Electronic Supplementary Material (ESI) for Biomaterials Science.

Supplementary information

Polymer/copper nanocomplex-induced lysosomal cell death promotes tumor lymphocyte infiltration and synergizes anti-PD-L1 immunotherapy for triple-negative breast cancer

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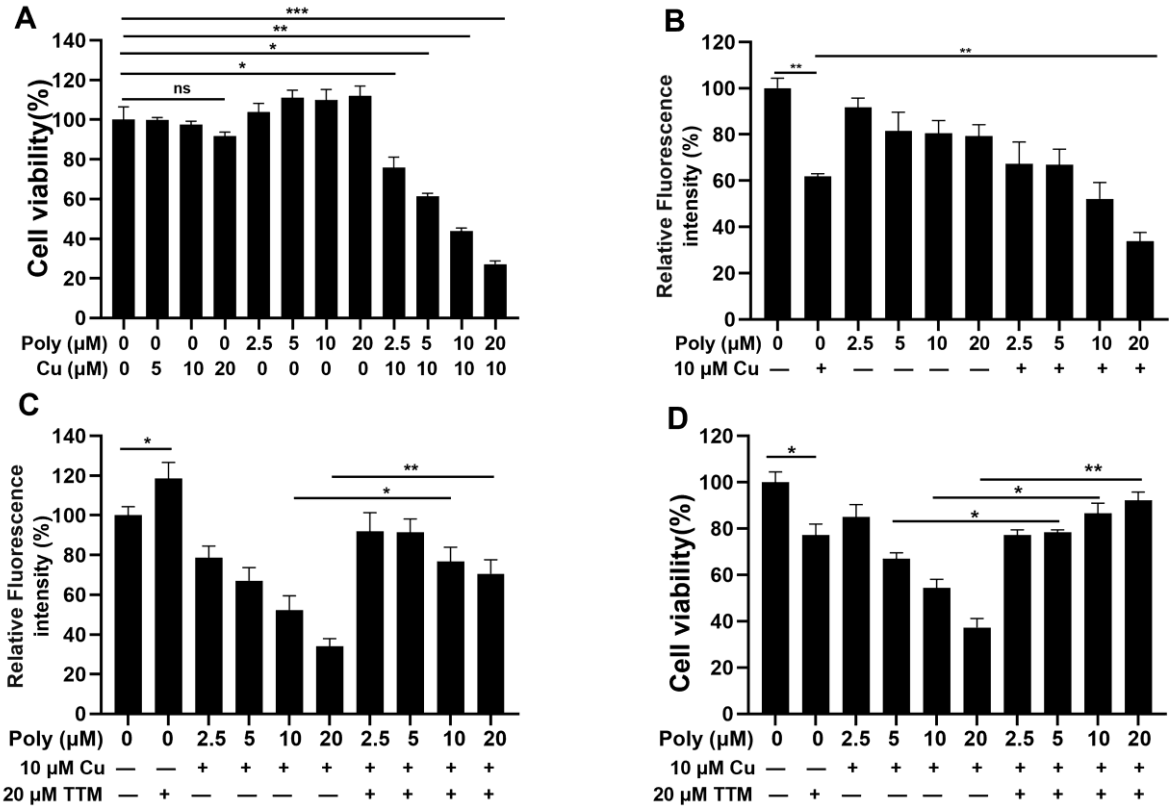

Figure S1. (A) The viability of 4T1 cells after receiving different treatments for 48 h. Cell viability was evaluated by MTT assay. (B, C) 4T1 cells were treated with different concentrations of polymer, TTM, and $CuCl_2$ for 6 h before being stained with Phen Green™ FL Diacetate dye for 30 min. The relative fluorescence intensity of the cells was determined by a microplate reader. (D) Cell viability of 4T1 cells after receiving different concentrations of polymer, TTM, and $CuCl_2$. Data were represented as mean ± SD, n=3 (*P < 0.05).

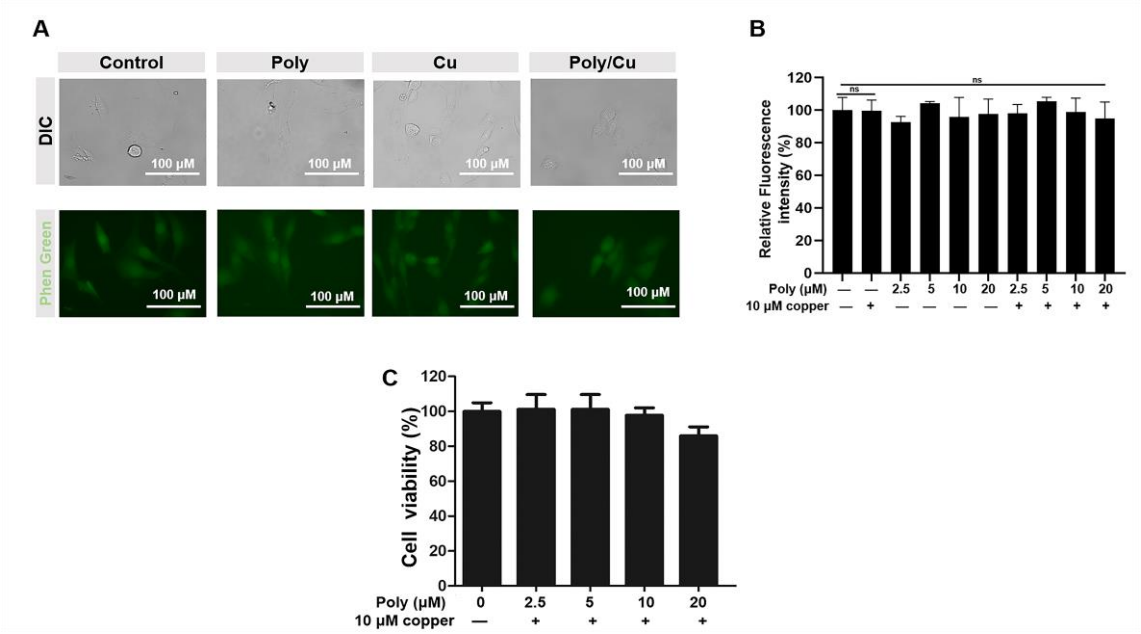

Figure S2. NIH 3T3 cells were treated with PDA-PEG, CuCl2, and Poly/Cu at the concentration of 20 μM for polymer and 10 μM for CuCl2 for 6 h. Cells were stained with Phen Green™ FL Diacetate dye, the copper concentration in the NIH3T3 cells were detected with confocal microscopy (A) and a microplate reader (B). (C) NIH3T3 cells were treated with different concentrations of PDA-PEG combined with 10 μM $CuCl_2$ for 48 h. Cell viability was evaluated by MTT assay. Data were represented as mean ± SD, n=3 (*P < 0.05).

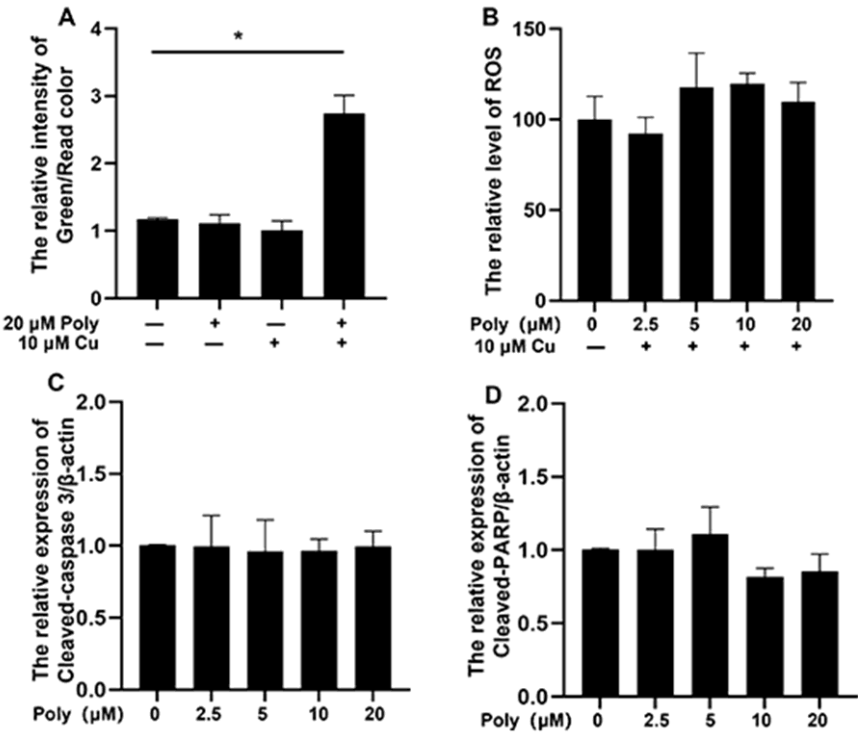

Figure S3. (A) 4T1 cells were treated with 20 μM Polymer, 10 μM $CuCl_2$ and their combination for 24 h, then stained with Acridine orange. The green to red color ratio of different treatments was analyzed by image J. Data were represented as mean ± SD, n=3 (*P < 0.05). (B) 4T1 cells were treated with different concentrations of Polymer combined with 10 μM $CuCl_2$ for 12 h. ROS in the cells were quantified by a microplate reader. Quantitative analysis of cleaved-PARP (C) and cleaved-caspase 3 (D) expression after receiving different treatments. Data were represented as mean ± SD, n=3 (*P < 0.05).

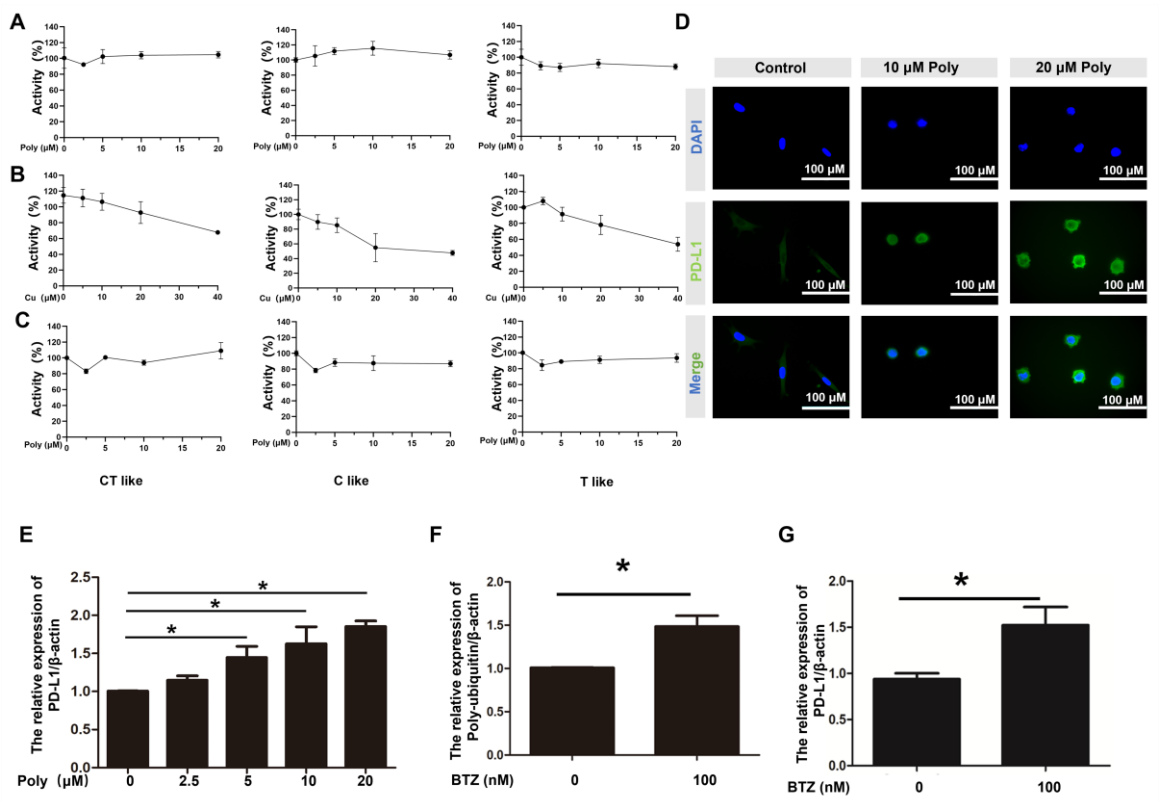

Figure S4. The activity of 20S proteasome with different substrates after receiving different treatments (A-C). 20S proteasomes were isolated from 4T1 cells and then treated with (A) Polymer, (B) $CuCl_2$, and (c) their combination. (D) PD-L1 expression after being treated with 10 μM Polymer/10 μM $CuCl_2$ and 20 μM Polymer/10 μM $CuCl_2$ for 48 h and determined by immunofluorescent staining. Quantitative analysis of PD-L1 (E-G) and Poly-ubiquitin expression after receiving different treatments. Data were represented as mean ± SD, n=3 (*$P < 0.05$).

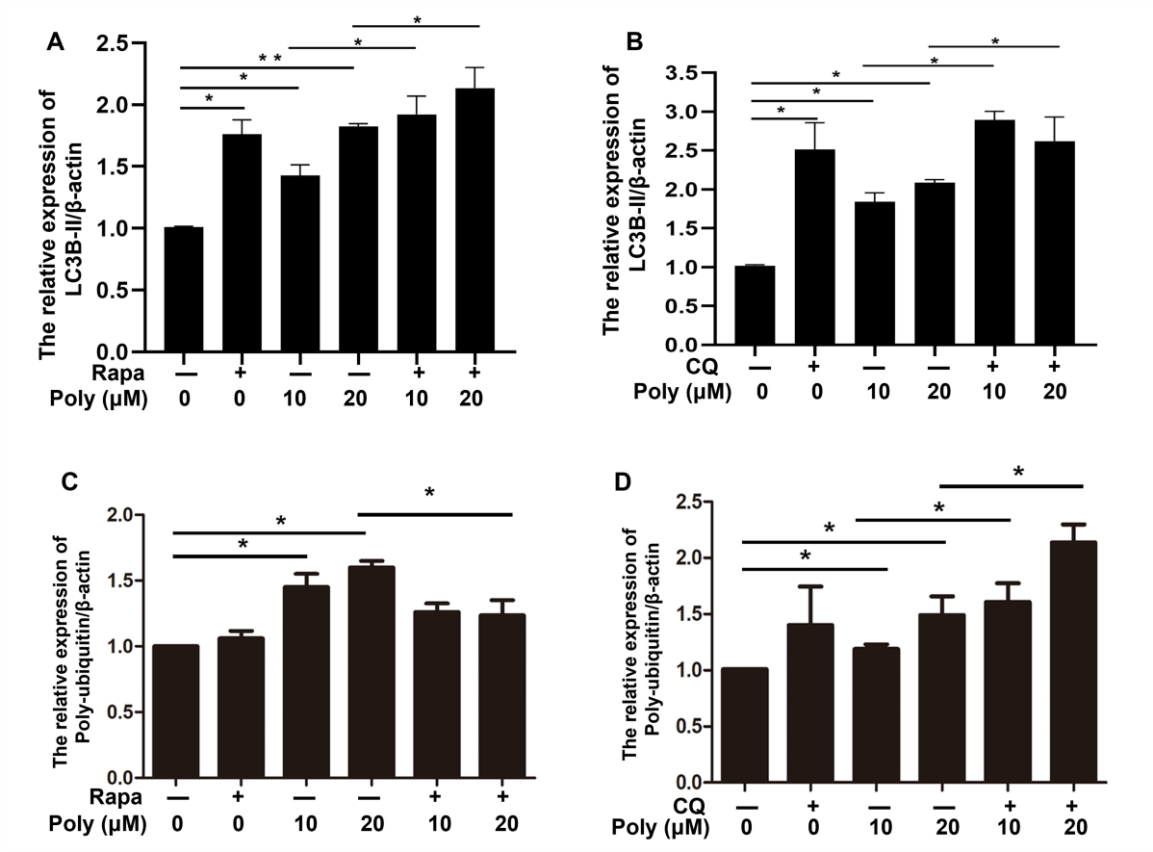

Figure S5. Quantitative analysis of LC3B-II (A, B) and Poly-ubiquitin (C, D) expression after receiving different treatments. Data were represented as mean ± SD, n=3 (*P < 0.05). 4T1 cells were treated with 31.25 nM Rapamycin combined with different concentrations of Polymer/10 μM $CuCl_2$ for 24 h in (A, C). 4T1 cells were treated with 3.125 μM CQ combined with different concentrations of Polymer/10 μM $CuCl_2$ for 24 h in (B, D).

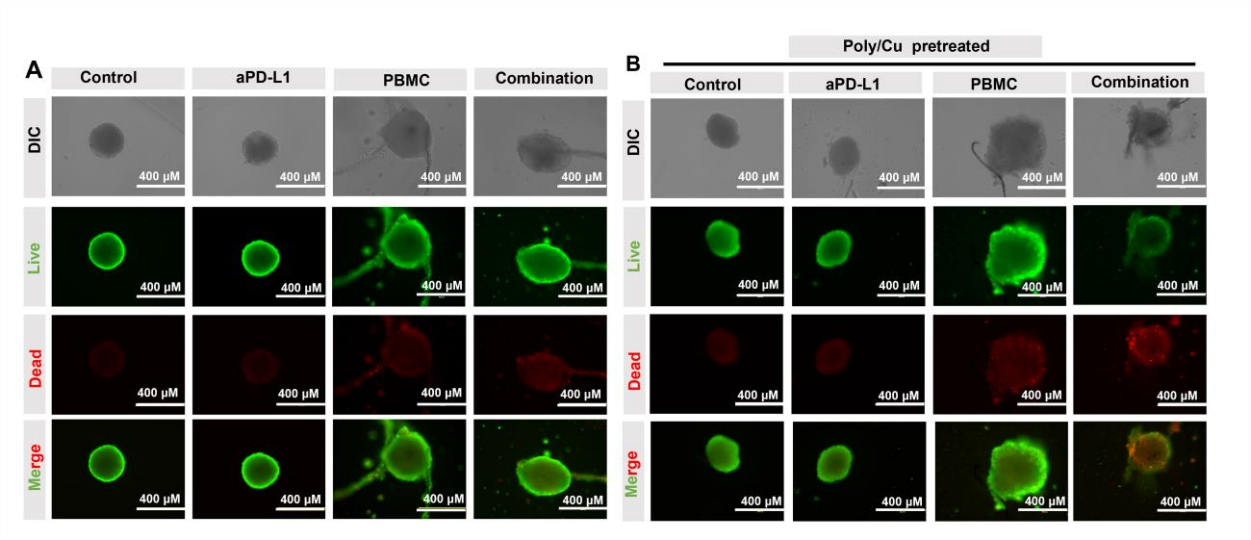

Figure S6. 4T1 tumor spheroid viability detected by live/dead staining. Tumor spheroids were pretreated without (A) or with (B) 20 μM Polymer/10 μM $CuCl_2$ for 2 days before the addition of aPD-L1, PBMC, or their combination for 5 days.

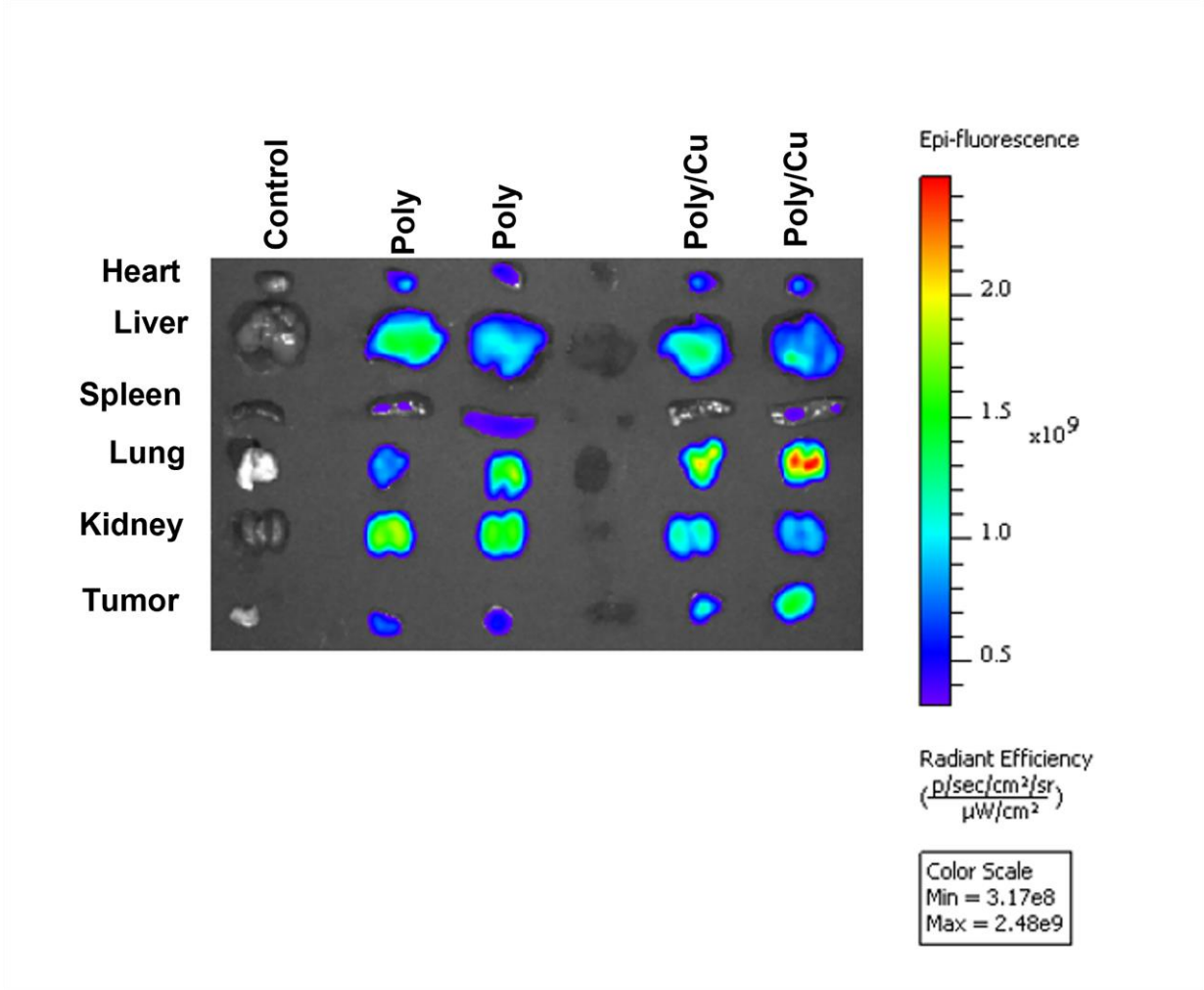

Figure S7. *Ex vivo* image of Poly/Cu distribution in different organs. Poly-Cy5 or Poly-Cy5/copper was i.v. injected to the 4T1 tumor-bearing mice. Animals were sacrificed 3 h after the treatments. Organs collected from different treatment groups were imaged with an IVIS Lumina III whole-body imaging system.

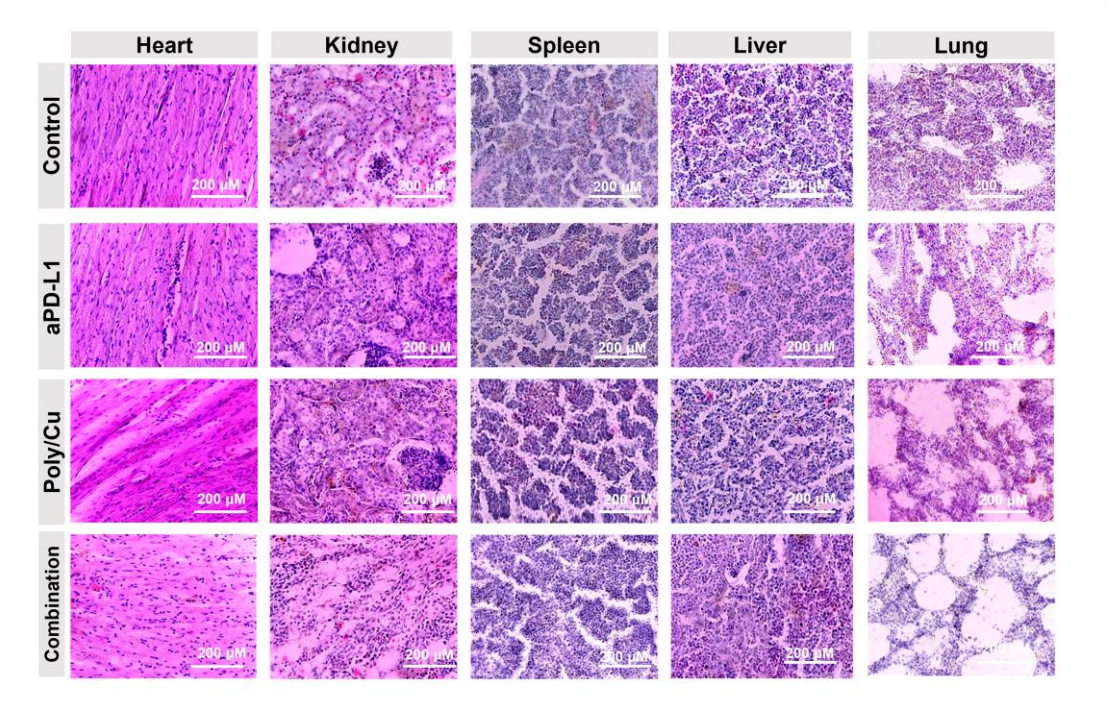

Figure S8. Representative images of HE stained tissue sections from different treatment groups.