

Figure S1. TC-HT treatment utilizing a modified PCR machine and temperature measurements conducted with a thermocouple. (A) Image of the TC-HT controller setup. (B) The thermocouple was inserted into the culture medium and its needle touched the surface where the cells were located to measure the actual temperature experienced by the cells. TC-HT, thermal cycling-hyperthermia.

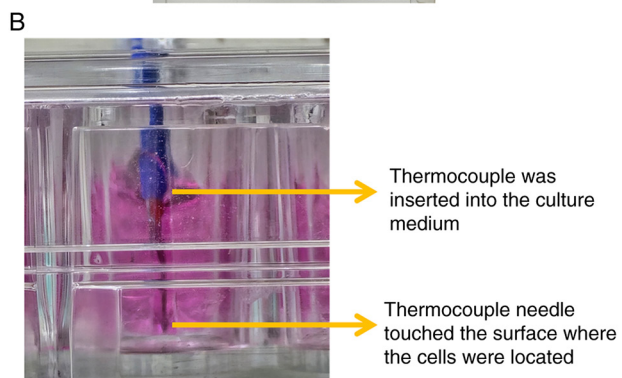


Figure S2. Effect of Erl or TC-HT or in combination on the viability of A549 and H460 cells at various time points. (A) MTT viability assay of A549 NSCLC cells treated with different concentrations of Erl or in combination with moderate temperature TC-HT treatment (41.5-43.0°C) at 24 and 48 h. (B) MTT viability assay of H460 NSCLC cells treated with various concentrations of Erl or in combination with moderate temperature TC-HT treatment (41.5-43.0°C) at 24, 48 and 72 h. Data are shown as mean  $\pm$  standard deviation (n=3). Statistical significance was determined using ANOVA followed by Tukey's post-hoc test. \*P<0.05, \*\*P<0.01, \*\*\*P<0.001. Erl, erlotinib; TC-HT, thermal cycling-hyperthermia; NSCLC, non-small cell lung cancer; ctrl, control.

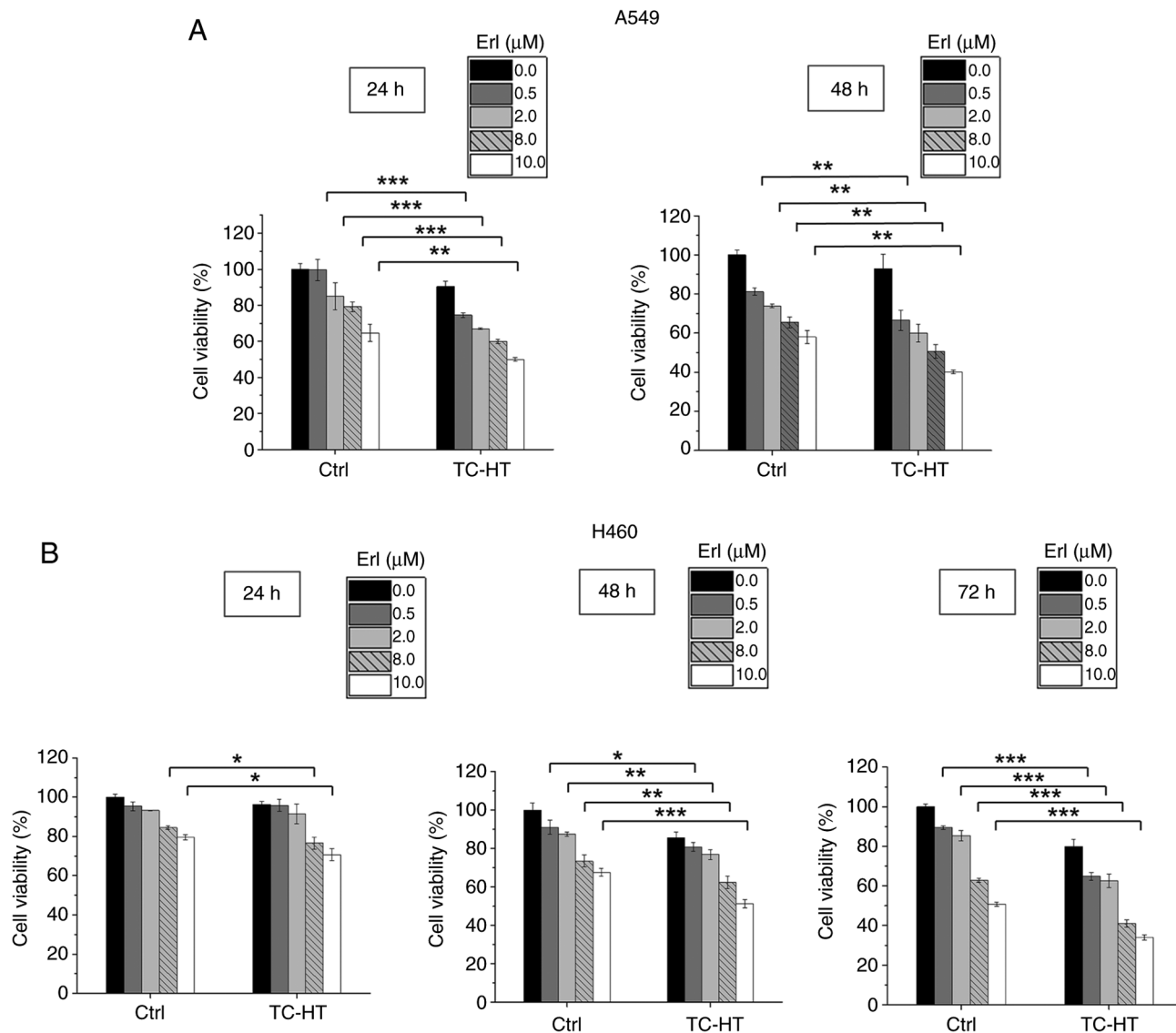


Figure S3. Effect of Erl combined with TC-HT on p-EGFR protein expression levels in A549 cells. Western blotting analysis of p-EGFR expression in A549 cells treated with 0.5 and 2  $\mu$ M Erl, as well as the combination treatment. The expression levels of p-EGFR were normalized to GAPDH. Each relative expression level was compared to the control and represented as a fold relative to the control. Data are shown as mean  $\pm$  standard deviation (n=3). Statistical significance was determined using ANOVA followed by Tukey's post-hoc test. \*P<0.05, \*\*P<0.01, \*\*\*P<0.001. Erl, erlotinib; TC-HT, thermal cycling-hyperthermia; p, phosphorylated; t, total; ctrl, control.

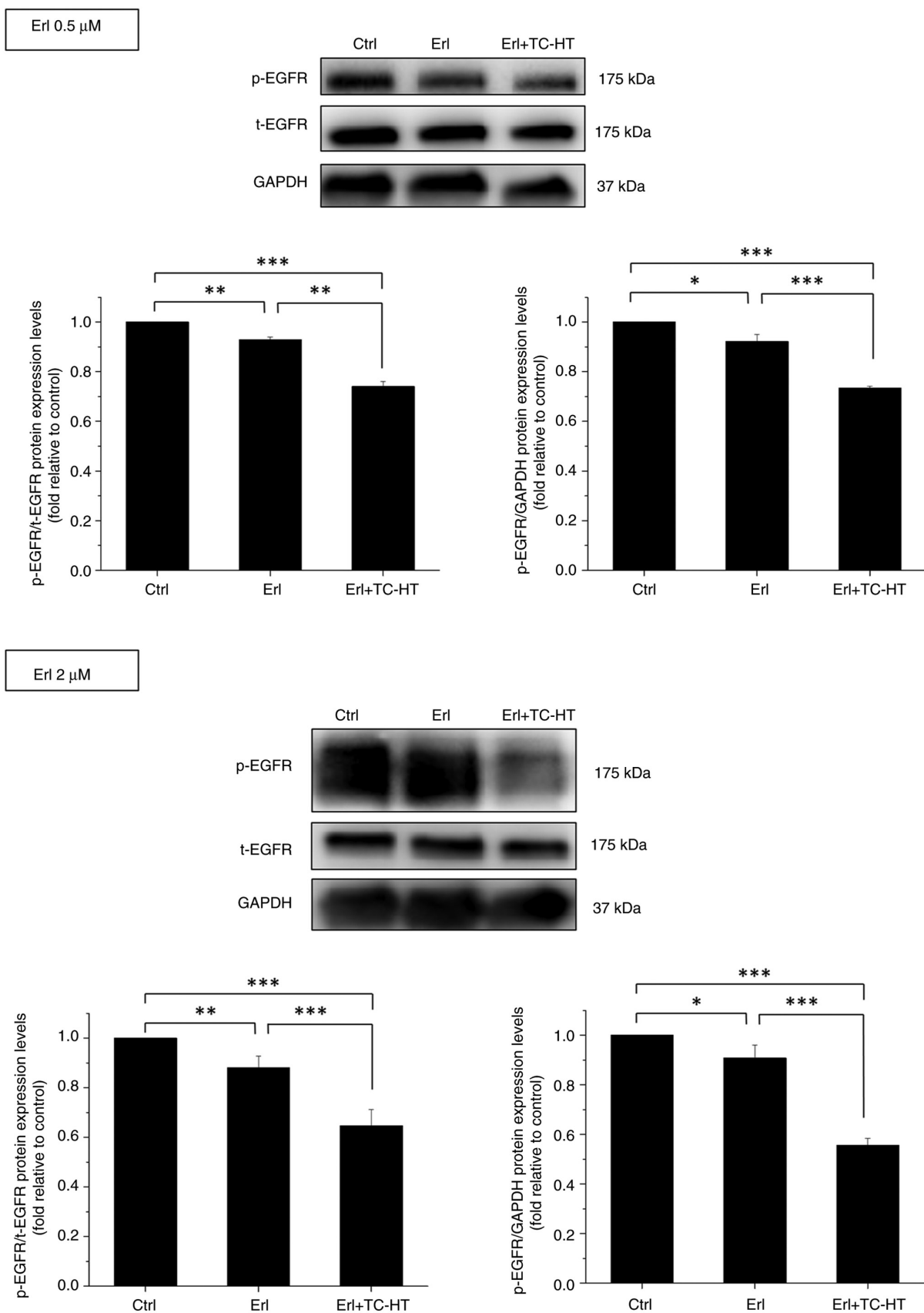


Figure S4. Flow cytometric analysis of MMP in A549 cells treated with Erl and/or TC-HT. A549 cells were treated with 10  $\mu$ M Erl, moderate temperature TC-HT and the combination of both treatments. (A) MMP was evaluated using the DiOC<sub>6</sub>(3) fluorescent dye and detected by flow cytometry. (B) Quantification of the cells with decreased MMP was measured. Data are shown as mean  $\pm$  standard deviation (n=3). Statistical significance was determined using one-way ANOVA followed by Tukey's post-hoc test. \*\*P<0.01. Erl, erlotinib; TC-HT, thermal cycling-hyperthermia; MMP, mitochondrial membrane potential; ctrl, control.

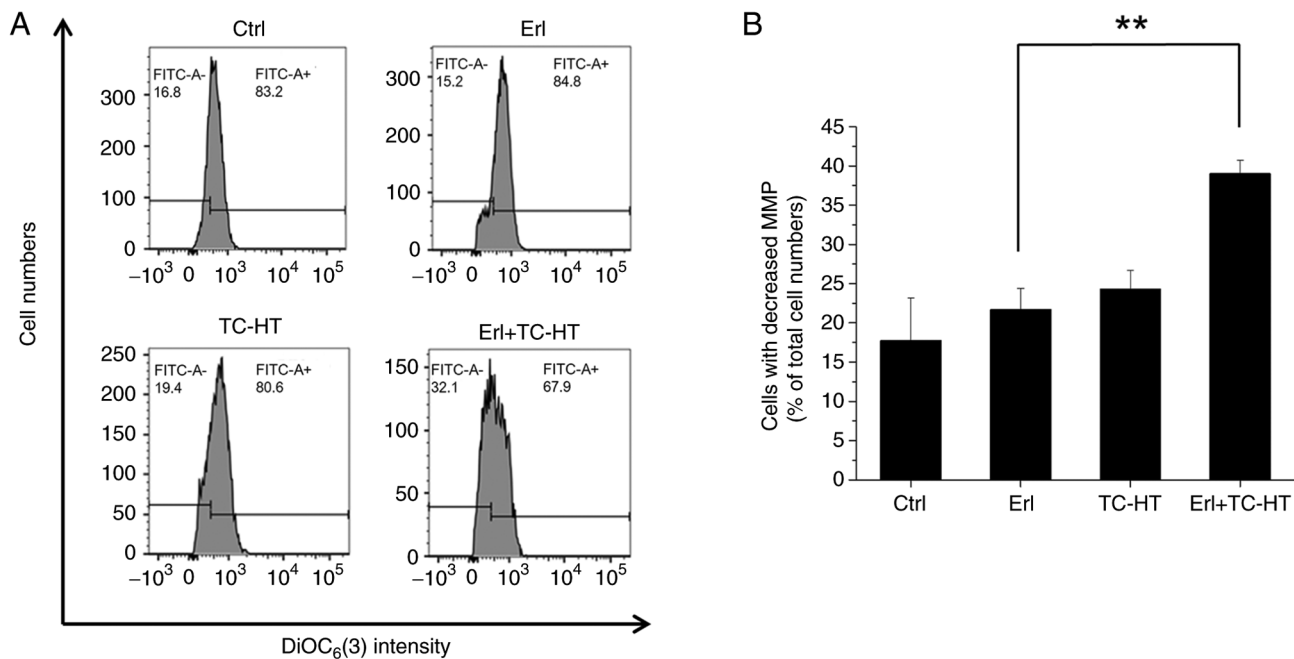


Figure S5. Effect of high temperature TC-HT and/or Erl on cell viability. The MTT viability assay was conducted on H460 non-small cell lung cancer cells treated with various concentrations of Erl, either alone or in combination with high temperature TC-HT treatment (42.5-45.6°C). Data are shown as mean  $\pm$  standard deviation (n=3). Statistical significance was determined by one-way ANOVA followed by Tukey's post-hoc test. \*\*\*P<0.001. Erl, erlotinib; TC-HT, thermal cycling-hyperthermia; ctrl, control.

