with and without 808 nm laser irradiation. (scale bar, 50 μm)

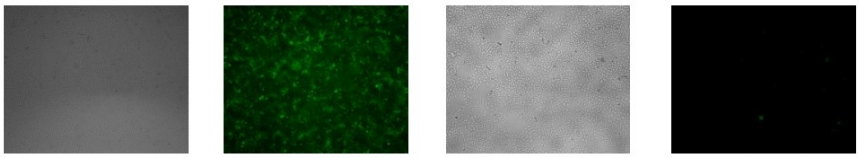
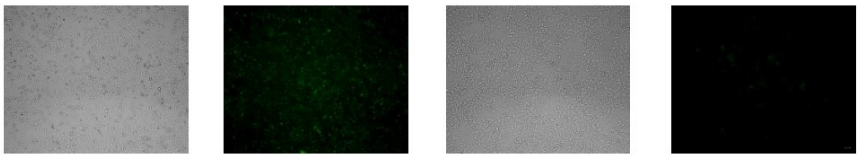
PTT trigger at 2.9 μM TST NPs	808 Laser		Dark	
	Bright field	Fluorescent channel	Bright field	Fluorescent channel
MCF-7-H				
A549-H				

Table S5. CLSM images of MCF-7-C and A549-C cells after the incubation with 2.9 μM of TST NPs with and without 808 nm laser irradiation. (scale bar, 50 μm)

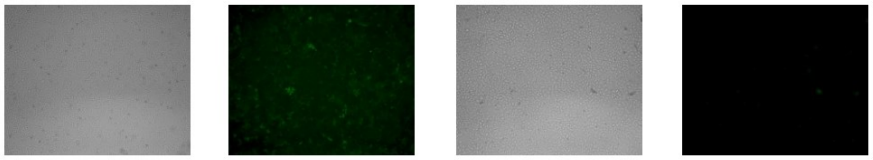
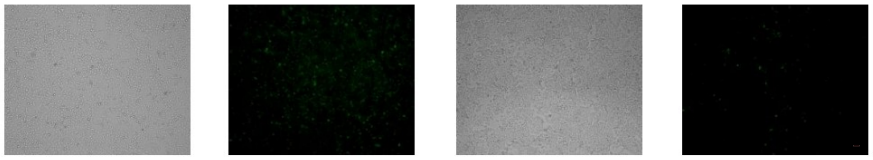
PTT trigger at 2.9 μM TST NPs	808 Laser		Dark	
	Bright field	Fluorescent channel	Bright field	Fluorescent channel
MCF-7-C				
A549-C				

Table S6. Exogenous gene sequences used in this study.

HSP90α knockdown groups

HSP70 promoter-EGFP-mir155(HSP90α)-EF1A-orange

CCTGCAGGGCCGCCCACTCCCCCTTCCTCTCAGGGTCCCTGTCCCCTCCAGTGAATCCCAGAAGACTC
TGGAGAGTTCTGAGCAGGGGGCGGCACTCTGGCCTCTGATTGGTCCAAGGAAGGCTGGGGGGCAGG
ACGGGAGGCGAAAACCCTGGAATATTCCCGACCTGGCAGCCTCATCGAGCTCGGTGATTGGCTCAGA
AGGGAAAAGGCGGGTCTCCGTGACGACTTATAAAAGCCCAGGGGCAAGCGGTCCGGATAACGGCTA
GCCTGAGGAGCTGCTGCGACAGTCCACTACCTTTTTCGAGAGTGACTCCCGTTGTCCCAAGGCTTCCC
AGAGCGAACCTGTGCGGCTGCAGGCACCGGCGCGTCGAGTTTCCGGCGTCCGGAAGGACCGAGCTCT
TCTCGCGGATCCAGTGTTCCGTTTCCAGCCCCCAATCTCAGAGCGGAGCCGACAGAGAGCAGGGAAC
CCCTCTAGACTCGAGCGGCCGCATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATC
CTGGTCGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGAT
GCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCA

CCCTCGTGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCCGCTACCCCGACCACATGAAGCAGCA
CGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGAC
GGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTG
AAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGC
CACAACGTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTGAACTTCAAGATCCGCCAC
AACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCCATCGGCGACGGC
CCCGTGCTGCTGCCCGACAACCACTACCTGAGCACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGA
AGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCT
GTACAAGTAACTGGAGGCTTGCTGAAGGCTGTATGCTGGAATGTTTCTTCACAATCTCCGTTTTGGCC
ACTGACTGACGGAGATTGAAGAAACATTCCAGGACACAAGGCCTGTTACTAGCACTCACATGGAAC
AAATGGCCCCTCGAGTCCATCGATACTAGTAAGGATCTGCGATCGCTCCGGTGCCCGTCAGTGGGCA
GAGCGCACATCGCCCACAGTCCCCGAGAAGTTGGGGGGAGGGGTCGGCAATTGAACGGGTGCCTAG
AGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTG
GGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAG
AACACAGCTGAAGCTTCGAGGGGCTCGCATCTCTCCTTCACGCGCCCGCCGCCCTACCTGAGGCCGC
CATCCACGCCGGTTGAGTCGCGTTCTGCCGCCTCCCGCCTGTGGTGCCTCCTGAACTGCGTCCGCCGT
CTAGGTAAGTTTAAAGCTCAGGTCGAGACCGGGCCTTTGTCCGGCGCTCCCTTGGAGCCTACCTAGA
CTCAGCCGGCTCTCCACGCTTTGCCTGACCCTGCTTGCTCAACTCTACGTCTTTGTTTCGTTTTCTGTT
CTGCGCCGTTACAGATCCAAGCTGTGACCGGCGCCTACTCTAGCCGCCACCATGGTGAGCAAGGGCG
AGGAGAATAACATGGCCATCATCAAGGAGTTCATGCGCTTCAAGGTGCGCATGGAGGGCTCCGTGA
ACGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCCGCCCCTACGAGGGCTTTCAGACCGCTA
AGCTGAAGGTGACCAAGGGTGGCCCCCTGCCCTTCGCCTGGGACATCCTGTCCCCTCAGTTCACCTAC
GGCTCCAAGGCCTACGTGAAGCACCCCGCCGACATCCCCGACTACTTCAAGCTGTCCTTCCCCGAGG
GCTTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGGCGGCGTGGTGACCGTGACCCAGGACTCCTC
CCTGCAGGACGGCGAGTTCATCTACAAGGTGAAGCTGCGCGGCACCAACTTCCCCTCCGACGGCCCC
GTAATGCAGAAGAAGACCATGGGCTGGGAGGCCTCCTCCGAGCGGATGTACCCCGAGGACGGCGCC
CTGAAGGGCGAGATCAAGATGAGGCTGAAGCTGAAGGACGGCGGCCACTACACCTCCGAGGTCAAG
ACCACCTACAAGGCCAAGAAGCCCGTGCAGCTGCCCGGCGCCTACATCGTCGGCATCAAGTTGGACA
TCACCTCCCACAACGAGGACTACACCATCGTGGAACAGTACGAACGCGCCGAGGGCCGCCACTCCAC
CGGCGGCATGGACGAGCTGTACAAGTAA

Control groups without HSP90α resistance gene transfection

HSP70 promoter-EGFP-mir155(Control)-EF1A-orange

CCTGCAGGGCCGCCCACTCCCCCTTCCTCTCAGGGTCCCTGTCCCCTCCAGTGAATCCCAGAAGACTC
TGGAGAGTTCTGAGCAGGGGGCGGCACTCTGGCCTCTGATTGGTCCAAGGAAGGCTGGGGGGCAGG
ACGGGAGGCGAAAACCCTGGAATATTCCCGACCTGGCAGCCTCATCGAGCTCGGTGATTGGCTCAGA
AGGGAAAAGGCGGGTCTCCGTGACGACTTATAAAAGCCCAGGGGCAAGCGGTCCGGATAACGGCTA
GCCTGAGGAGCTGCTGCGACAGTCCACTACCTTTTTCGAGAGTGACTCCCGTTGTCCCAAGGCTTCCC
AGAGCGAACCTGTGCGGCTGCAGGCACCGGCGCGTCGAGTTTCCGGCGTCCGGAAGGACCGAGCTCT
TCTCGCGGATCCAGTGTTCCGTTTCCAGCCCCCAATCTCAGAGCGGAGCCGACAGAGAGCAGGGAAC
CCCTCTAGACTCGAGCGGCCGCATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATC
CTGGTCGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGAT
GCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCA
CCCTCGTGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCCGCTACCCCGACCACATGAAGCAGCA

CGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGAC
GGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTG
AAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGC
CACAACGTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTGAACTTCAAGATCCGCCAC
AACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCCATCGGCGACGGC
CCCGTGCTGCTGCCCGACAACCACTACCTGAGCACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGA
AGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCT
GTACAAGTAACTGGAGGCTTGCTGAAGGCTGTATGCTGACGTGACACGTTCGGAGAAGTTTTGGCCA
CTGACTGACTTCTCCGAGTGTCACGTCAGGACACAAGGCCTGTTACTAGCACTCACATGGAACAAAT
GGCCCCTCGAGTCCATCGATACTAGTAAGGATCTGCGATCGCTCCGGTGCCCGTCAGTGGGCAGAGC
GCACATCGCCCACAGTCCCCGAGAAGTTGGGGGGAGGGGTCGGCAATTGAACGGGTGCCTAGAGAA
GGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGGG
AGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACA
CAGCTGAAGCTTCGAGGGGCTCGCATCTCTCCTTCACGCGCCCGCCGCCCTACCTGAGGCCGCCATCC
ACGCCGGTTGAGTCGCGTTCTGCCGCCTCCCGCCTGTGGTGCCTCCTGAACTGCGTCCGCCGTCTAGG
TAAGTTTAAAGCTCAGGTCGAGACCGGGCCTTTGTCCGGCGCTCCCTTGGAGCCTACCTAGACTCAG
CCGGCTCTCCACGCTTTGCCTGACCCTGCTTGCTCAACTCTACGTCTTTGTTTCGTTTTCTGTTCTGCG
CCGTTACAGATCCAAGCTGTGACCGGCGCCTACTCTAGCCGCCACCATGGTGAGCAAGGGCGAGGAG
AATAACATGGCCATCATCAAGGAGTTCATGCGCTTCAAGGTGCGCATGGAGGGCTCCGTGAACGGCC
ACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCCGCCCCTACGAGGGCTTTCAGACCGCTAAGCTGA
AGGTGACCAAGGGTGGCCCCCTGCCCTTCGCCTGGGACATCCTGTCCCCTCAGTTCACCTACGGCTCC
AAGGCCTACGTGAAGCACCCCGCCGACATCCCCGACTACTTCAAGCTGTCCTTCCCCGAGGGCTTCA
AGTGGGAGCGCGTGATGAACTTCGAGGACGGCGGCGTGGTGACCGTGACCCAGGACTCCTCCCTGCA
GGACGGCGAGTTCATCTACAAGGTGAAGCTGCGCGGCACCAACTTCCCCTCCGACGGCCCCGTAATG
CAGAAGAAGACCATGGGCTGGGAGGCCTCCTCCGAGCGGATGTACCCCGAGGACGGCGCCCTGAAG
GGCGAGATCAAGATGAGGCTGAAGCTGAAGGACGGCGGCCACTACACCTCCGAGGTCAAGACCACC
TACAAGGCCAAGAAGCCCGTGCAGCTGCCCGGCGCCTACATCGTCGGCATCAAGTTGGACATCACCT
CCCACAACGAGGACTACACCATCGTGGAACAGTACGAACGCGCCGAGGGCCGCCACTCCACCGGCG
GCATGGACGAGCTGTACAAGTAA