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Supporting Information

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Supplementary information

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Keywords: cancer immunotherapy, ferroptosis, hydrogel, malignant ascites, tumor microenvironment

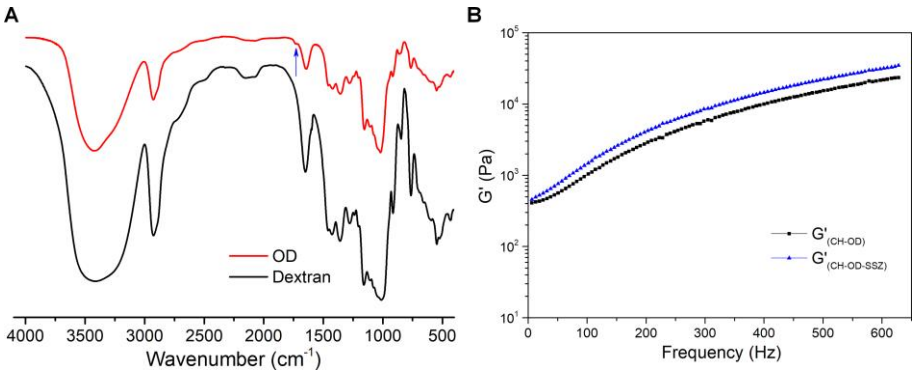

Figure S1. A) Fourier transform infrared spectra of OD and dextran. B) Frequency dependence of storage modulus (*G'*) for a 3.5% CH-OD and CH-OD-SSZ hydrogel.

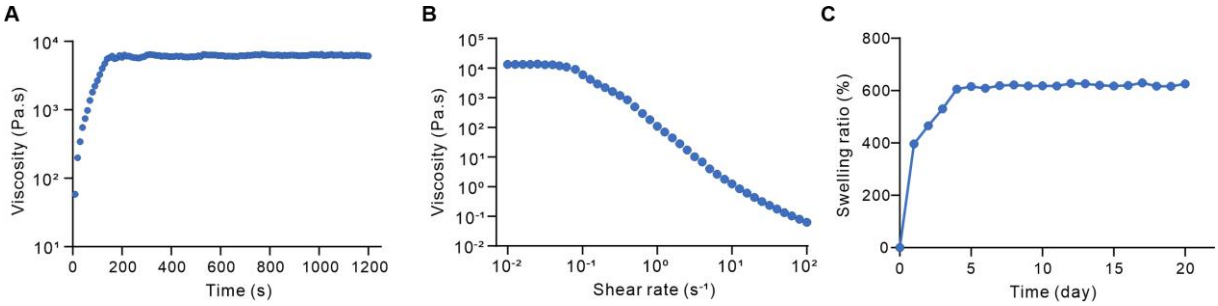

Figure S2. The rheological characteristics and swelling ratio of the hydrogels. A) Flow peak hold measurements with a fixed shear rate of 0.1 s^{-1}. B) Steady shear rheology of the hydrogel. C) Swelling ratio of the hydrogel.

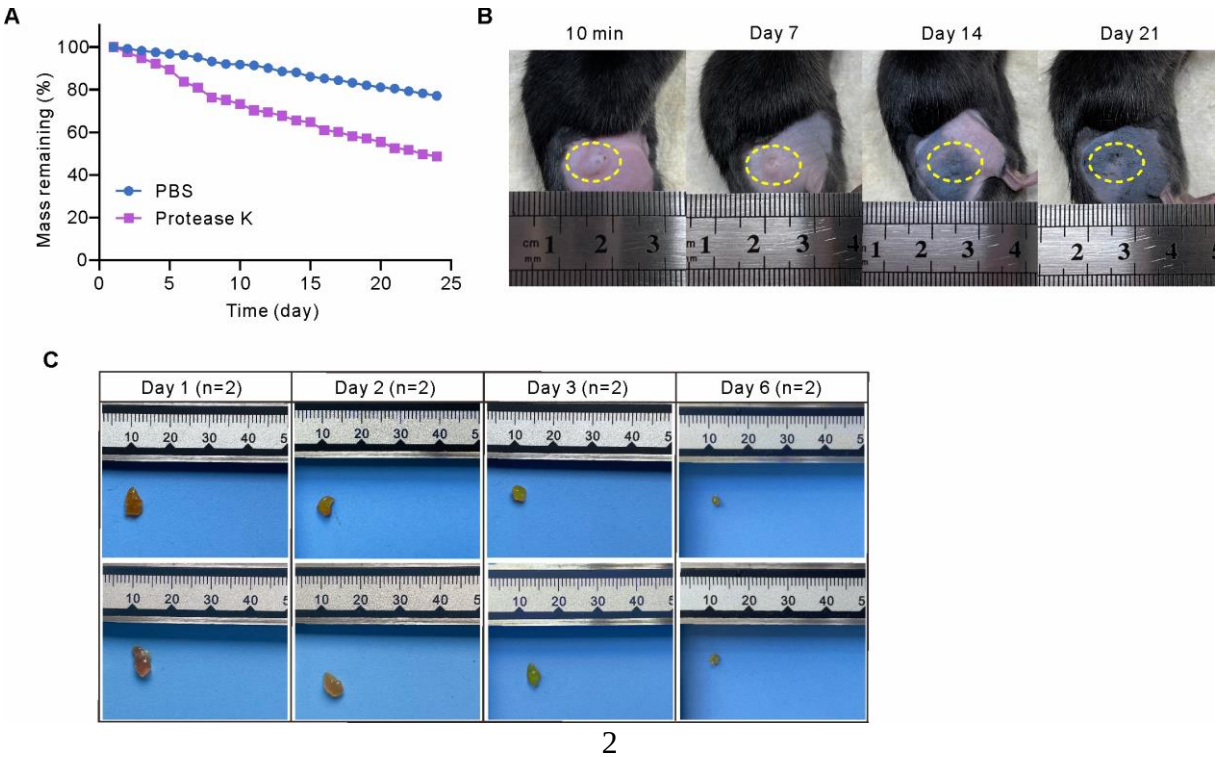

Figure S3. Degradation of hydrogels *in vitro* and *in vivo*. A) Percentage of remaining mass of hydrogel *in vitro*. B) *In vivo* degradation of hydrogel. C) Remained CH-OD-SSZ hydrogels in mice with formed malignant ascites at day 1, 2, 3 and 6 after peritoneal injection.

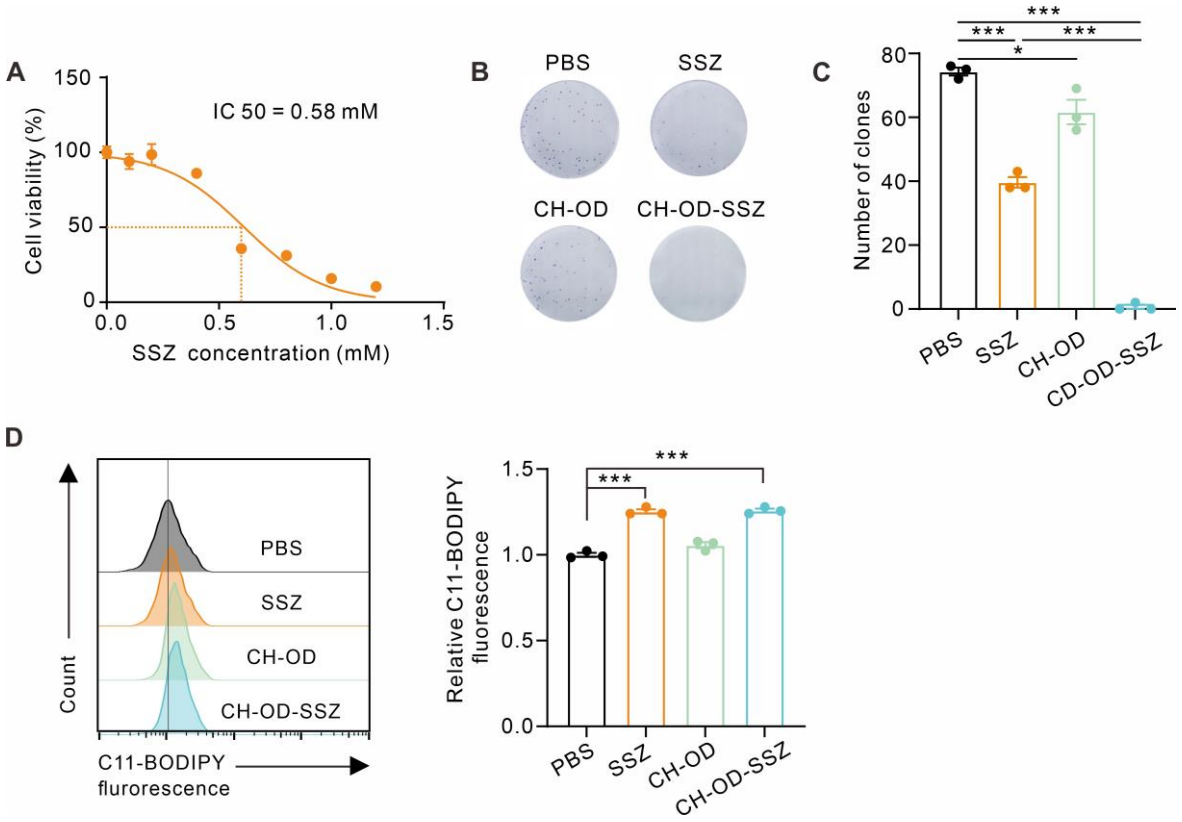

Figure S4. In vitro killing effect of different formulations. A) The IC50 value of SSZ in H22 cells (24 h). B) Representative images showing cell clones of H22 cells treated with PBS, SSZ, CH-OD or CH-OD-SSZ hydrogel. C) Quantification of colony formation analysis. D) Lipid peroxidation was assessed by flow cytometry using BODIPY 581/591 C11 fluorescent probe. The indicated results represent the means ± SEM of three independent experiments. * $P < 0.05$, *** $P < 0.001$, analyzed by one-way ANOVA.

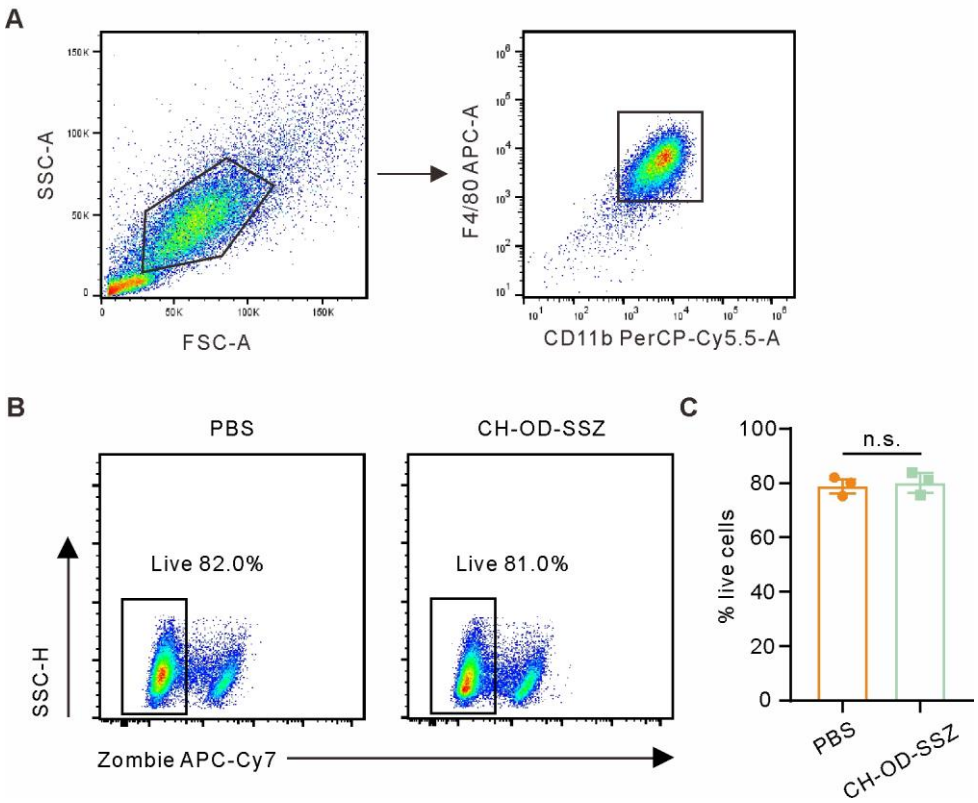

Figure S5. A) Gating strategy for the flow cytometry analysis of BMDMs. BMDMs were characterized as $CD11b^{+}F4/80^{+}$ cells. B,C) Cell viability of BMDMs treated with CH-OD-SSZ for 24 h was detected by Zombie dye staining with flow cytometry. The indicated results represent the means ± SEM of three replicates, n.s., not significant.

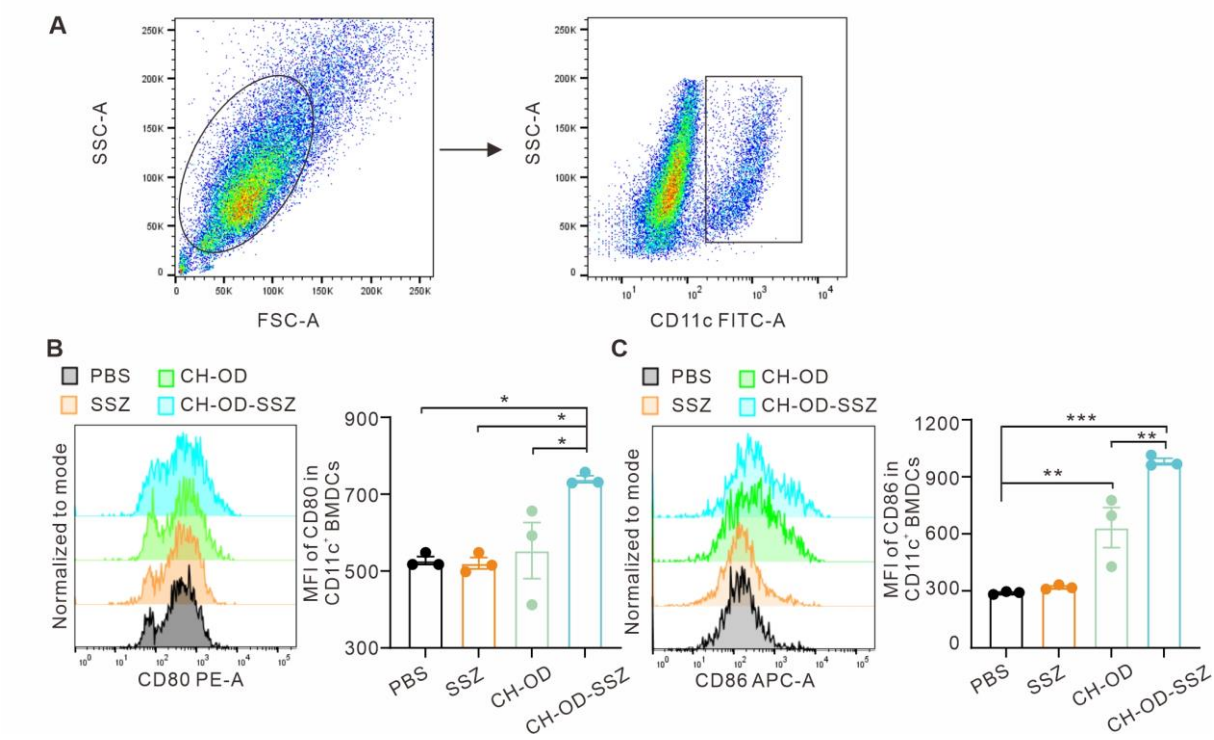

Figure S6. A) Gating strategy for the flow cytometry analysis of BMDCs. BMDCs were characterized as $CD11c^+$ cells. B,C) Flow cytometry analysis for the expression of CD80 and CD86 in BMDCs. BMDCs were cocultured with PBS, SSZ, CH-OD or CH-OD-SSZ-treated H22 cells for 24 h. The indicated results represent the means ± SEM of three independent experiments. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, analyzed by one-way ANOVA.

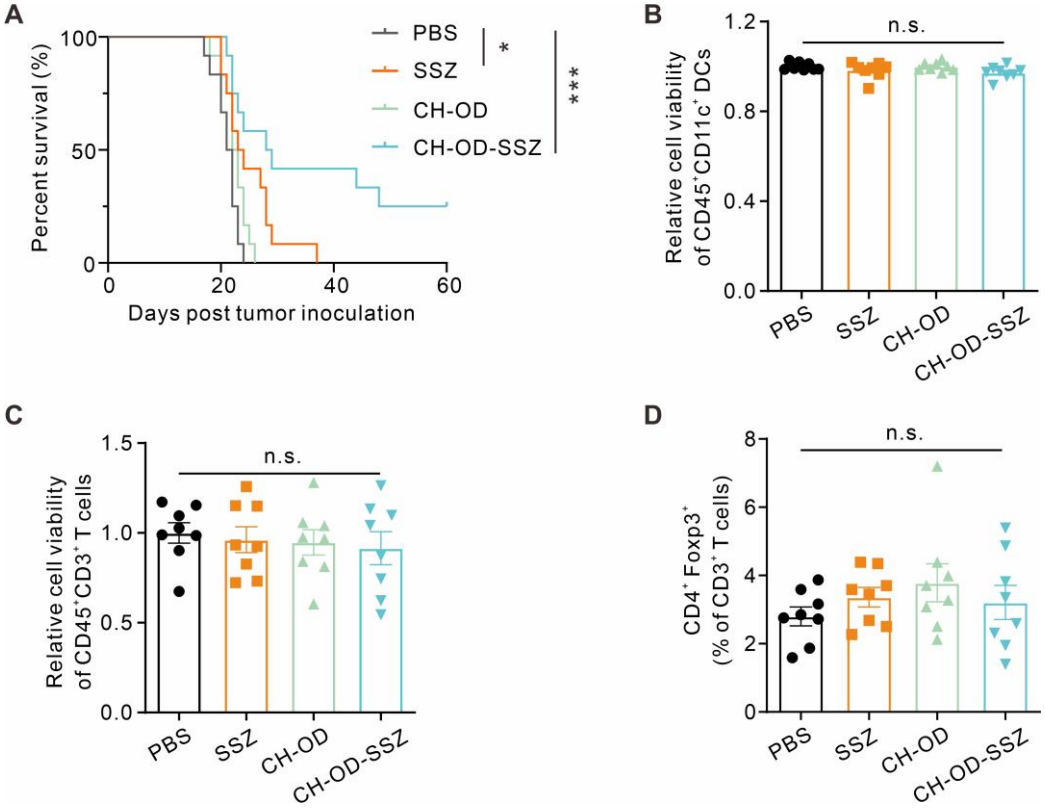

Figure S7. A) Survival curves of mice with H22-luc malignant ascites in different treatment groups (n = 12 mice per group). Day 0 indicates mice random grouping; Day 1 indicates treatment beginning. B,C) Cell viability of DCs ($CD45^+CD11c^+$) (B) and T cells ($CD45^+CD3^+$) (C) within ascites of each group were determined according to Zombie dye negative staining (n=8 mice per group). D) Percentages of Tregs ($CD4^+Foxp3^+$) within ascites of each group (n=8 mice per group). The indicated results represent the means ± SEM of three independent experiments. * $P < 0.05$, *** $P < 0.001$, n.s., not significant.

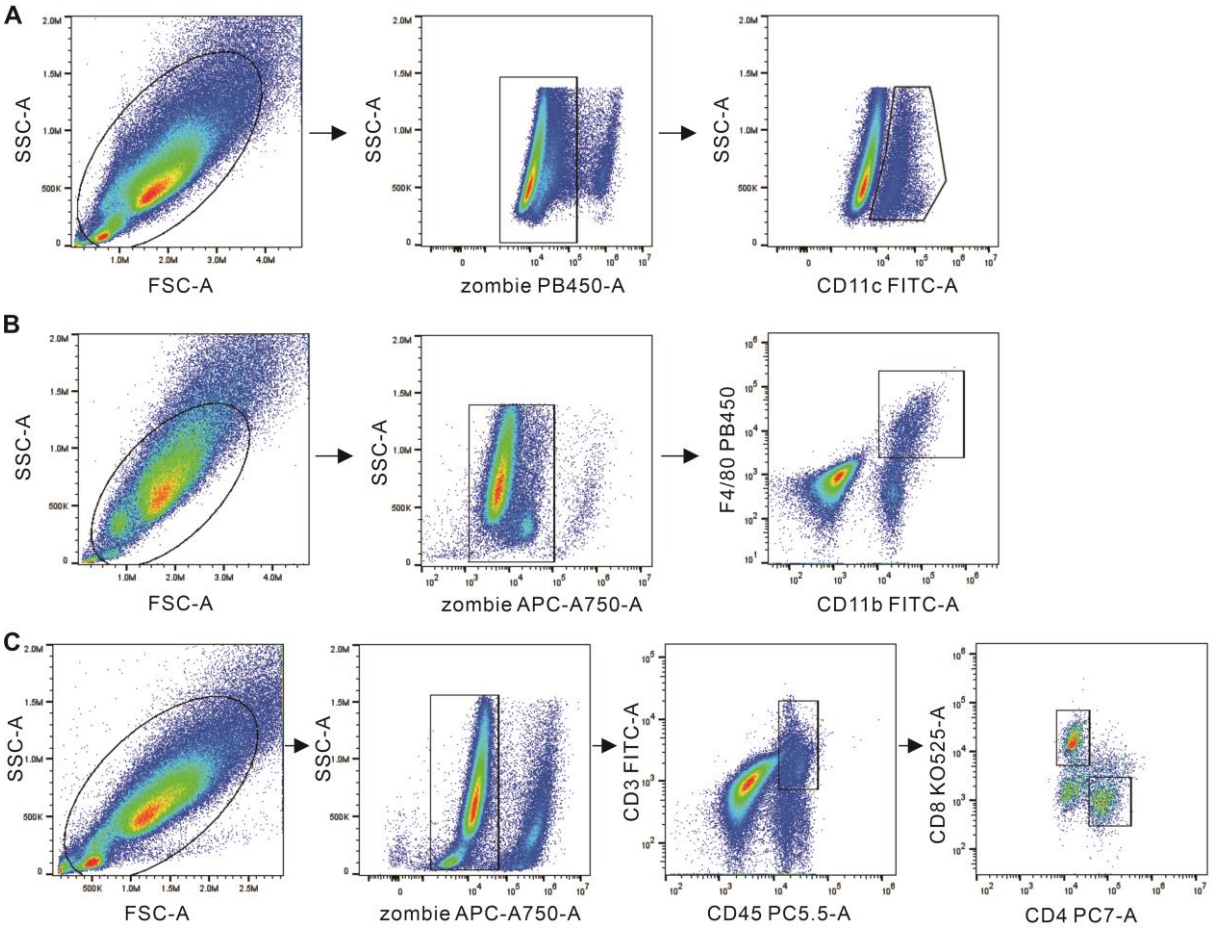

Figure S8. Gating strategy for the analysis of immune cells within the peritoneal lavage. A) DCs, B) macrophages, C) $CD4^+$ T cells and $CD8^+$ T cells.

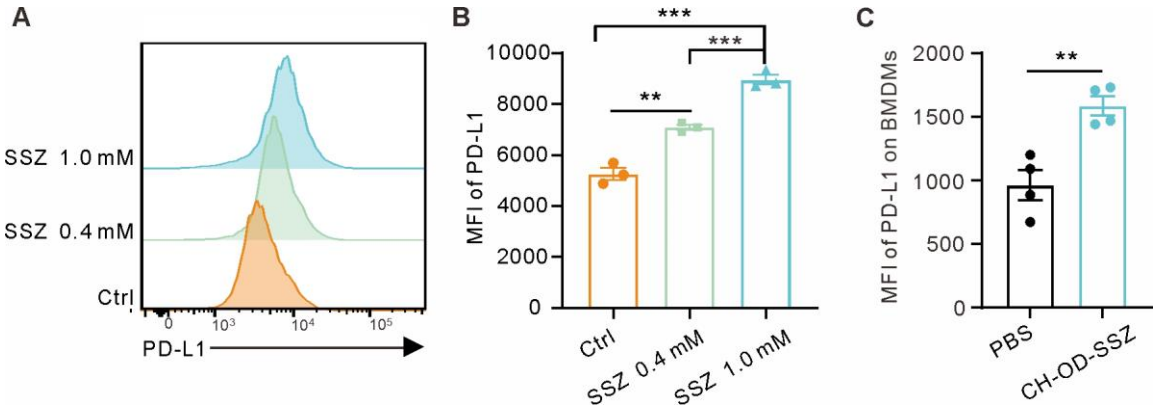

Figure S9. A,B) The expression of PD-L1 on the surface of H22 cells treated with SSZ (0.4 and 1 mM) for 24 h was detected by flow cytometry. C) The expression of PD-L1 on the surface of BMDMs treated with PBS or CH-OD-SSZ hydrogel for 24 h was detected by flow cytometry. The indicated results represent the means ± SEM of three replicates, ** $P < 0.01$, *** $P < 0.001$.

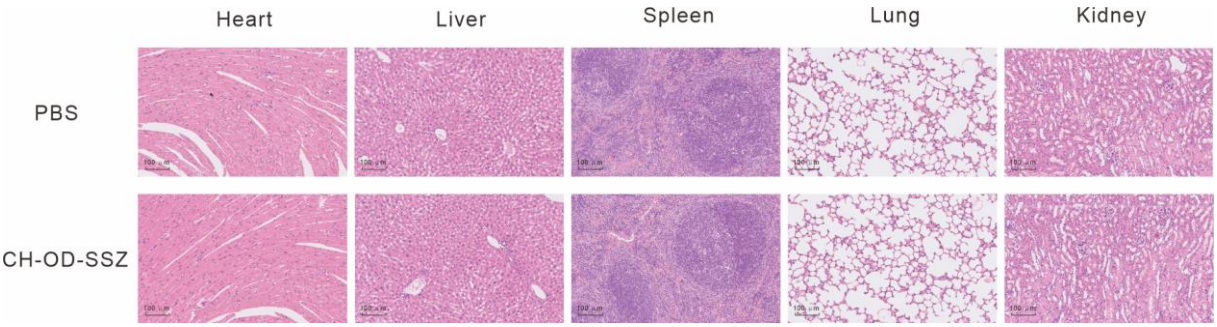

Figure S10. Representative images of HE staining of the heart, liver, spleen, lung and kidney of mice receiving PBS or CH-OD-SSZ hydrogels treatment. Scale bar, 100μm.