Supporting information of

Poly(ester-thioether) microspheres co-loaded with erlotinib and α-tocopheryl succinate for combinational therapy of non-small cell lung cancer

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Materials. Poly(vinyl alcohol) 1788 (PVA), D-α-tocopherol succinate (α-TOS) and erlotinib were purchased from Aladdin (China). Curcumin was purchased from Heowns (China). PHBD, PHBDT, and PHBDT-*g*-C3 were synthesized according to our previous study[1] and the synthetic routes were shown in Scheme S1. Actin-Tracker Green, reactive oxygen species assay kit, Annexin V-FITC apoptosis detection kit, mitochondrial membrane potential assay kit with JC-1, and ATP assay kit were purchased from Beyotime Biotechnology. Hoechst 33342 was purchased from Shanghai Yuanye Bio-Technology Co., Ltd (China). A calcein-AM/PI double staining kit was purchased from Dojindo Laboratories. PI/RNase staining buffer was purchased from BD Pharmingen. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Energy Chemical Co. (China). Dulbecco's modified Eagle's medium (DMEM), Roswell Park Memorial Institute (RPMI) 1640 medium, 100 U mycillin, and fetal bovine serum (FBS) were purchased from HyClone Inc. All other chemical reagents were purchased from Chengdu Kelong Chemical Co. (China) and purified before use.

Characterizations. Gel permeation chromatography (GPC) was performed on Waters 1515 equipped with a refractive index detector (Waters 2414), with THF as eluent at a flow rate of 1 mL/min at 35 °C and polystyrene as standards. The thermal properties of the poly(ester-thioether)s and drug-loaded microspheres were measured by differential scanning calorimetry (DSC, TA Instruments Q2000); all the experiments were carried out under nitrogen atmosphere at a flow rate of 50 mL min^{-1} and a cooling rate of 10 °C min^{-1}. Scanning electron microscopy (SEM, S-4800, HITACHI) was used to observe the morphology of microspheres.

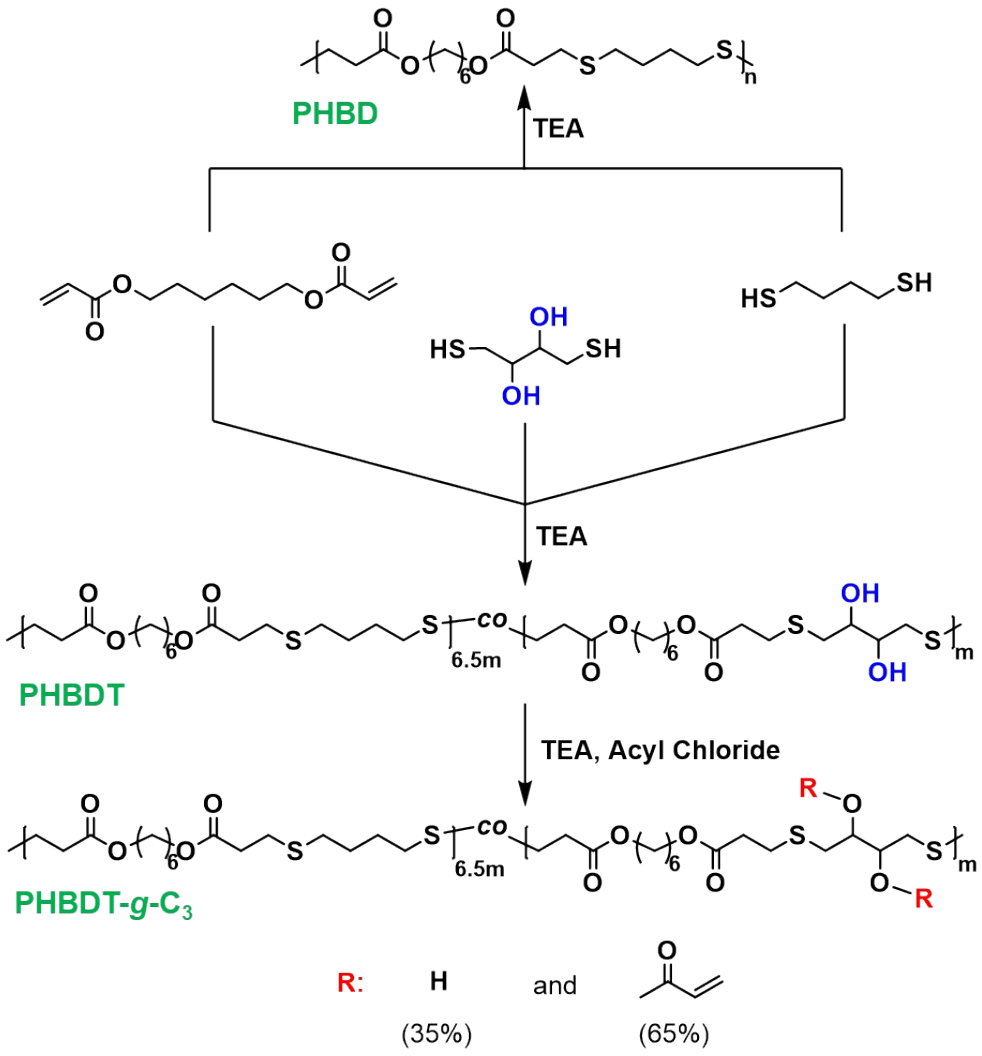
***In vitro* microsphere degradation study.** The degradation behaviors of blank microspheres (NPM and PM) were evaluated by *in vitro* hydrolysis in acidic condition. To a solution of microspheres

(80 mg mL^{-1}) in acetate buffer solution (pH 5.0, 0.1 M, 7 mL) was placed in a shaking bed (37 °C, 120 rpm). After incubated for 6 days, the mixture was withdrawn and freeze dried, and the molecular weights of the obtained samples were characterized by GPC.

Cell Culture. NIH/3T3 (mouse embryonic fibroblast) and A549 (human non-small cell lung cancer) cells were obtained from Cell Bank of the Chinese Academy of Sciences (Shanghai, China). NIH/3T3 and A549 cells were respectively cultured in DMEM and RPMI 1640 medium with 10% FBS and 1% penicillin-streptomycin solution in a humidified atmosphere containing 5% CO_2 at 37 °C.

References

1. F. Cheng, T. Su, Y. Pu, W. Gao and B. He, *Macromol. Biosci.*, 2019, 1900171.



Scheme S1. Synthetic illustration of PHBD, PHBDT, and PHBDT-*g*-C_3.

Table S1. Molecular and thermal properties of poly(ester-thioether)s.

Samples	M_n (kDa) [a]	PDI [a]	T_m (^oC) [b]	ΔH (J g^{-1}) [b]
PHBD	13.0	1.90	54.4	50.9
PHBDT	19.9	1.87	49.6	45.2
PHBDT-*g*-C_3	18.0	1.96	41.9	31.4

[a] Determined by GPC; [b] Tested by DSC.

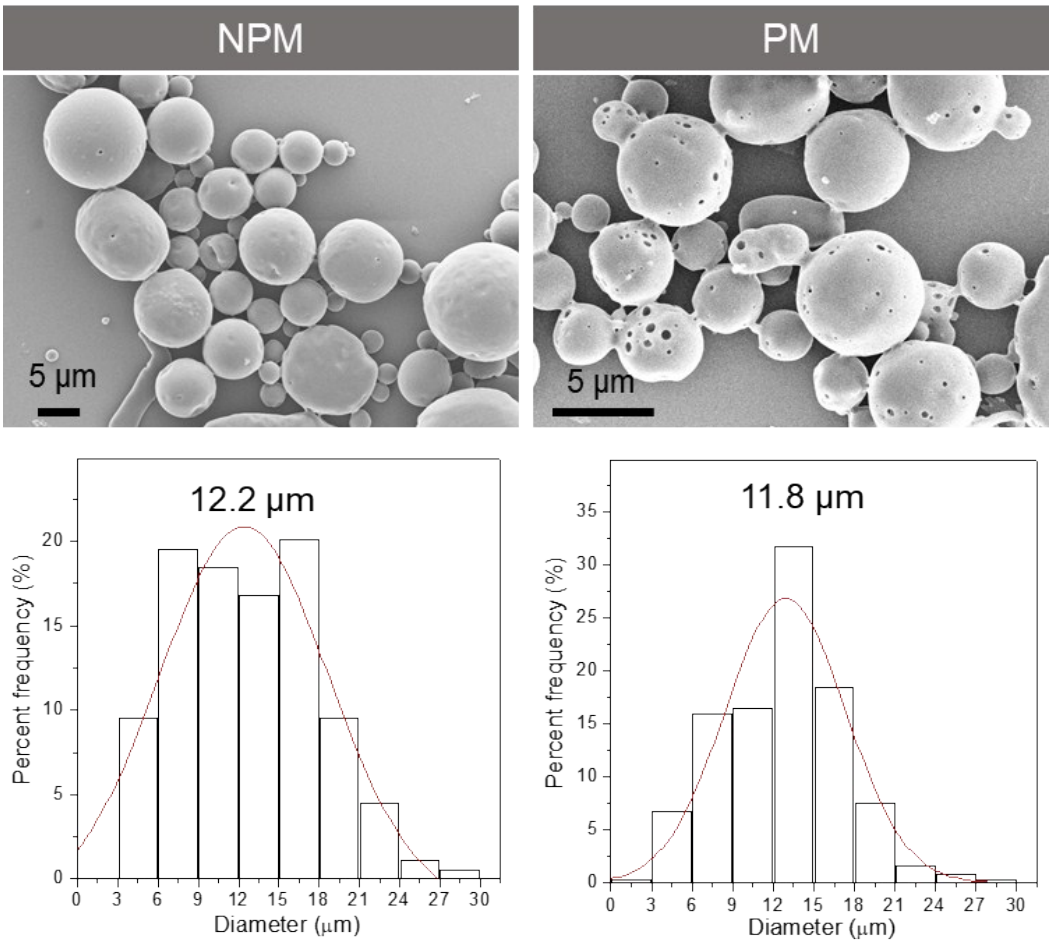


Figure S1. SEM images (top) and size distributions (down) of blank microspheres NPM and ET/PM.

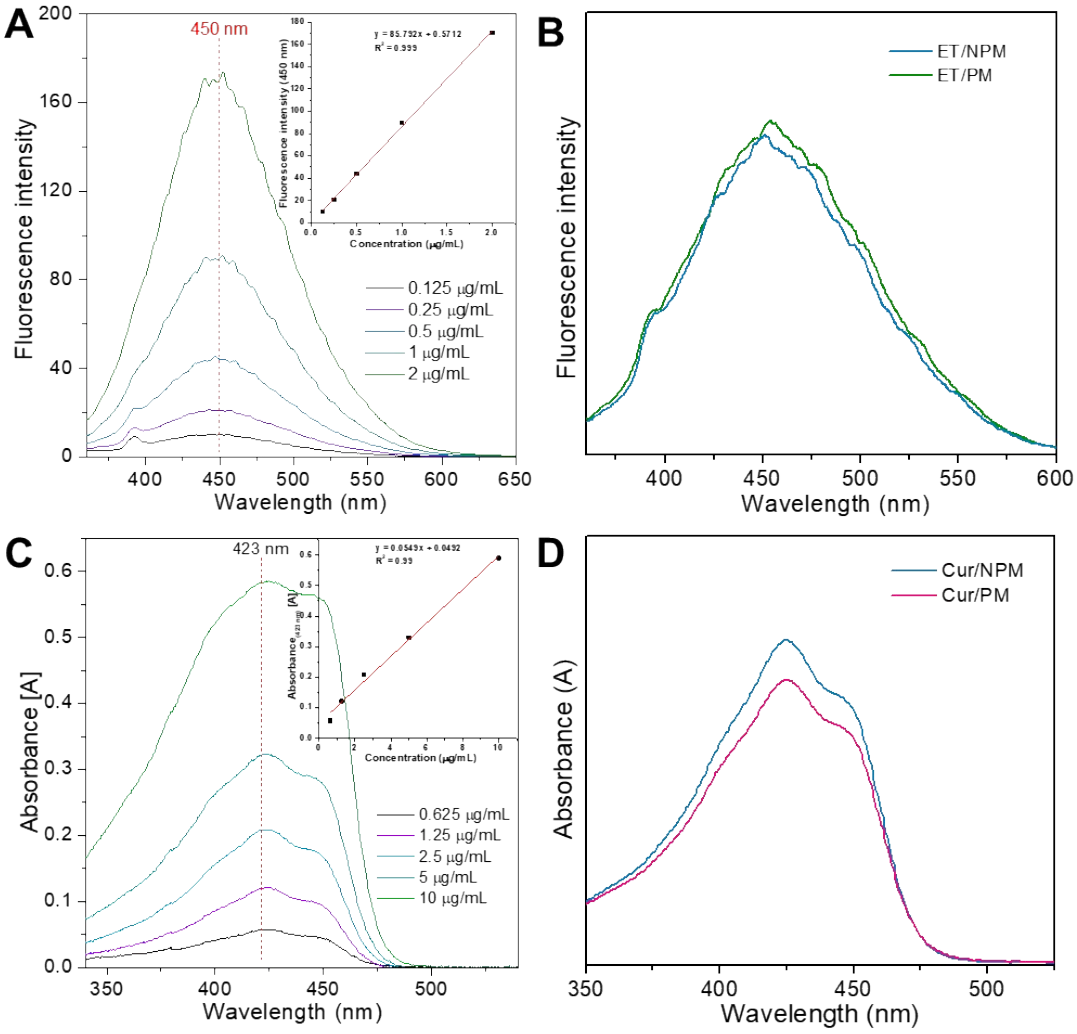


Figure S2. Fluorescence spectroscopy of free erlotinib (A) and drug-loaded microspheres (B). The UV spectra of free curcumin (C) and curcumin-loaded microspheres (D). The insets in Figure S2A and C were the corresponding standard curves.

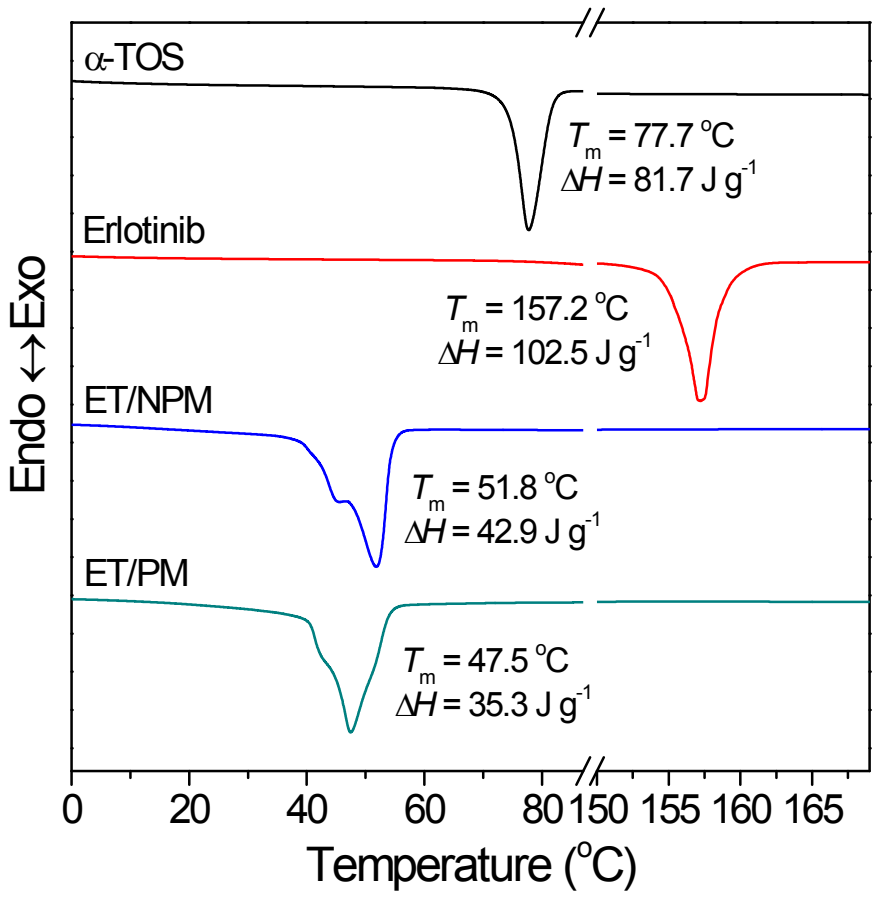


Figure S3. DSC curves of α-TOS, erlotinib, ET/NPM, and ET/PM.

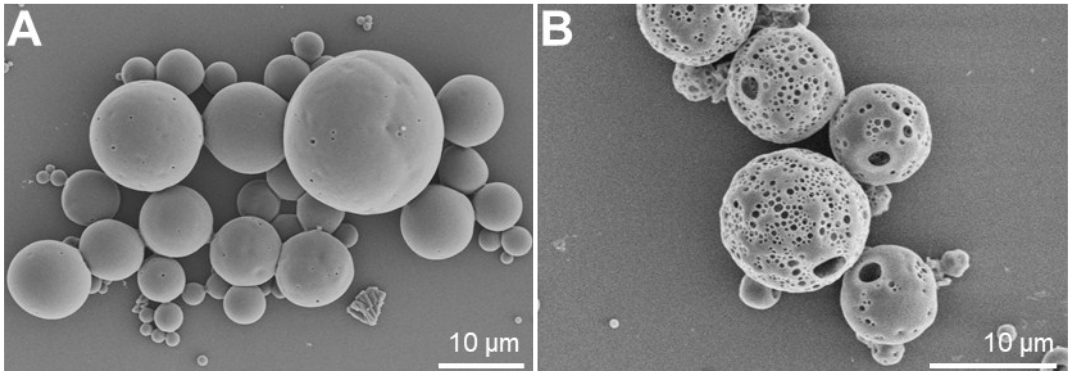


Figure S4. SEM images of Cur/NPM (A) and Cur/PM (B).

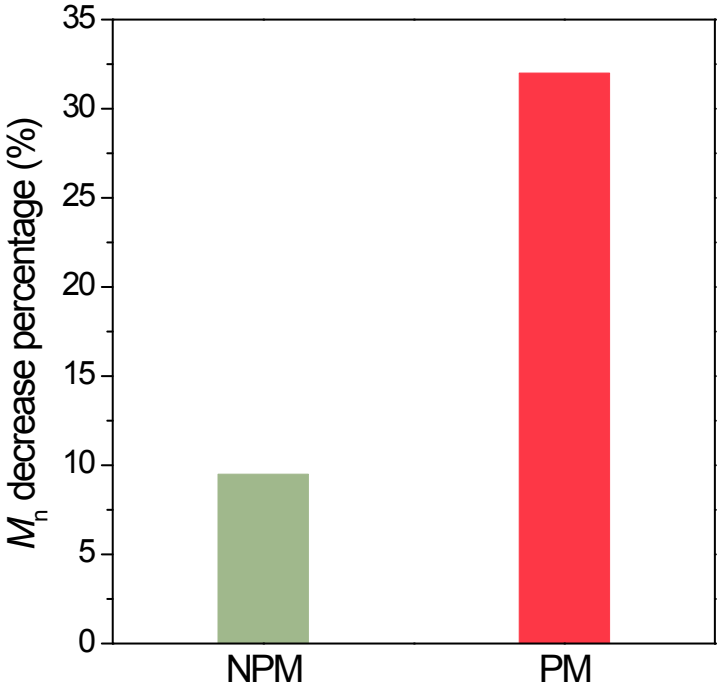


Figure S5. Degradation study of NPM and PM after treated with acetate buffer solution (pH 5.0) for 6 d.

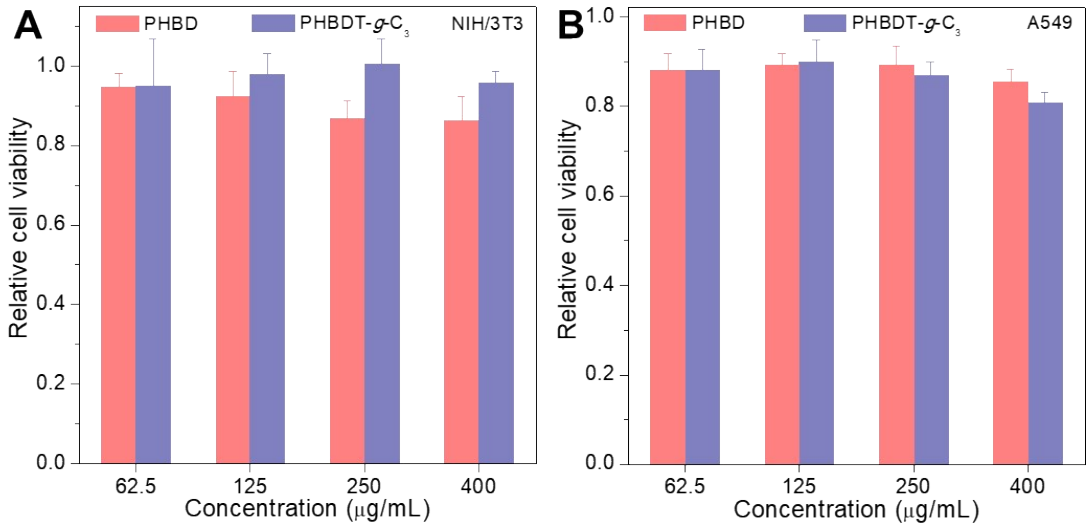


Figure S6. Cell viability of NIH/3T3 (A) and A549 (B) cells after incubation with blank microspheres for 72 h (n = 5).

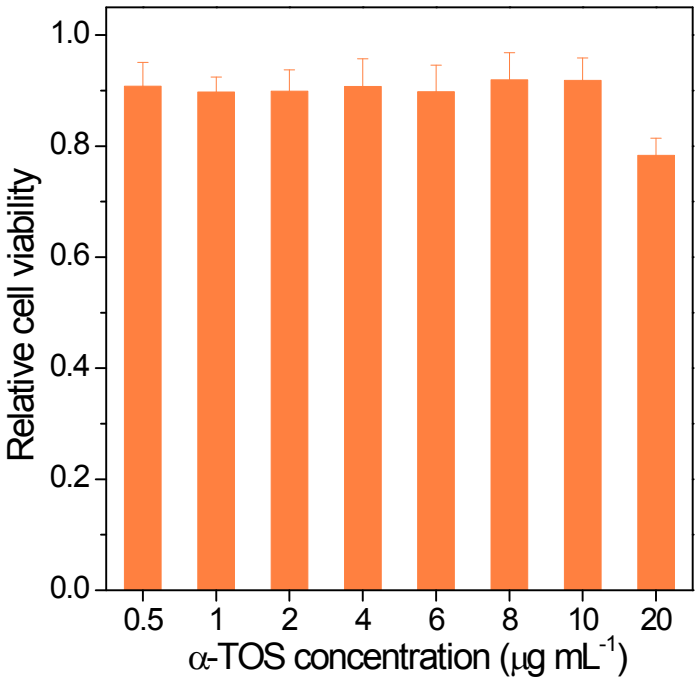


Figure S7. Relative cell viability of A549 cells which co-cultured with different concentrations of α-TOS for 24 h and then replaced with fresh medium further co-incubation for 48 h.

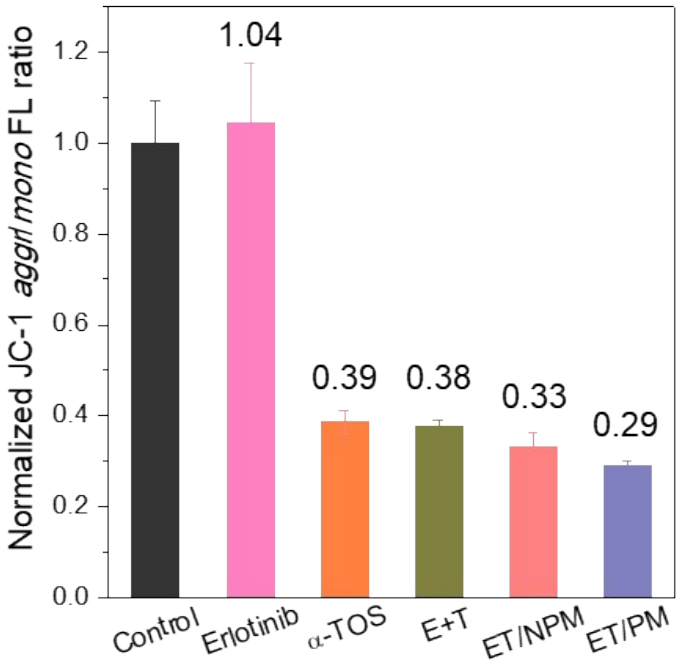


Figure S8. The normalization of fluorescence (FL) ratio detected by CLSM of A549 cells stained by JC-1 after treated with different treatments.

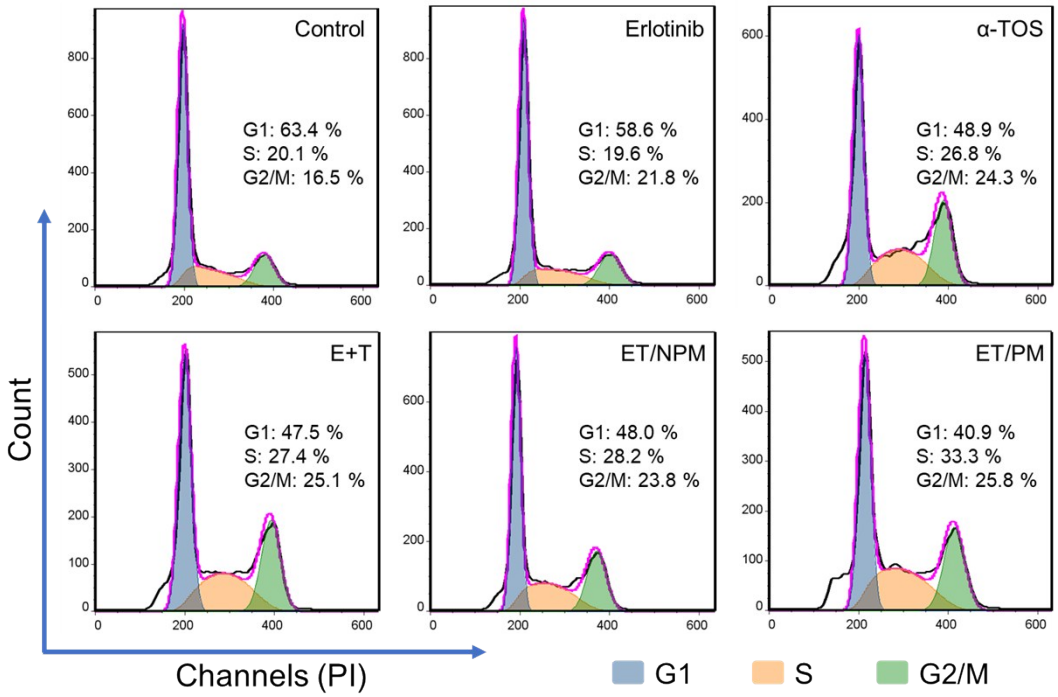


Figure S9. Cell cycle analysis of A549 cells treated with erlotinib, α-TOS, E+T, ET/NPM and ET/PM for 24 h.

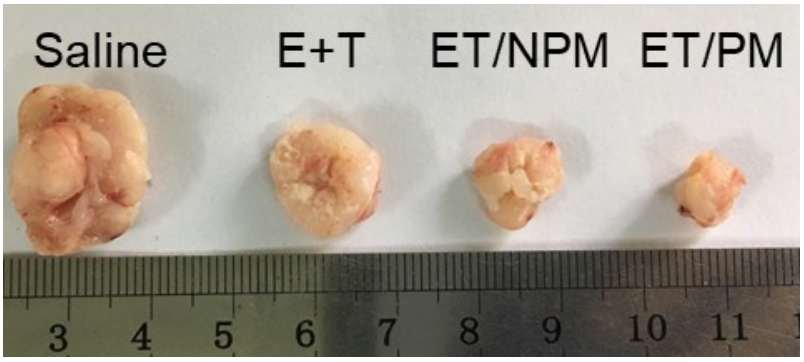


Figure S10. Image of excised A549 solid tumors from different treatment groups on the 18th day.