Supplementary Materials

Hypoxia-responsive soft biohybrid bacteria for breast cancer therapy

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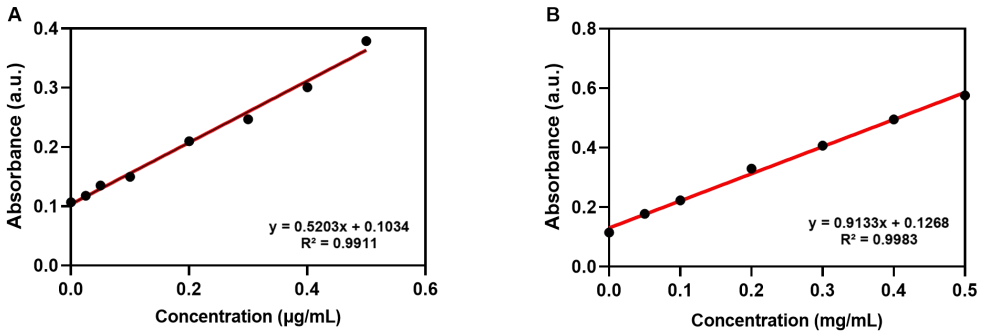
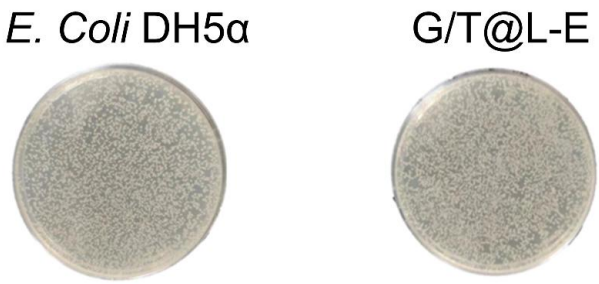
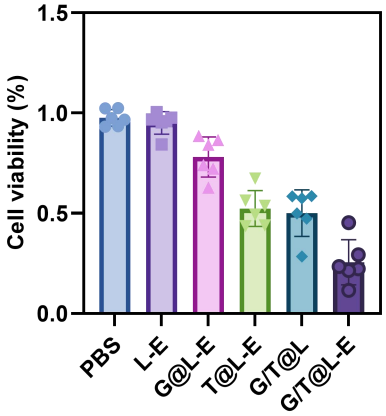


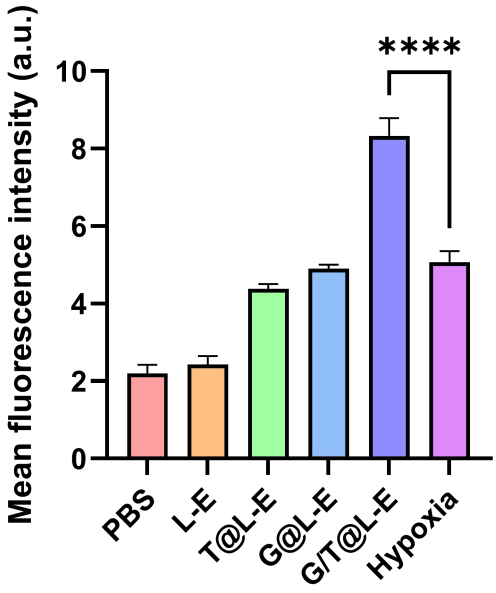
Supplementary Figure 1. (A) The standard curve showing the linear relation between the absorbance of protein at λ =560 nm and its concentration. (B)The standard curve showing the linear relation between the absorbance of TPZ at λ =475 nm and its concentration. It can be calculated that the encapsulation efficiency of GOx is 63.09% and the loading efficiency of TPZ is 57.14%. The loading capacity of GOx and TPZ were respectively 4.75% and 3.08%.



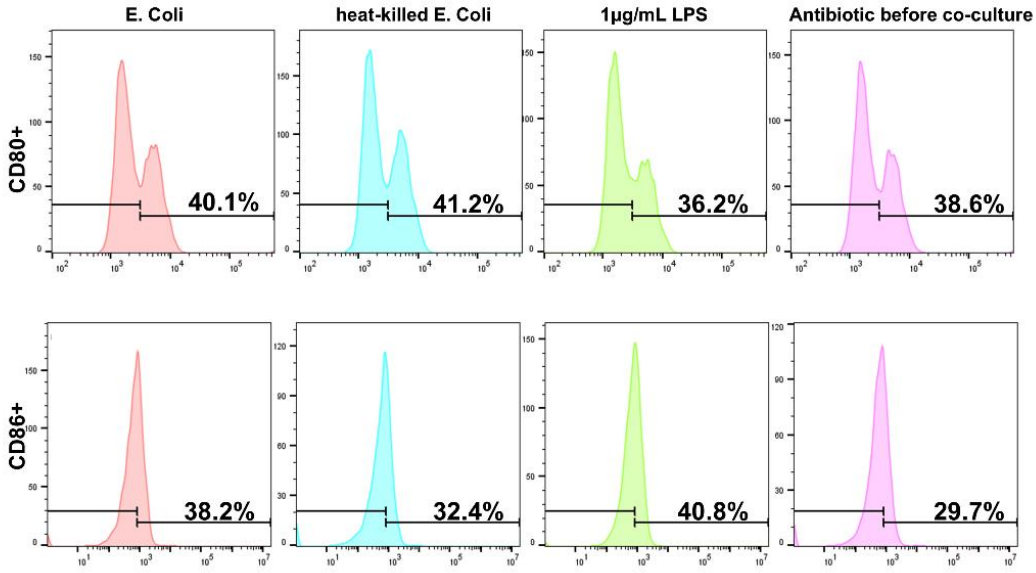
Supplementary Figure 2. Photographs of chromogenic LB agar of *E. Coli* DH5α and G/T@L-E after cultured 24 h in 37 ℃.



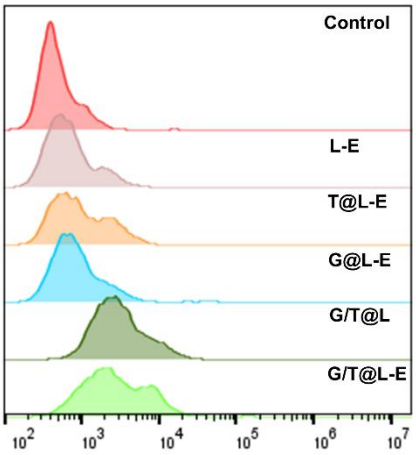
Supplementary Figure 3. Cell viability of 4T1 cells following different treatments. n = 6.



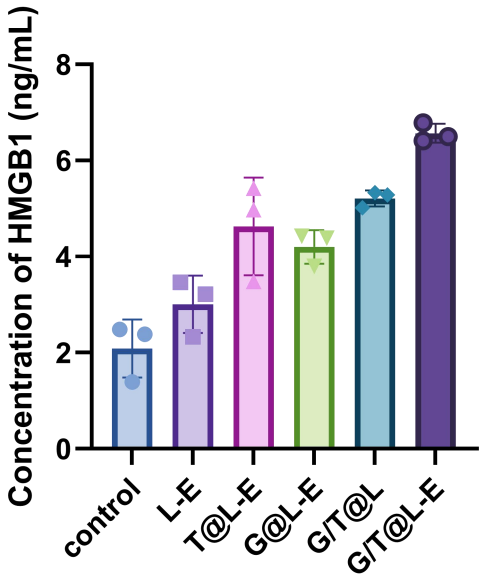
Supplementary Figure 4. The corresponding fluorescence intensities of HIF1-α quantified by ImageJ software. Data are presented as mean ± SD. The significances were compared by one-way ANOVA followed (Dunnett's multiple comparisons test). ****$p < 0.0001$.



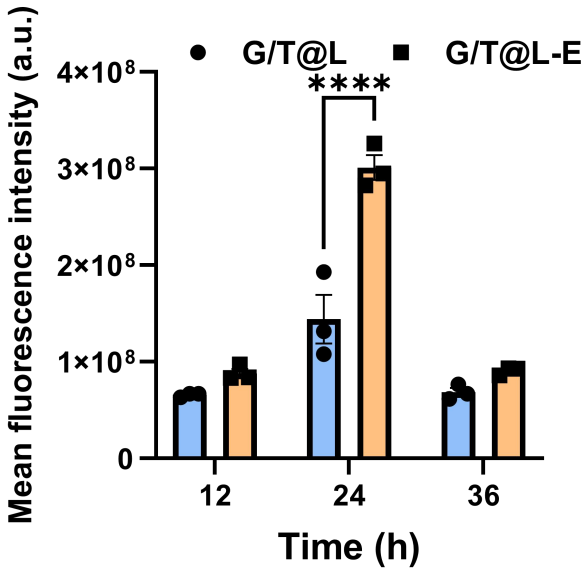
Supplementary Figure 5. FCM analyses of mature DCs (CD11c+ CD80+ CD86+) percentages following various treatments.



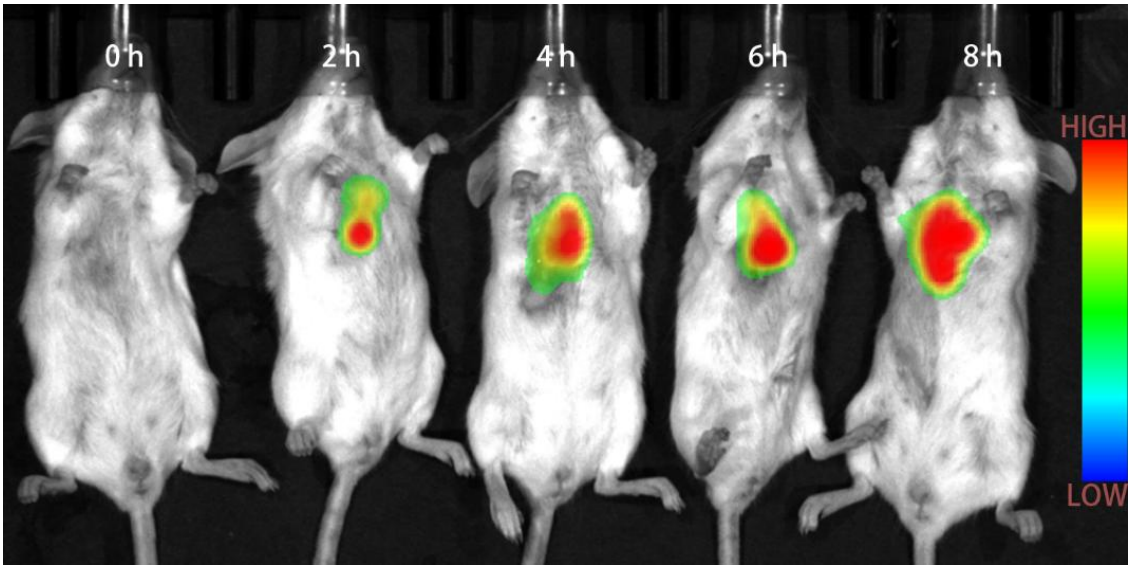
Supplementary Figure 6. Flow cytometry analysis of CRT expression on the surface of 4T1 cells with different treatments after 12 h.



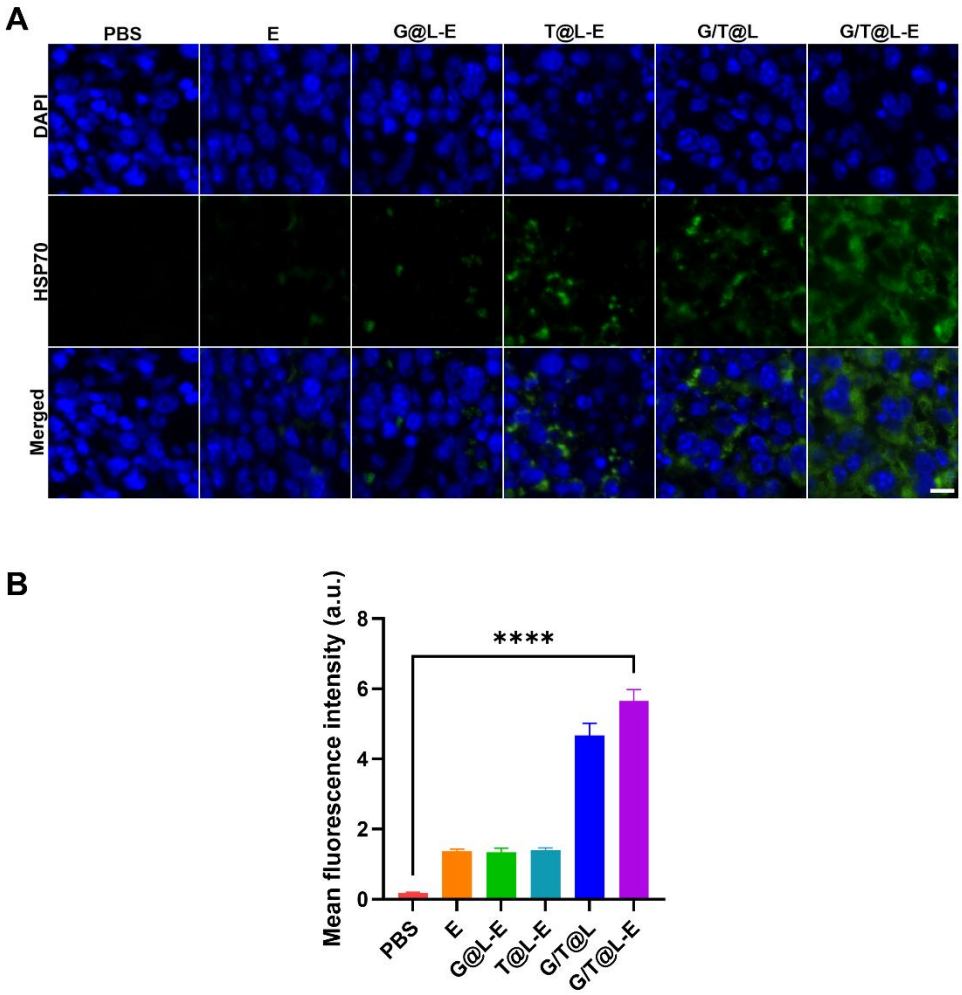
Supplementary Figure 7. HMGB1 release from 4T1 cells in the supernatant 24 h after different treatment. n = 3. Data are presented as mean ± SD.



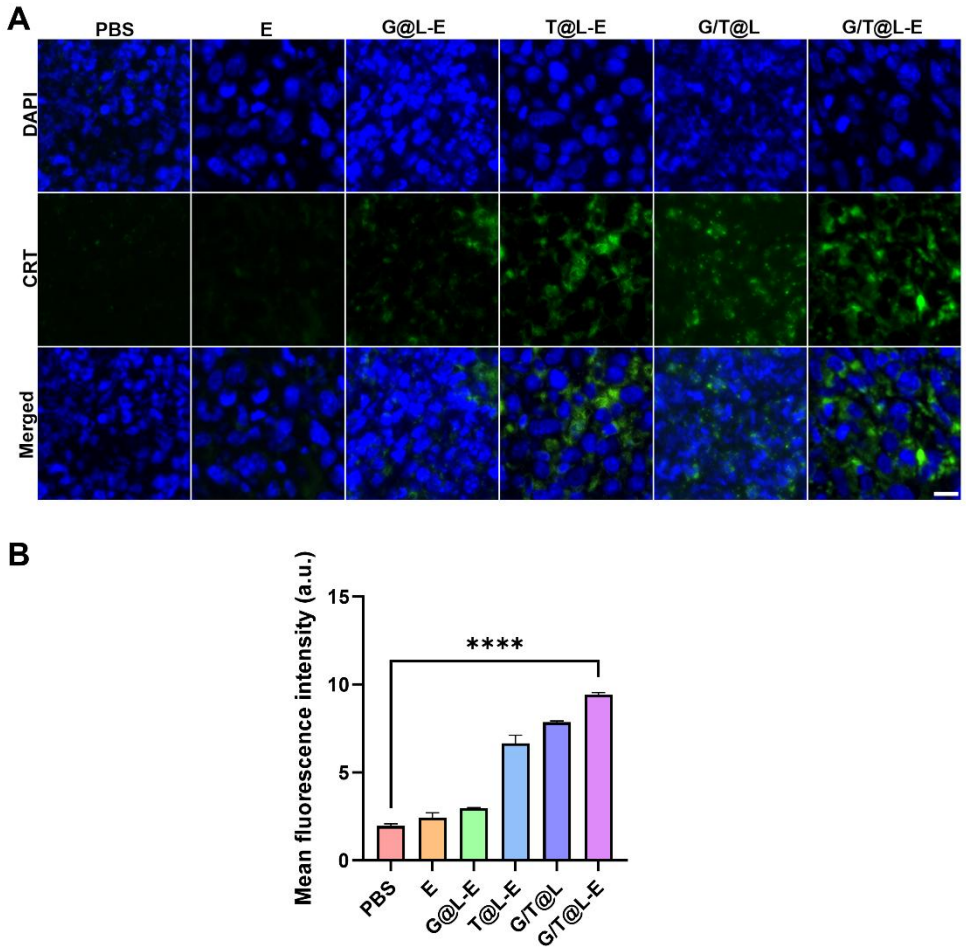
Supplementary Figure 8. Corresponding mean fluorescence intensity of DiI-labeled G/T@L-E and G/T@L in tumor after injection in 12 h, 24 h, and 36 h. Data are presented as mean ± SD. The significances were compared by two-way ANOVA followed by Tukey's multiple comparisons test. ****$p < 0.0001$.



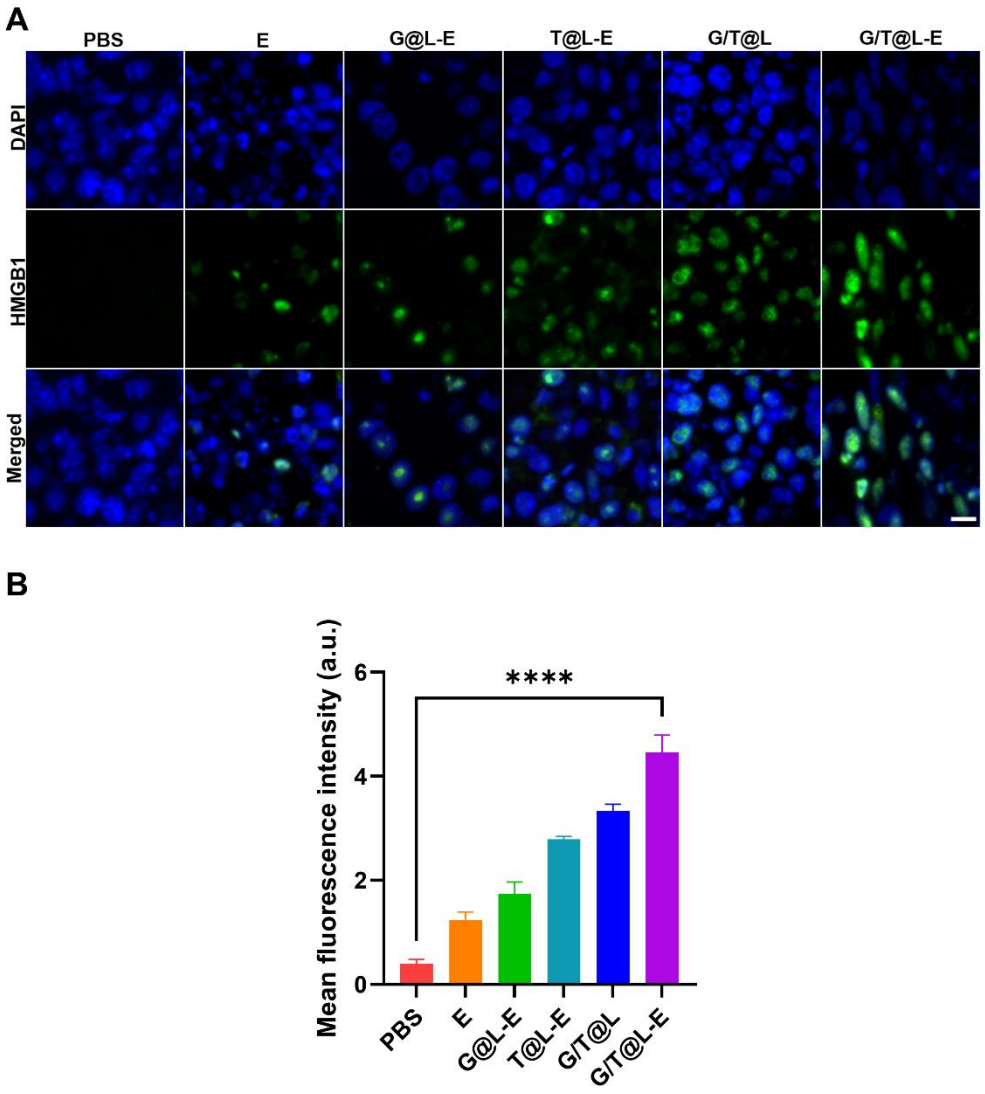
Supplementary Figure 9. *In vivo* imaging of DiI-labeled G/T@L-E in mice after injection of 0 h, 2 h, 4 h, 6 h and 8 h.



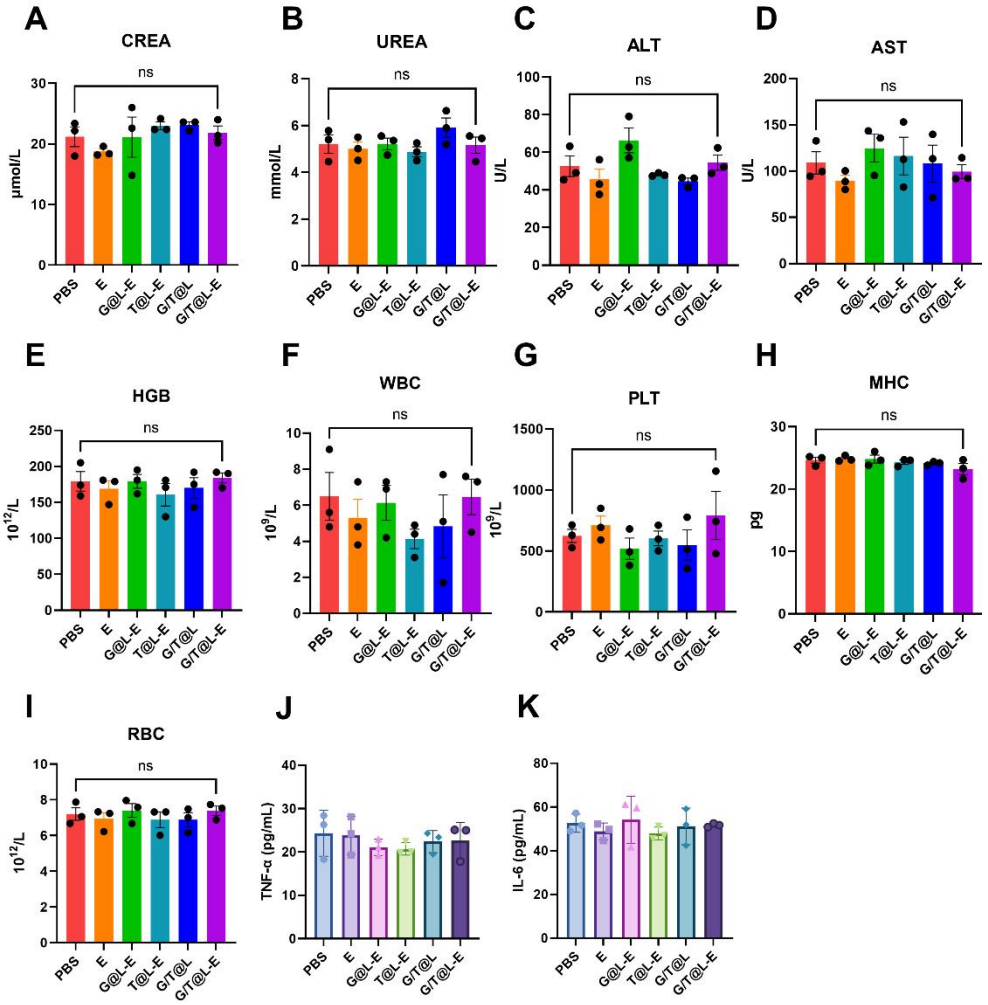
Supplementary Figure 10. (A) Immunofluorescence images of HSP70 from tumor tissue after different treatments. Scale bar = 100 μm (B) The corresponding fluorescence intensities of HSP70 quantified by ImageJ software. Data are presented as mean ± SD. The significances were compared by one-way ANOVA followed (Dunnett's multiple comparisons test). ****$p < 0.0001$.



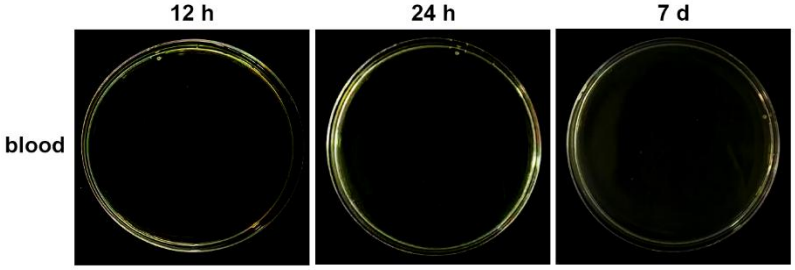
Supplementary Figure 11. (A) Immunofluorescence images of CRT form tumor tissue after different treatments. Scale bar = 100 μm (B) The corresponding fluorescence intensities of CRT quantified by ImageJ software. Data are presented as mean ± SD. The significances were compared by one-way ANOVA followed (Dunnett's multiple comparisons test). ****$p < 0.0001$.



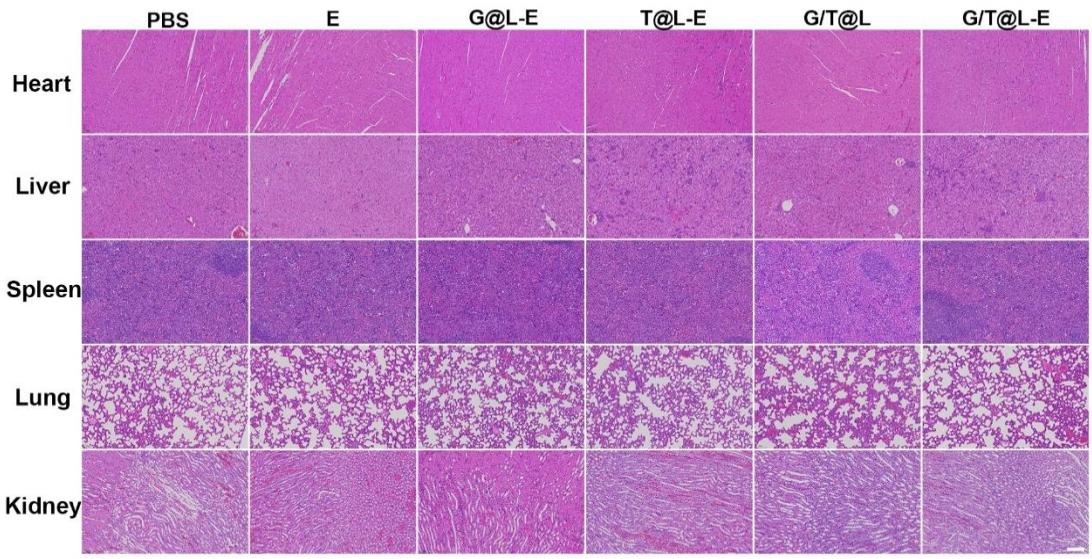
Supplementary Figure 12. (A)Immunofluorescence images of HMGB1 from tumor tissue after different treatments. Scale bar=100 μm (B)The corresponding fluorescence intensities of HMGB1 quantified by ImageJ software. Data are presented as mean ± SD. The significances were compared by one-way ANOVA followed (Dunnett's multiple comparisons test). ****$p < 0.0001$.



Supplementary Figure 13. *In vivo* toxicology study of different treatments. Blood levels of (A) Creatinine (CREA), (B) Blood urea nitrogen (UREA), (C) Alanine aminotransferase (ALT), (D) Aspartate Aminotransferase (AST), (E) Hemoglobin (HGB), (F) White blood cell count (WBC), (G) Platelet (PLT), (H) Mean corpuscular hemoglobin (MCH) and (I)red blood cell count (RBC). (J) TNF-α levels in serum at 24 h after different treatments. (K) IL-6 levels in serum at 24 h after different treatments. Data are presented as mean ± SD. The significances were compared by two-way ANOVA followed by Tukey's multiple comparisons test). ns ≥ 0.05.



Supplementary Figure 14. Photographs of chromogenic LB agar of blood samples collected at 12 h, 24 h, and 7 d after injecting G/T@L-E.



Supplementary Figure 15. H&E stained images obtained from heart, liver, spleen, lung, and kidney of 4T1-bearing Balb/c mice after injection of G/T@L-E in 24 h. Scale bar = 50 μm