

**Verteporfin-loaded hydrogel targeting YAP-mediated MDSCs recruitment for the treatment of residual tumors after incomplete radiofrequency ablation**

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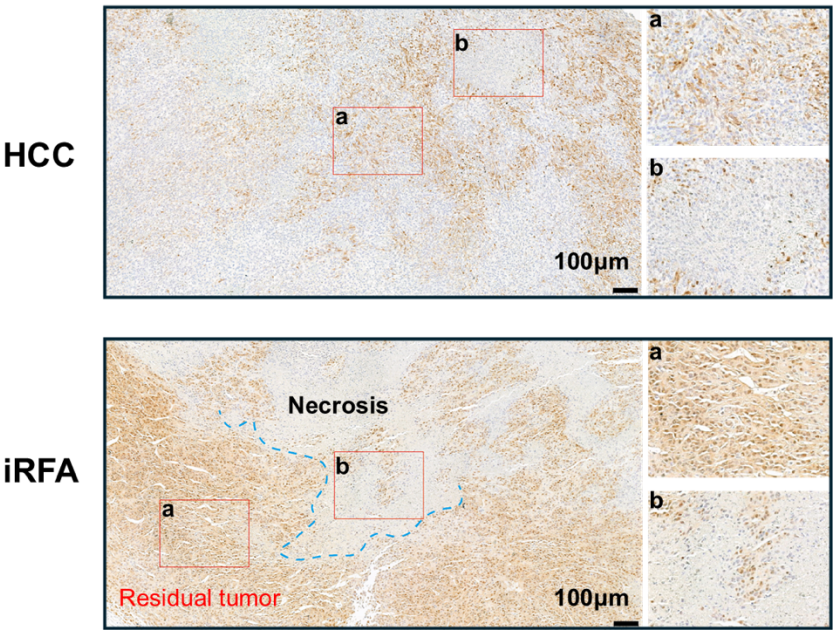
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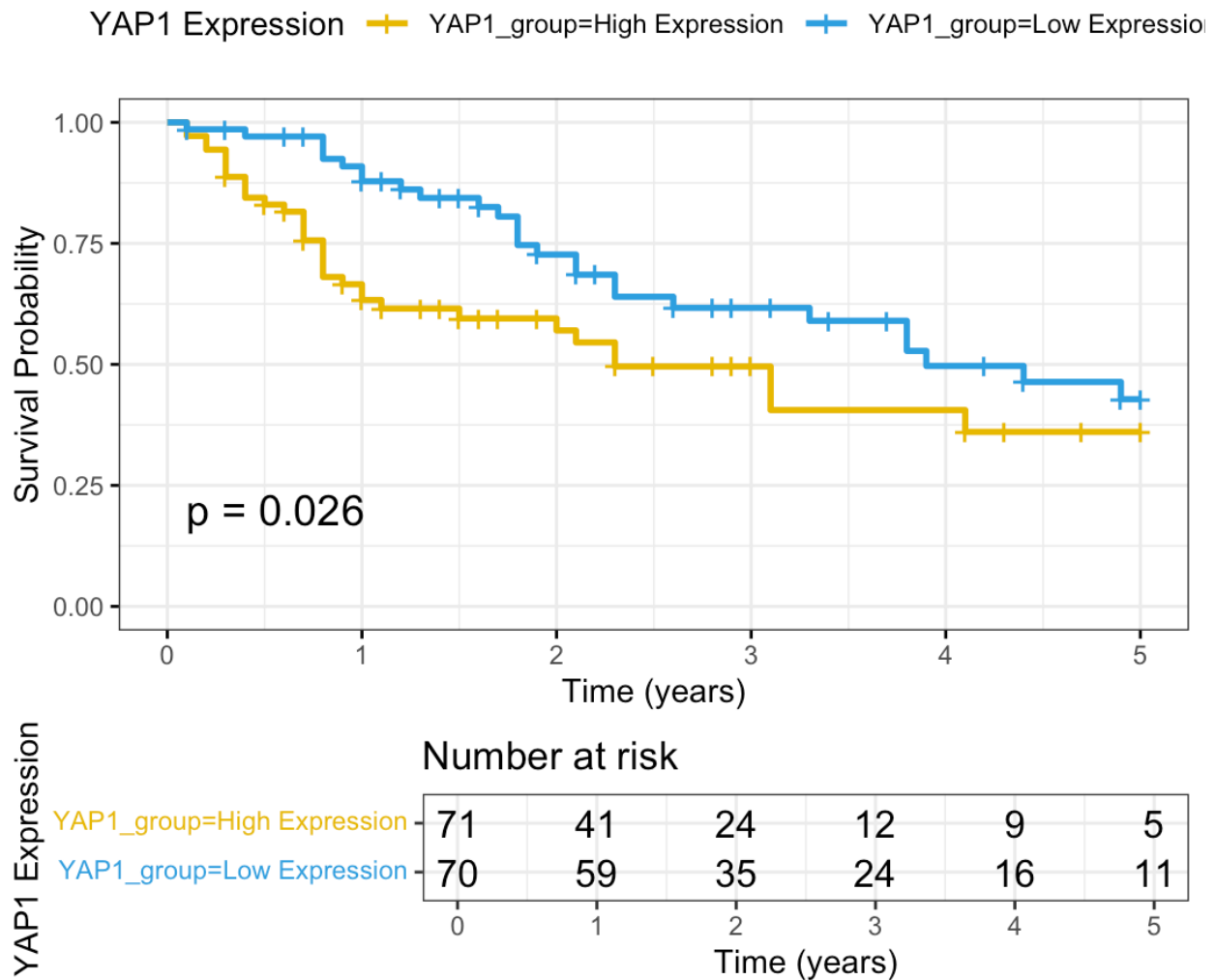
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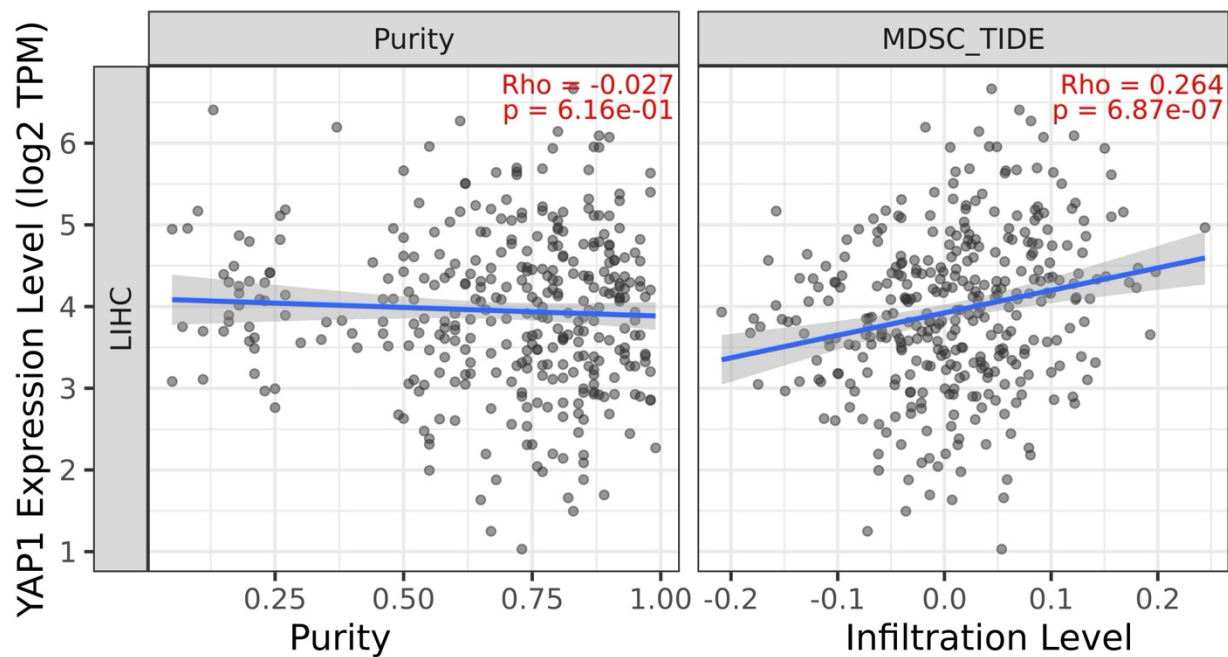
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**Figure. S1.** Immunohistochemical assessment of tumor tissue after iRFA Immunohistochemical staining revealed disrupted tissue architecture, loss of cellular morphology, and pallor in staining in the iRFA group (b), compared to the untreated HCC group.

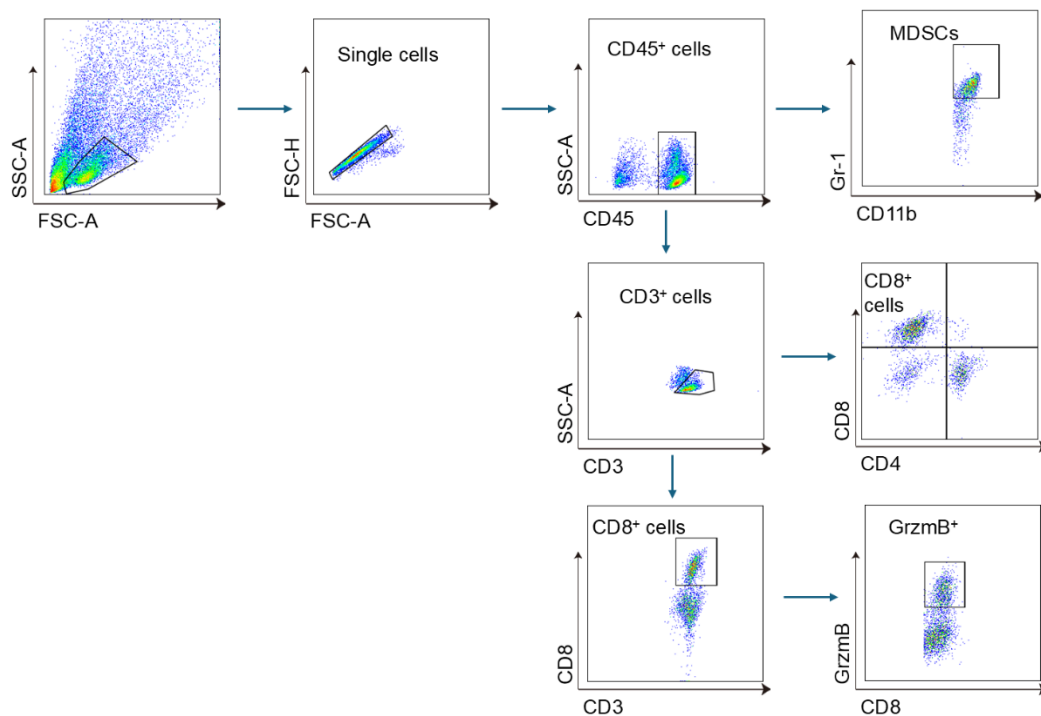
## 5-Year Survival of T2-T4 Patients by YAP1 Expression



**Figure. S2.** Kaplan-Meier survival analysis of YAP expression in advanced HCC (T2-T4 stages).

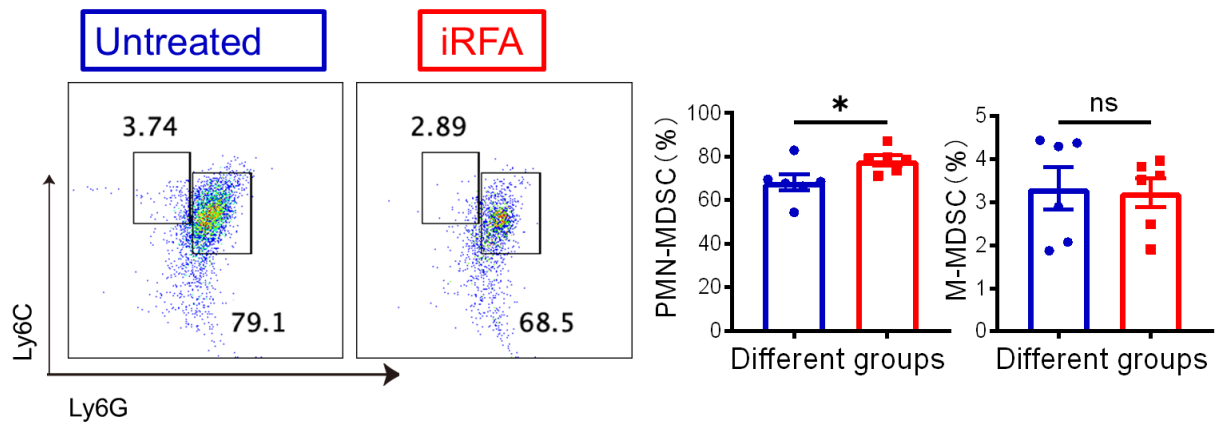


**Figure. S3.** Correlation between YAP1 expression with MDSCs infiltration. \*\*\*: p-value < 0.001.

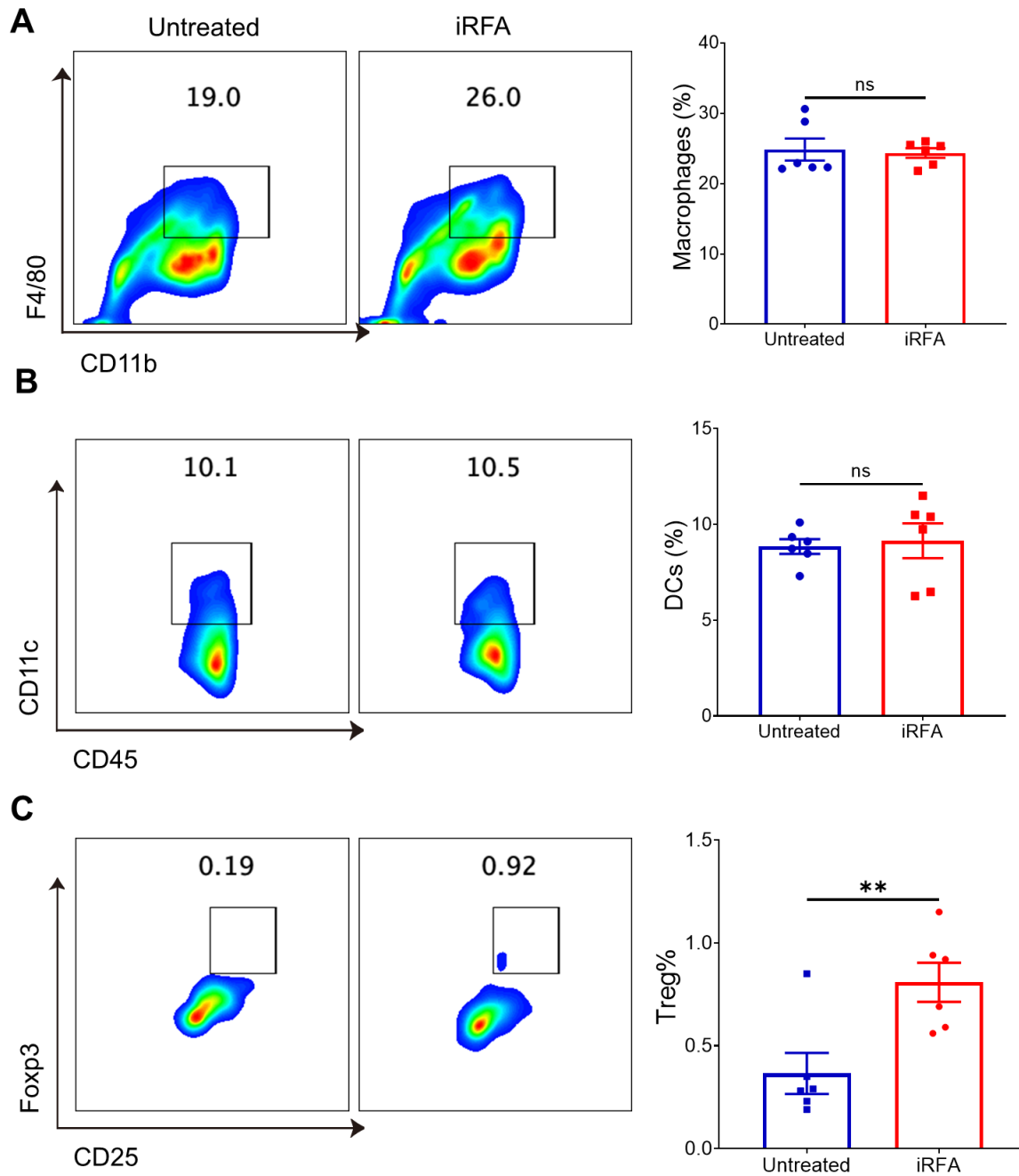


**Figure. S4.** The gating strategy for key flow cytometric analysis.



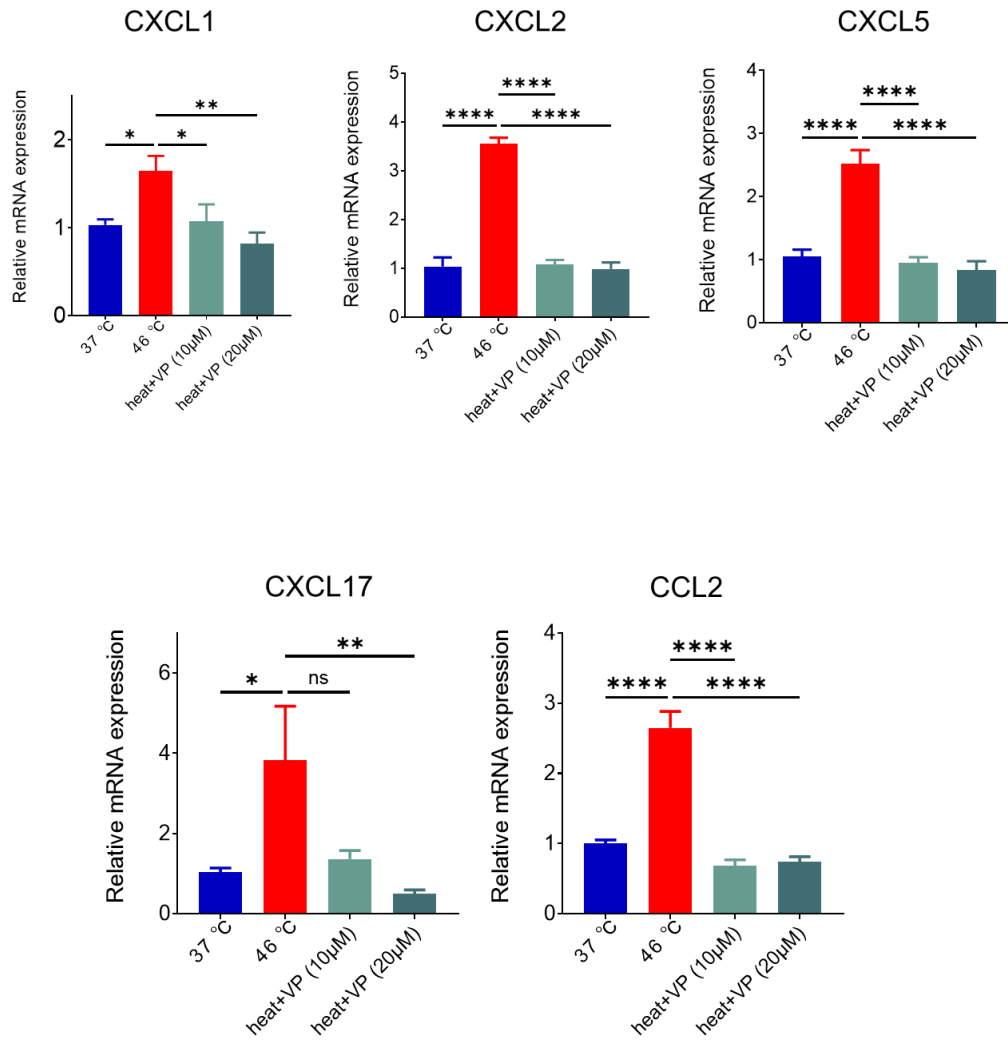


**Figure. S5.** Representative flow cytometry and Statistical plots illustrating MDSC subpopulation.

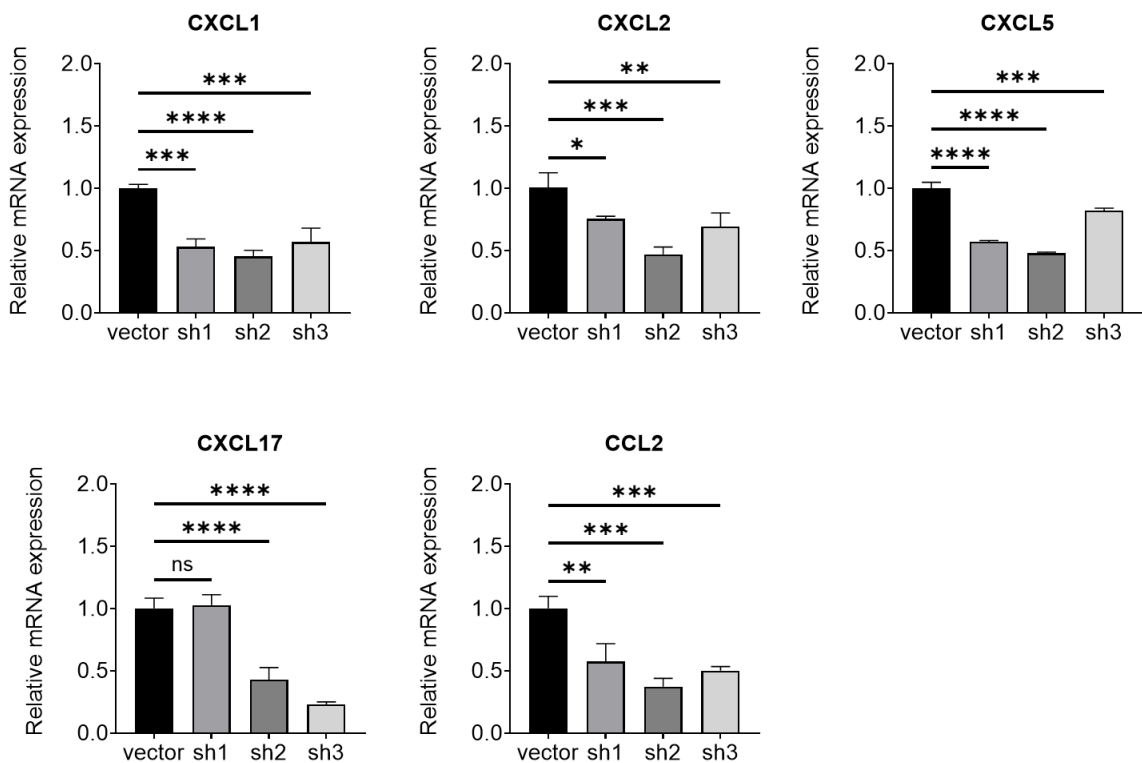


**Figure. S6.** (A) Representative flow cytometry and Statistical plots illustrating macrophages (F4/80<sup>+</sup> CD11b<sup>+</sup>) in residual tumors in untreated (Ctrl) and iRFA groups. (B) Representative flow cytometry and Statistical plots illustrating DCs (CD11c<sup>+</sup> CD45<sup>+</sup>) in residual tumors in untreated

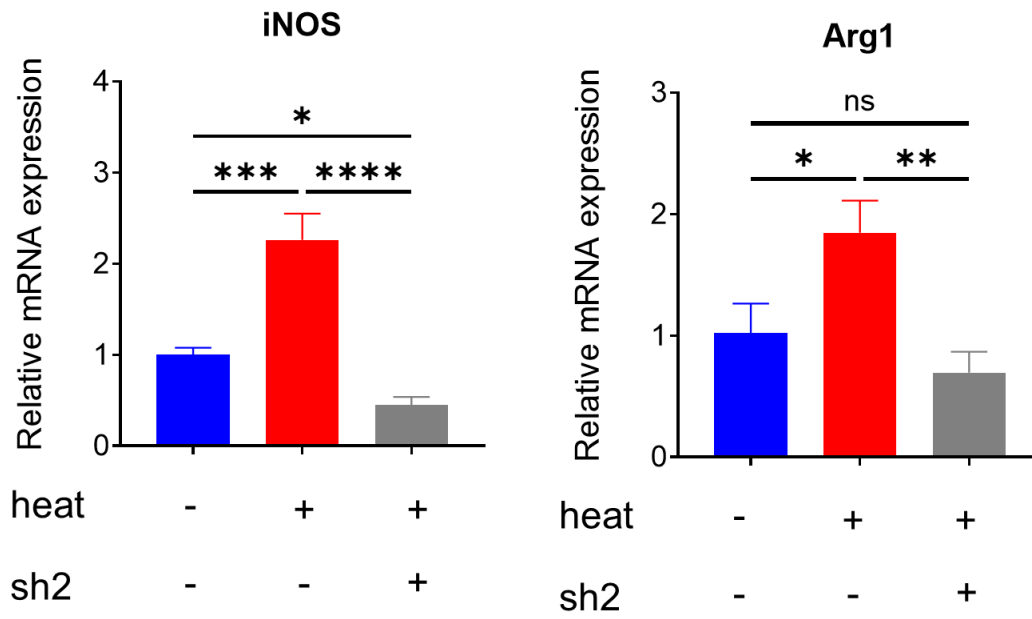
55 (Ctrl) and iRFA groups. (C) Representative flow cytometry and Statistical plots illustrating Tregs  
56 (Foxp3<sup>+</sup> CD25<sup>+</sup>) in residual tumors in untreated (Ctrl) and iRFA groups.  
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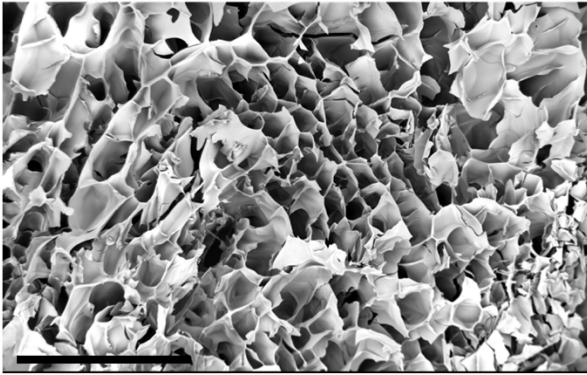
**Figure. S7.** qPCR analysis showing relative expression MDSCs-related cytokines following VP treatment in heated Hep3B cells. \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001, \*\*\*\*P < 0.0001



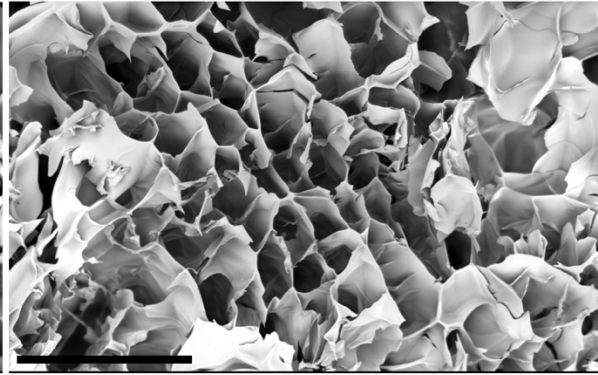
**Figure. S8.** qPCR analysis showing relative expression MDSCs-related cytokines following knockdown YAP



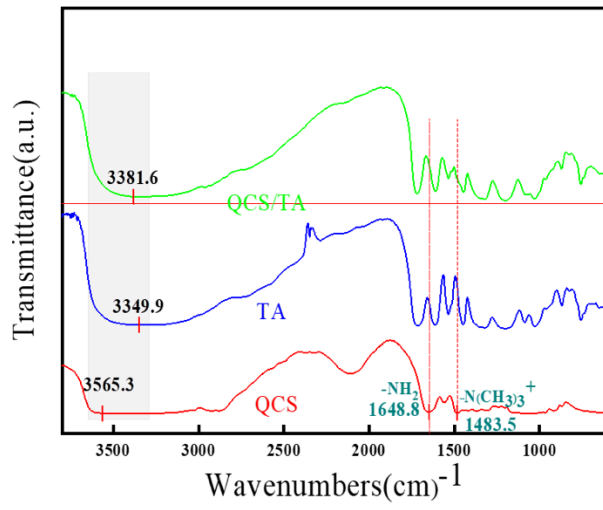
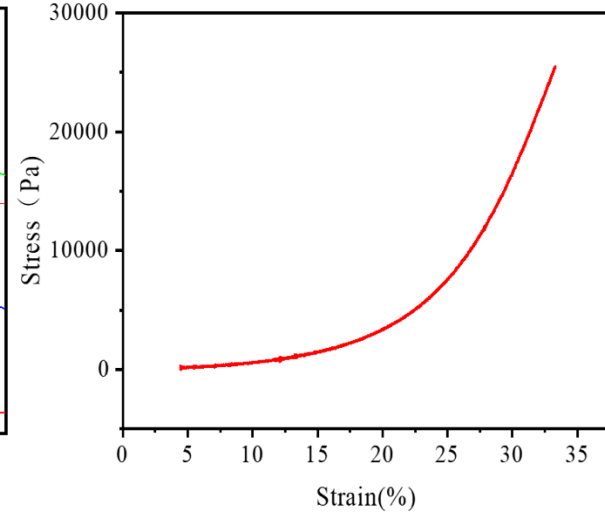
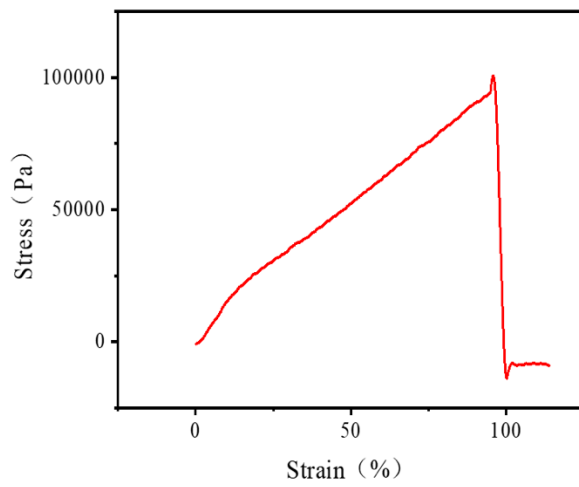
**Figure. S9.** qPCR analysis showing relative expression of iNOS and Arg1 following different treatment in MDSCs. \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001, \*\*\*\*P < 0.0001

**A**

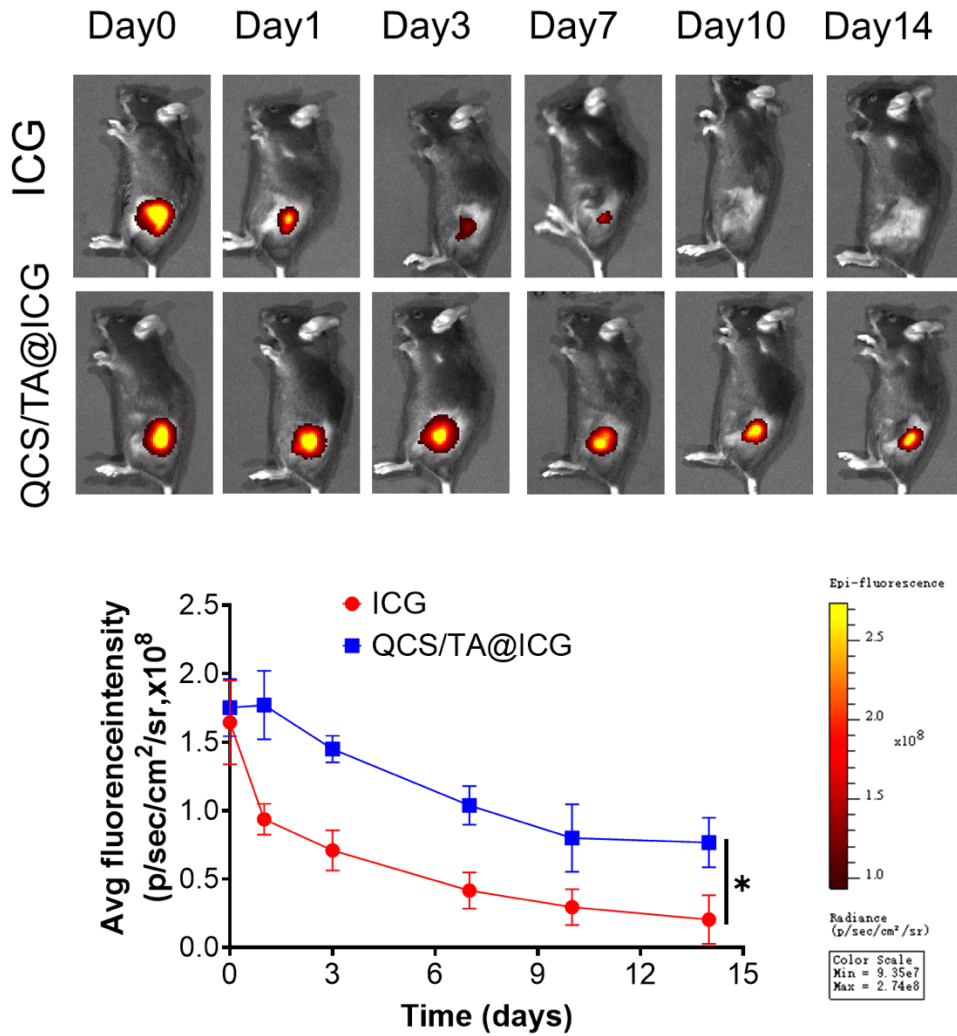
500μm

**B**

300μm

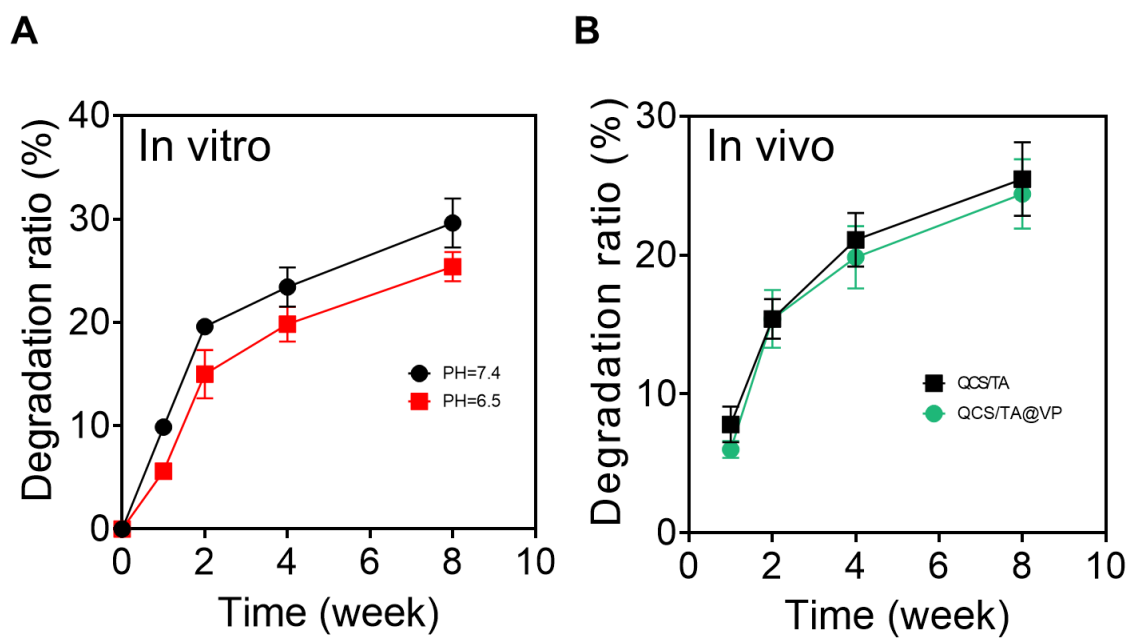
**C****D****E**

**Figure. S10.** The characterization of hydrogel. A.B The morphology of QCS/TA hydrogel was observed by SEM. (Scale bar: 500  $\mu\text{m}$  (left) and 300  $\mu\text{m}$  (right)). C. Fourier transform infrared (FTIR) spectra of the QCS, TA, and QCS/TA gel. D. Compression of QCS/TA hydrogels. E. Tensile testing of QCS/TA hydrogels

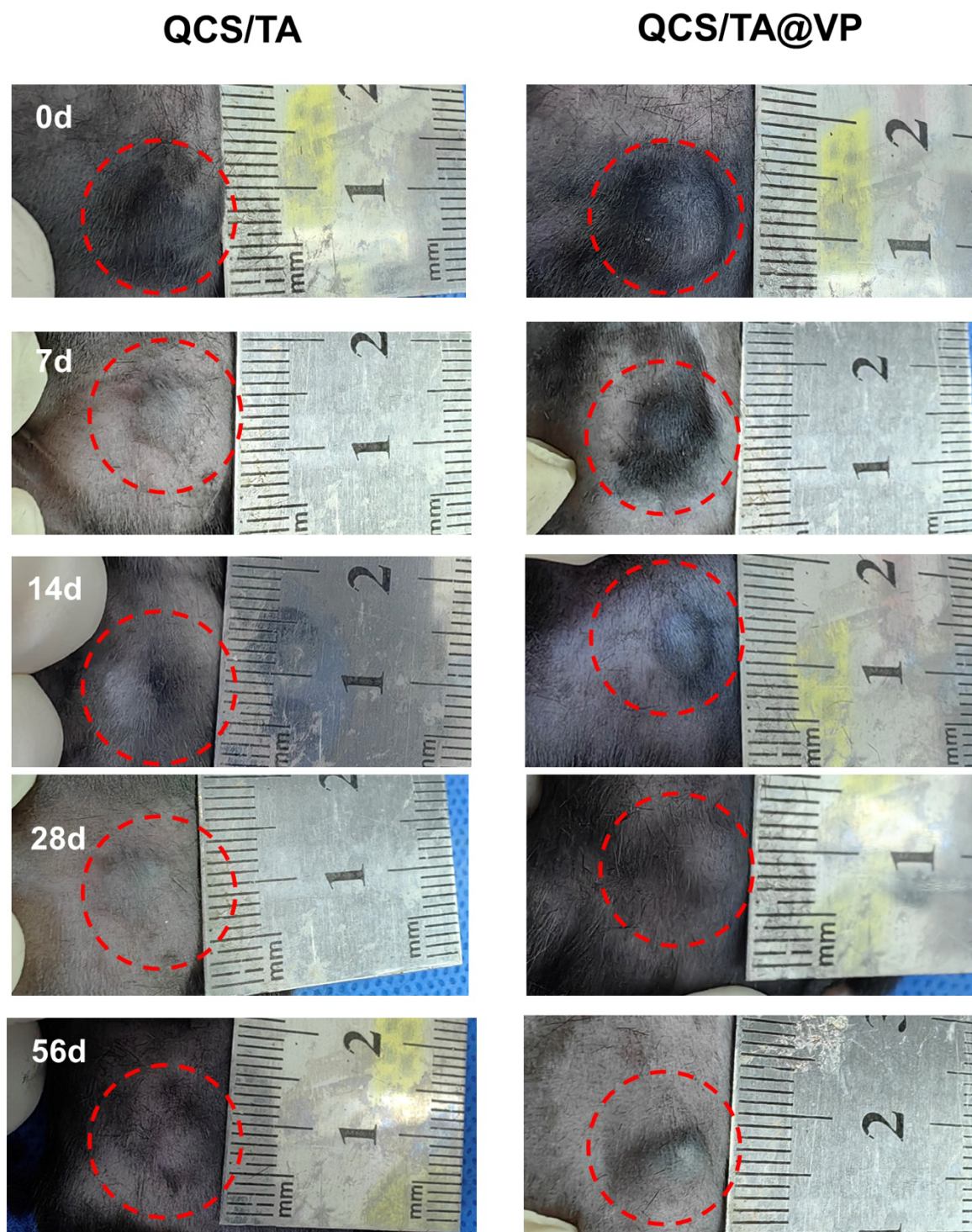


**Figure. S11.** IVIS images and statistical analysis of fluorescence signal recorded at different times after injection of ICG and ICG@Gel (n = 3).

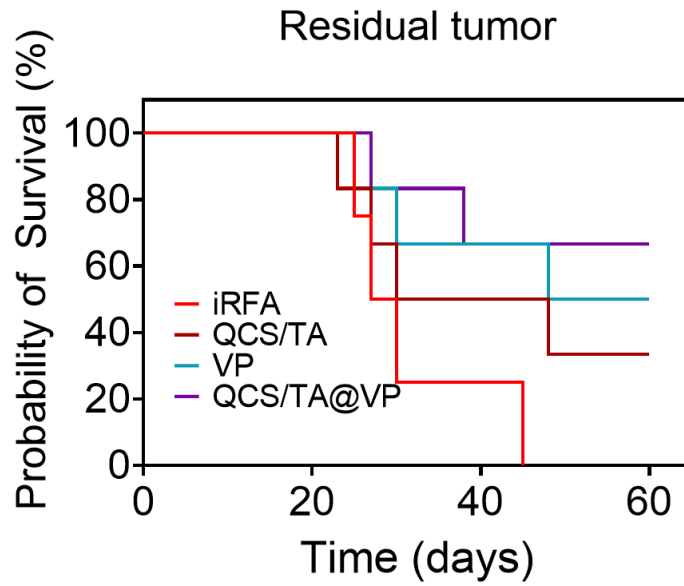




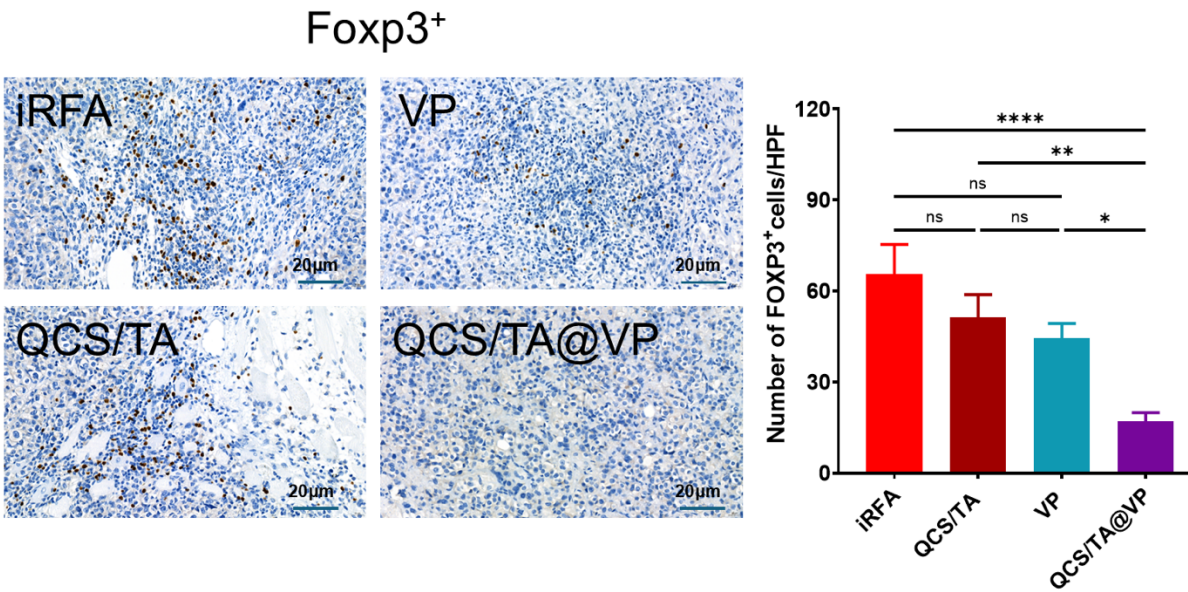
**Figure. S12.** Degradation of the QCS/TA and QCS/TA@VP hydrogel.



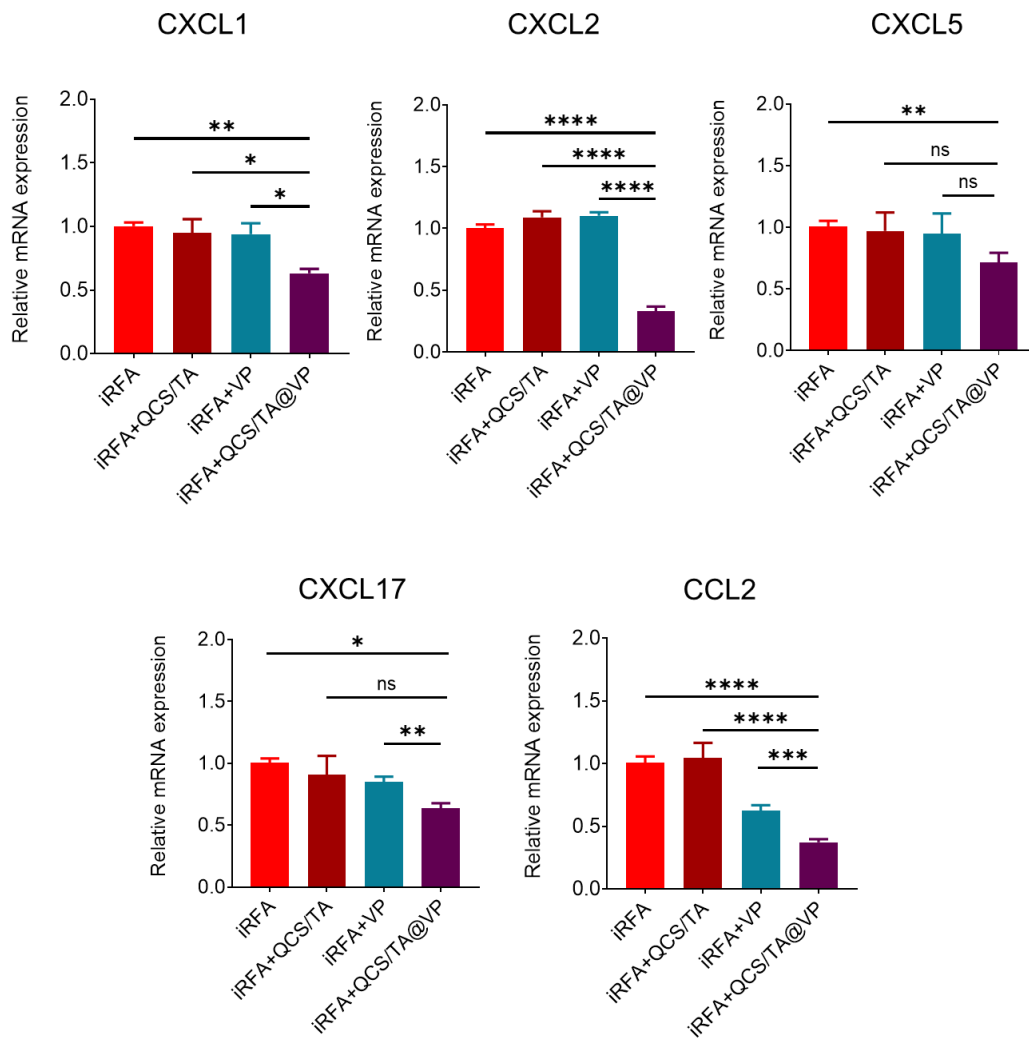
**Figure. S13** Macroscopic images of the mice receiving QCS/TA and QCA/TA@VP hydrogel



**Figure. S14.** Survival analysis of experimental mice in different groups (n = 6)

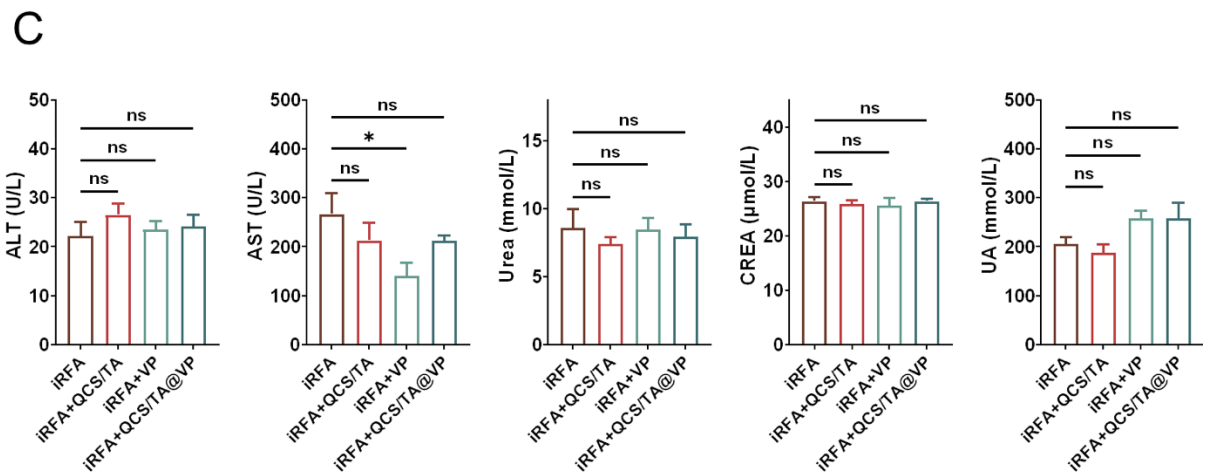
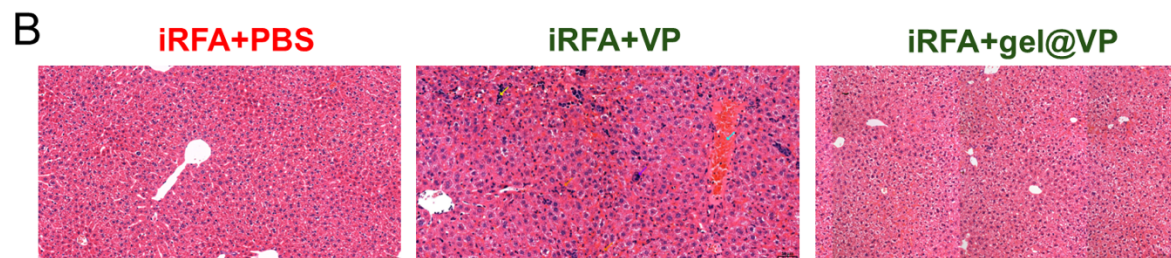
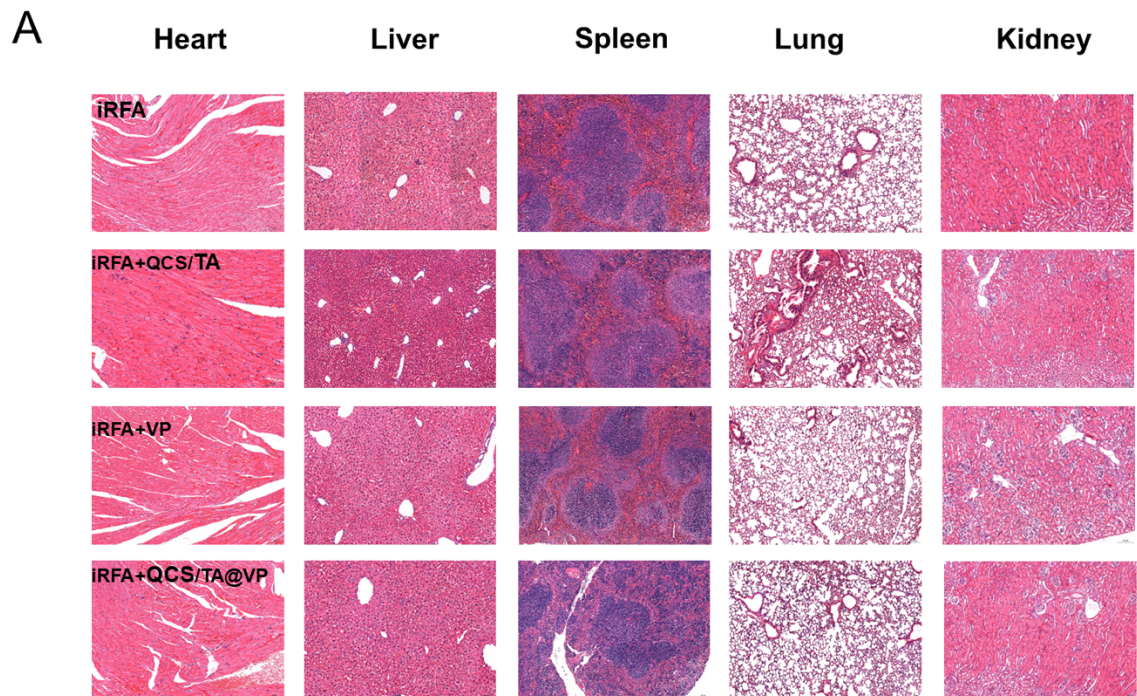


**Figure. S15.** IHC staining of Treg in residual tumor tissue, scale bar: 20  $\mu$ m.



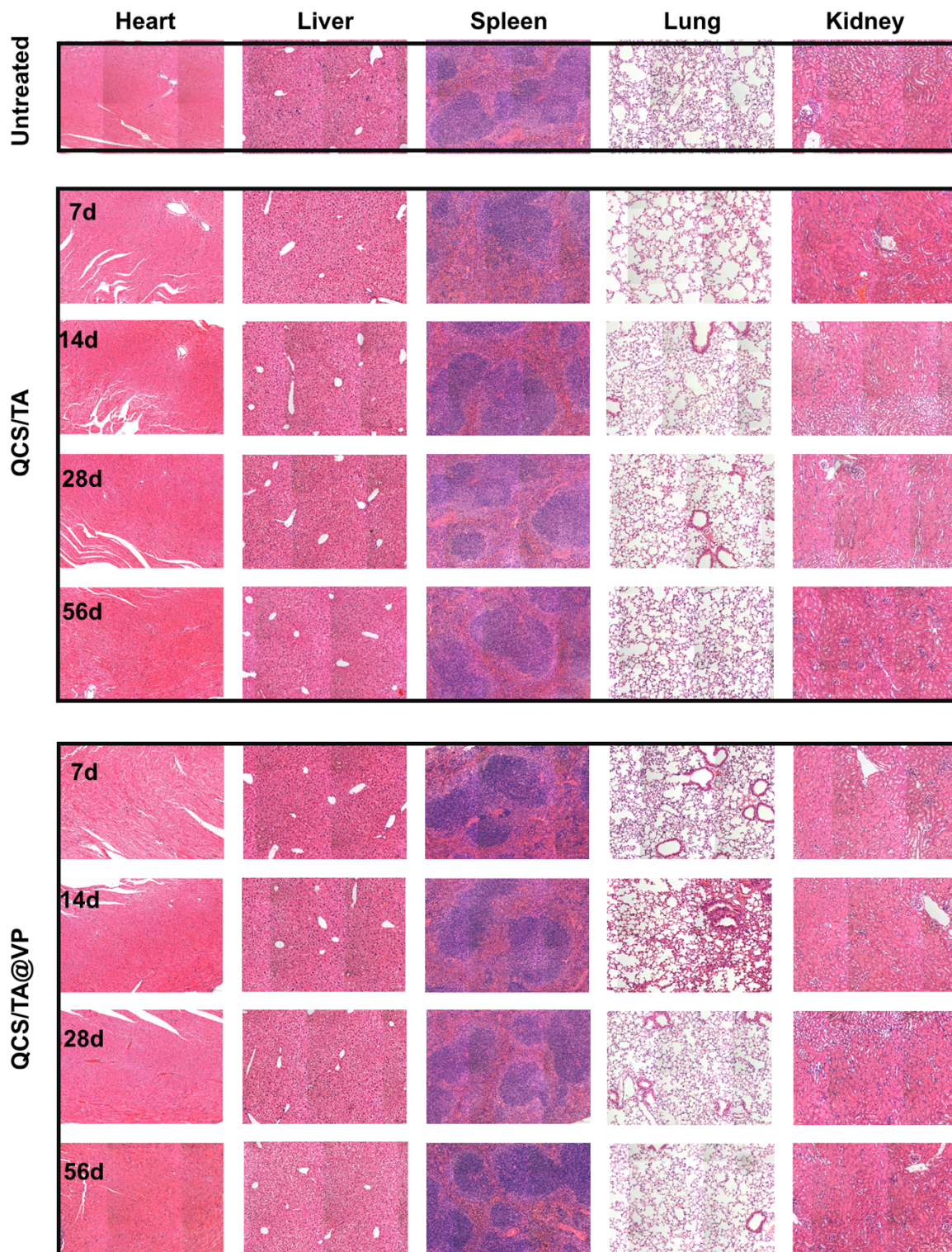
**Figure. S16.** qPCR analysis showing relative expression MDSCs-related cytokines following various treatments in residual cancer. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , \*\*\*\* $P < 0.0001$





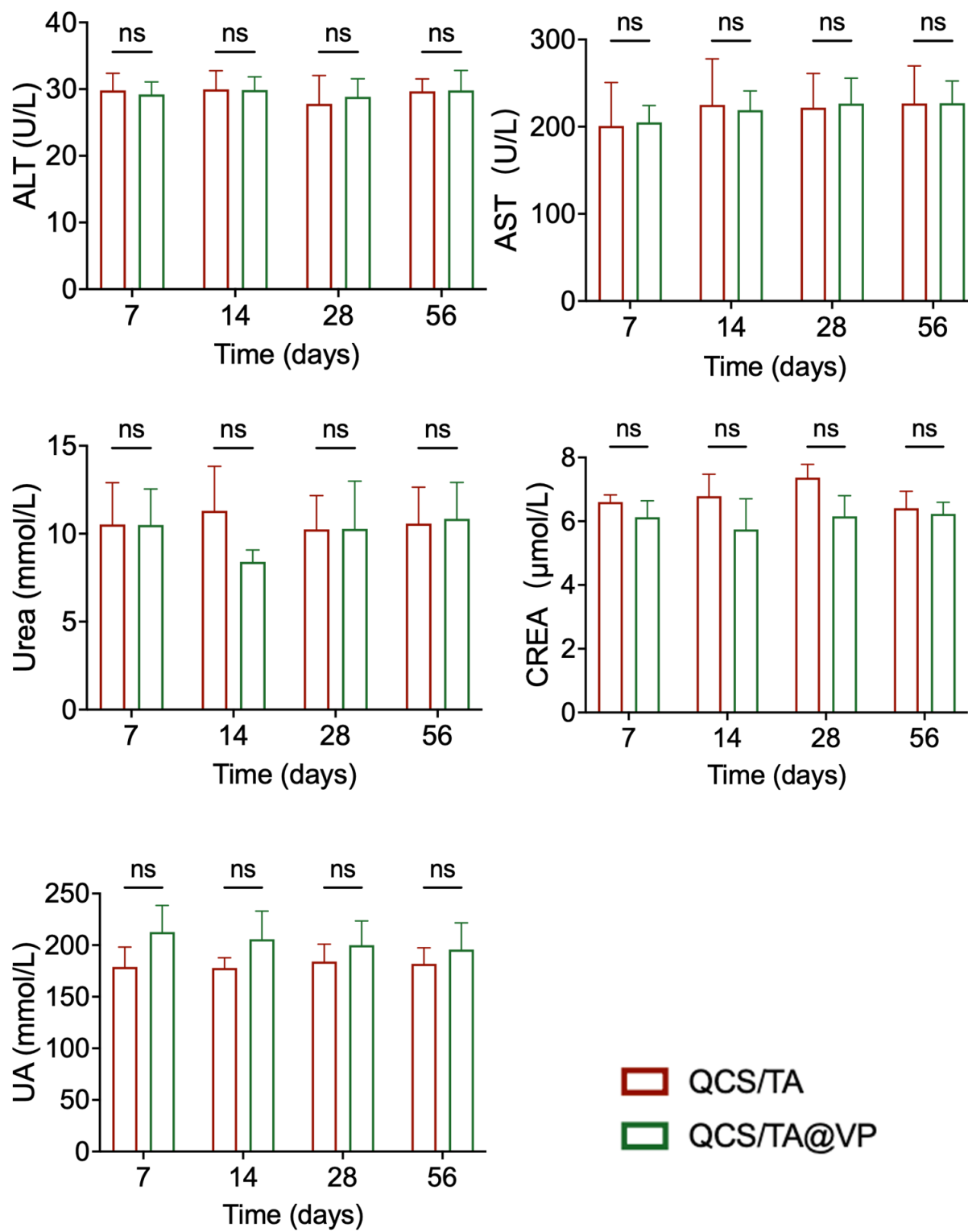
**Figure. S17.** (A) Representative HE staining images of heart, spleen, lung, and kidney in iRFA, iRFA + QCS/TA, iRFA + VP, and iRFA + QCS/TA@VP groups (scale bar: 100  $\mu$ m). (B) Liver injury by H&E staining in different treatment groups. Scar bar: 500 $\mu$ m. (C) Blood analysis of Aspartate aminotransferase (AST), alanine aminotransferase (ALT), uric acid (UA), urea nitrogen (Urea), creatinine (CREA). \*P < 0.05, \*\*P < 0.01



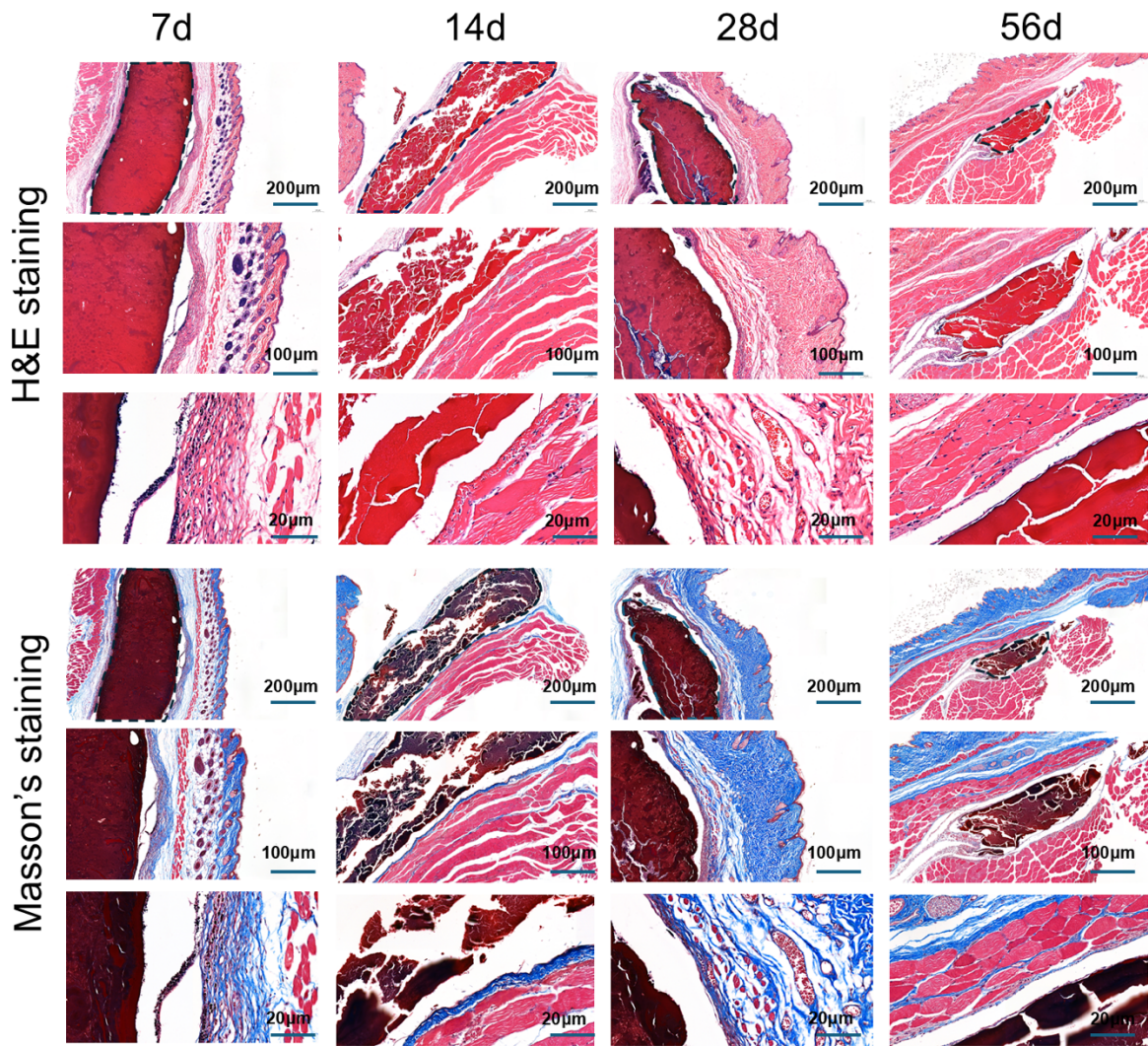


111 **Figure. S18.** Representative HE staining images of heart, spleen, lung, and kidney in Untreated,  
112 QCS/TA, and QCS/TA@VP groups (scale bar: 100  $\mu\text{m}$ ).  
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**Figure. S19.** Blood analysis of Aspartate aminotransferase (AST), alanine aminotransferase (ALT), uric acid (UA), urea nitrogen (Urea), creatinine (CREA).



**Figure.S20.**Hematoxylin-eosin (H&E) staining (above) and Masson's staining (below) representative images of the tissues

Table S1. The Primer Sequence of Gene for RT-qPCR Analysis

| Gene          | Forward primer sequence (5'-3') | Reverse primer sequence (5'-3') |
|---------------|---------------------------------|---------------------------------|
| <i>Cxcl1</i>  | GGCTGGGATTACCTCAAGAACATC        | TGAGTGTGGCTATGACTTCGGTTTG       |
| <i>CXCL1</i>  | GAACATCCAAAGTGTGAACGTGAAG       | CTTAACCTATGGGGGATGCAGGATTG      |
| <i>Cxcl2</i>  | CAGACAGAAGTCATAGCCACTCTCAAG     | CAGTTAGCCTTGCCTTTGTTCAGTATC     |
| <i>CXCL2</i>  | CACACTCAAGAATGGGCAGAAAG         | TTCTGGTCAGTTGGATTTGCCATTT       |
| <i>Cxcl5</i>  | TGTGTTTGCTTAACCGTAACTCCA        | ACAGACCTCCTTCTGGTTTTTCAG        |
| <i>CXCL5</i>  | AGTAATCTGCAAGTGTCGCCATAG        | CTTTAGAAAAGGGGCTTCTGGATCA       |
| <i>Cxcl17</i> | CCTCTCCCTTCCTTCTGTTGC           | GCGACTTCCTGTGGTGCTTT            |
| <i>CXCL17</i> | ACCGAGGCCAGGCTTCTA              | GGCTCTCAGGAACCAATCTTT           |
| <i>Ccl2</i>   | TGATCCCAATGAGTAGGCTGGA          | GACCTCTCTCTTGAGCTTGGTG          |
| <i>CCL2</i>   | CTCATAGCAGCCACCTTCATTCC         | GAAGATCACAGCTTCTTTGGGACA        |
| <i>Cyr61</i>  | GAGTTACCAATGACAACCCAGAGTG       | TTTCTTGGTCTTGCTGCATTTCTTG       |
| <i>CYR61</i>  | CTCGCCTTAGTCGTCACCC             | CGCCGAAGTTGCATTCCAG             |
| <i>Ctgf</i>   | ACCCGAGTTACCAATGACAATACC        | GGATGCACTTTTTGCCCTTCTTAAT       |
| <i>CTGF</i>   | CAGCATGGACGTTCGTCTG             | AACCACGGTTTGGTCCTTGG            |
| <i>Gapdh</i>  | TGACCTCAACTACATGGTCTACA         | CTTCCCATTCTCGGCCTTG             |
| <i>GAPDH</i>  | ACAACCTTGGTATCGTGGAAGG          | GCCATCACGCCACAGTTTC             |