Supplementary Information for:

Injectable Hydrogel-Encapsulating Pickering Emulsion for Overcoming Lenvatinib-Resistant Hepatocellular Carcinoma via Cuproptosis Induction and Stemness Inhibition

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Supplementary Figure

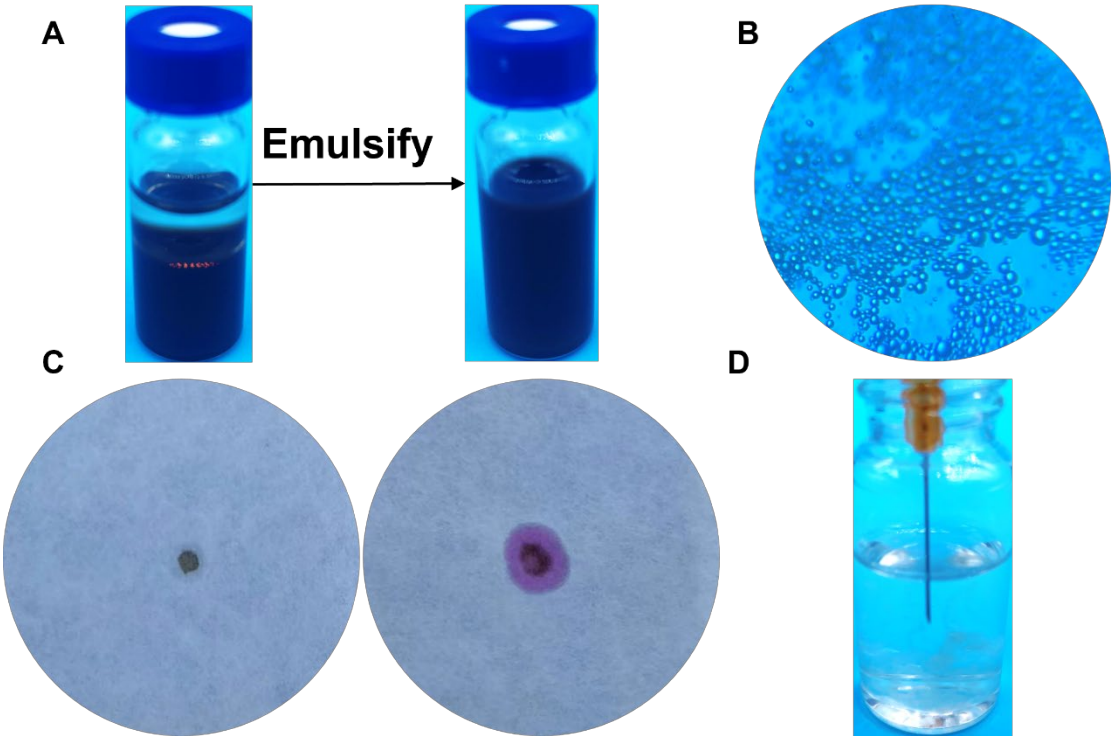


Figure S1. Characterization of injectable hydrogel DSF@CuO Gel. A) Photos before and after the formation of DSF@CuO; B) Images of DSF@CuO under a conventional optical microscope; C) Disk diffusion method used to characterize the morphology of DSF@CuO; D) Needle penetration test of DSF@CuO Gel in calcium ion solution



Figure S2. DSF@CuO Gel Gelation behavior in vivo after subcutaneous injection in mice.

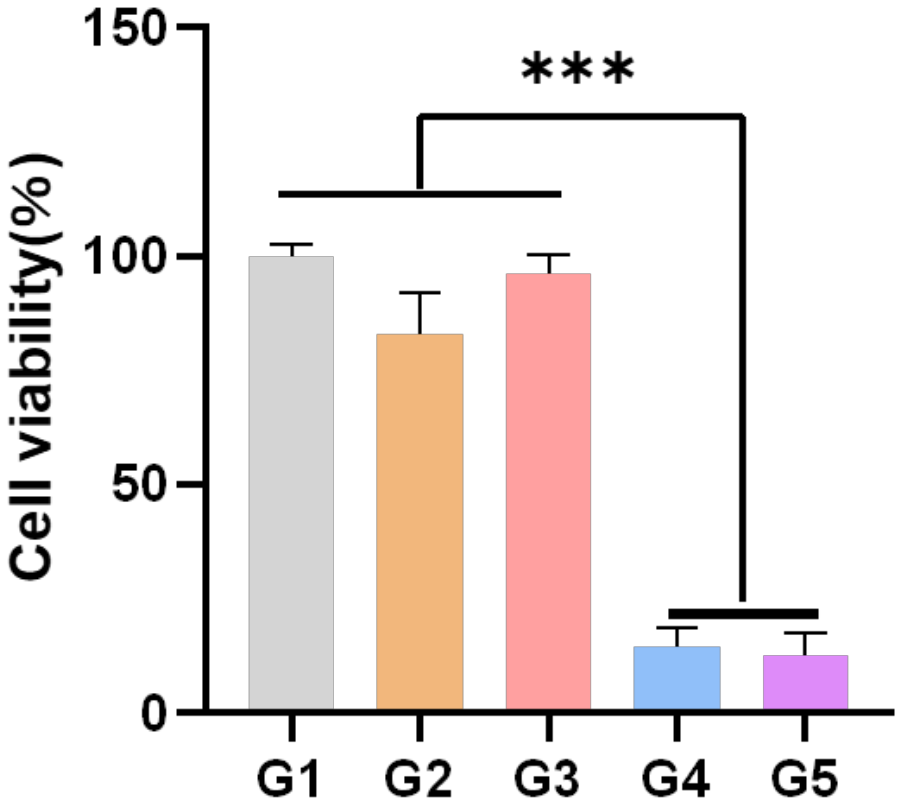


Figure S3. CCK-8 assay used to assess the cytotoxic effects of different treatment groups on LR Hep 3B. (G1: corn oil, G2: DSF, G3: CuO NPs, G4: Len@CuO, and G5: DSF@CuO). Data are presented as mean ± SD and are representative of three independent experiments. ***$p < 0.001$.

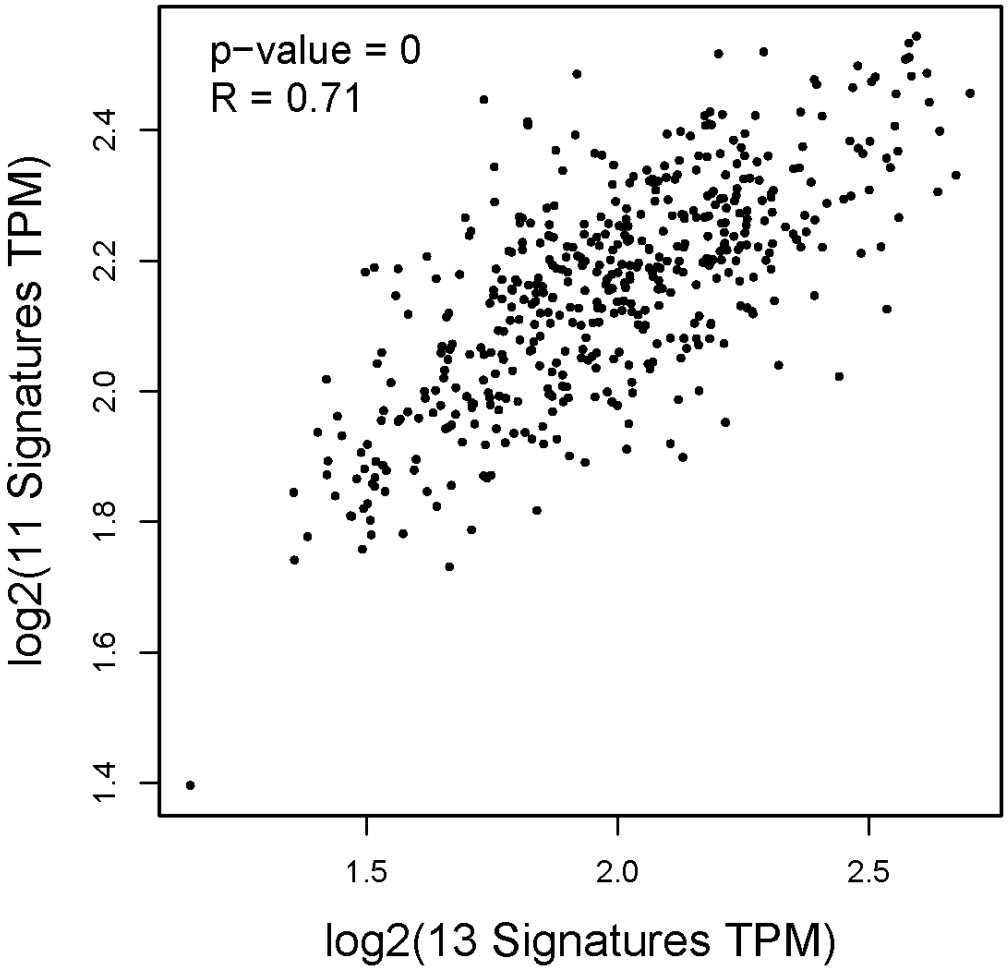


Figure S4. The correlation analysis between key cuproptosis genes and genes associated with effector T cells.

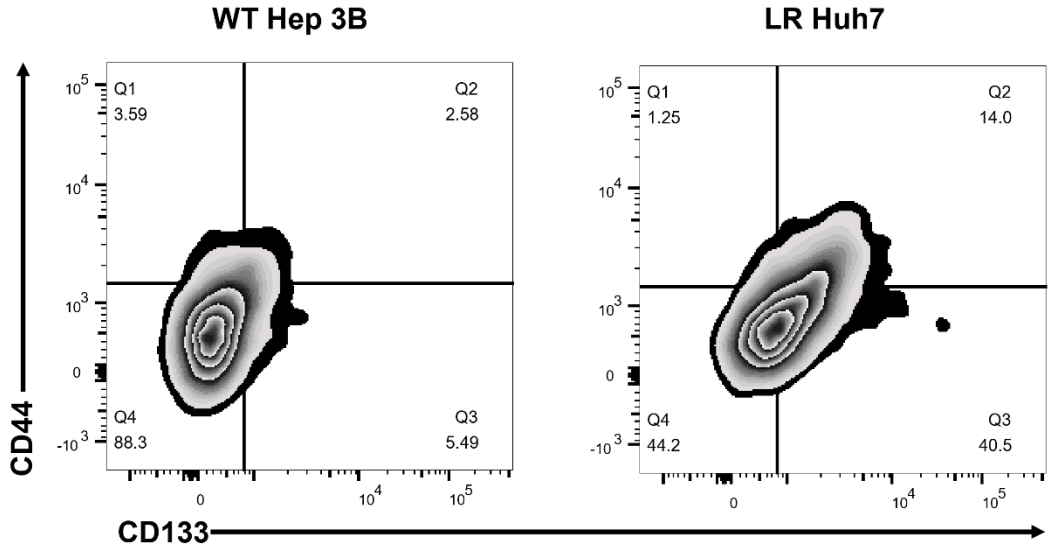


Figure S5. The proportion of CSCs in Hep 3B WT and LR strains.

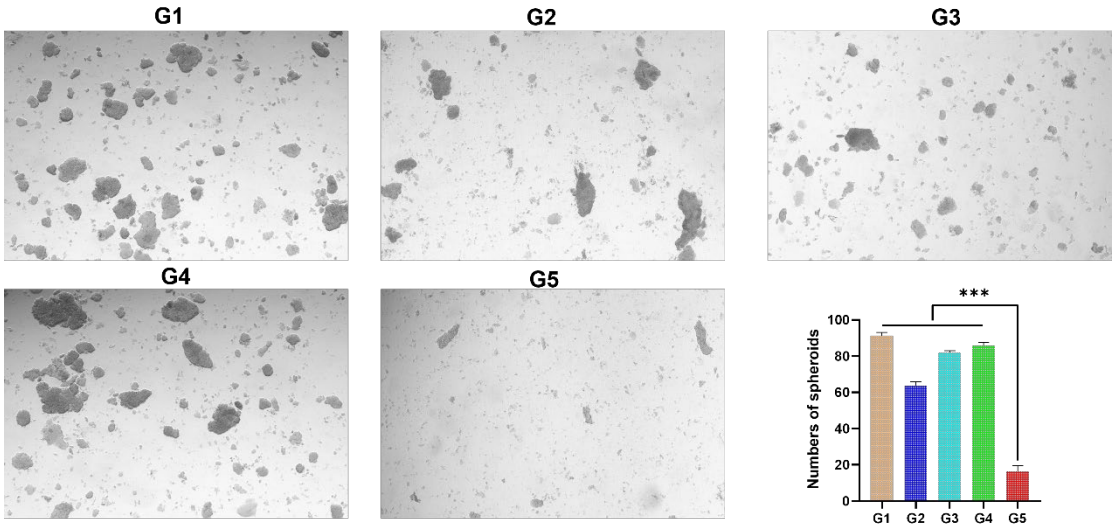


Figure S6. The impact of different treatment groups on the sphere formation ability of LR Hep 3B tumors. (G1: corn oil, G2: DSF, G3: CuO NPs, G4: Len@CuO, and G5: DSF@CuO). Data are presented as mean ± SD and are representative of three independent experiments. ***$p < 0.001$.

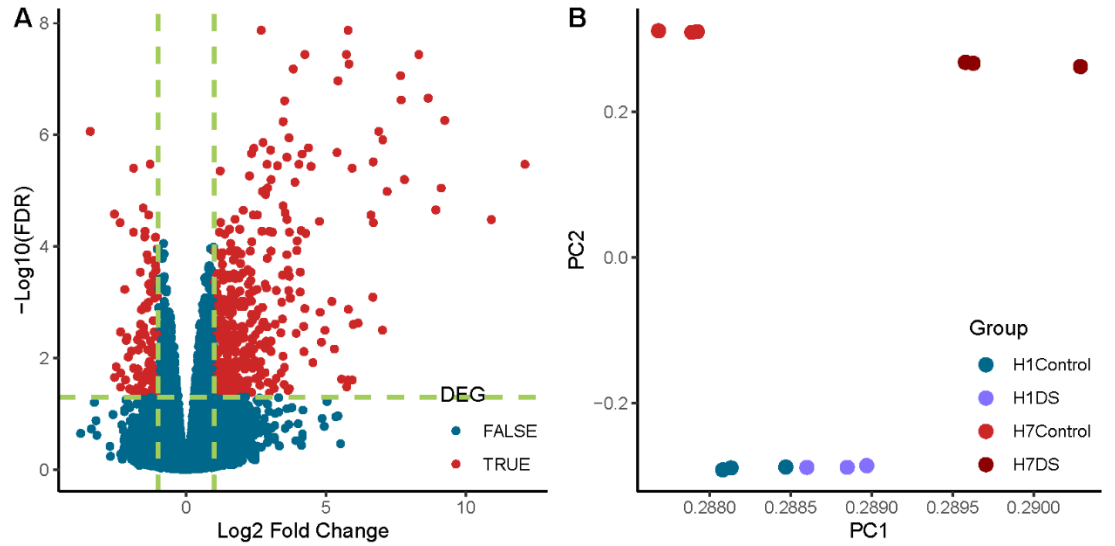


Figure S7. Volcano and PCA plot of gene expression of upregulated and downregulated genes between PBS and DSF groups (log2FC≥2, FDR DSF≤ 0.5).

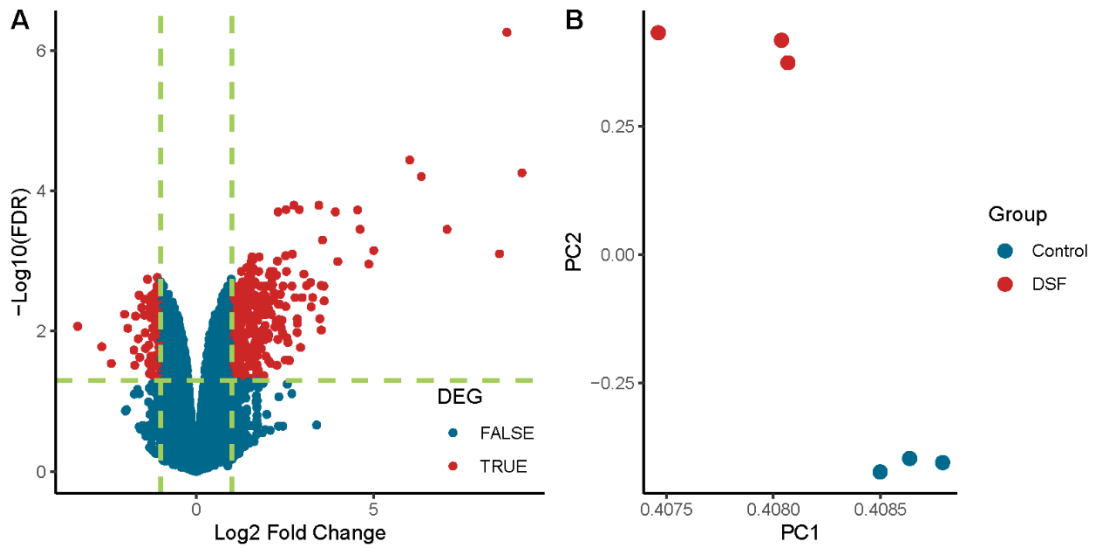


Figure S8. Volcano and PCA plot of gene expression of upregulated and downregulated genes between PBS and DSF+Cu^{2+} groups (log2FC≥2, FDR ≤ 0.5)

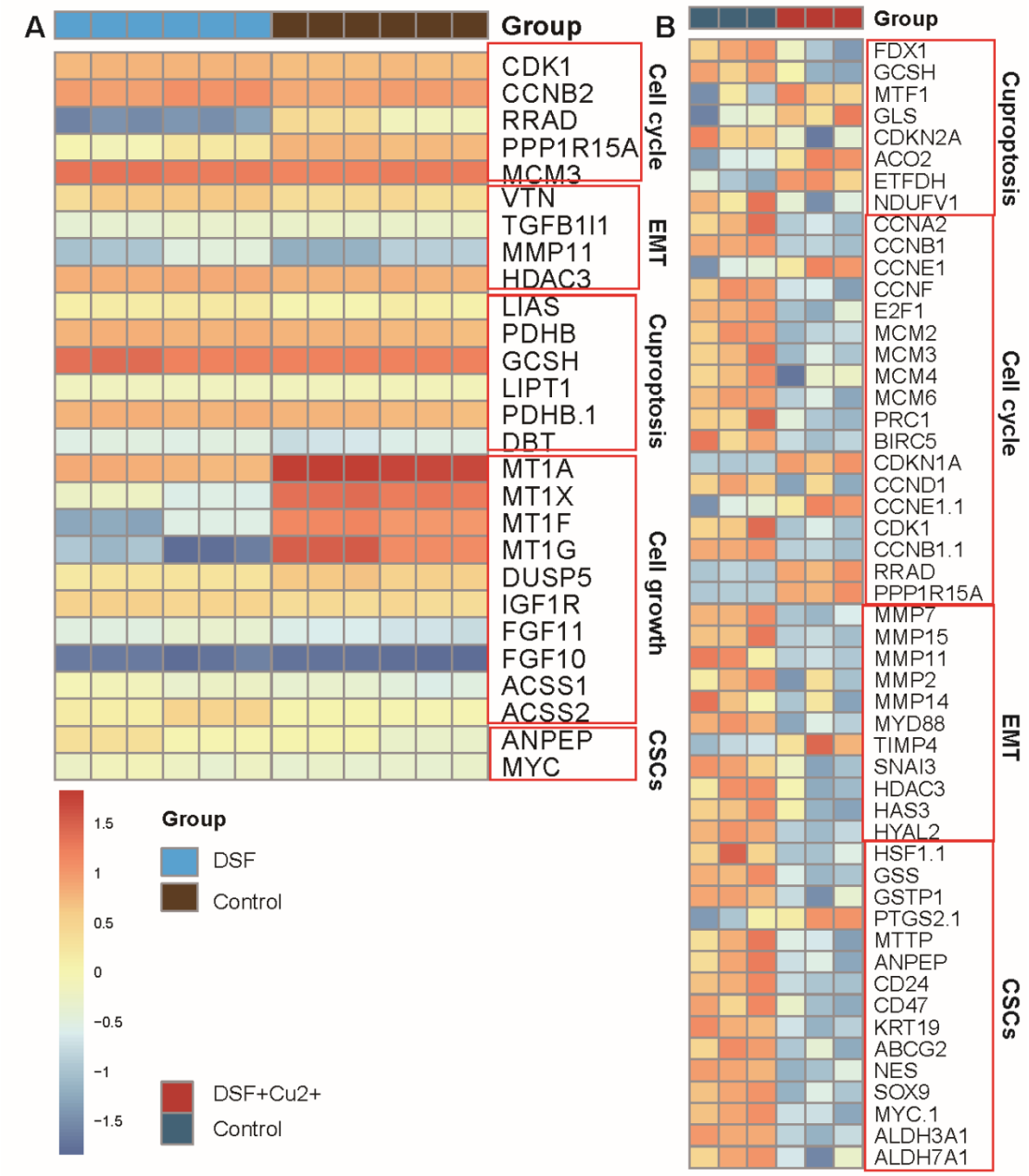


Figure S9. A heatmap analysis DEGs among different treatment groups.

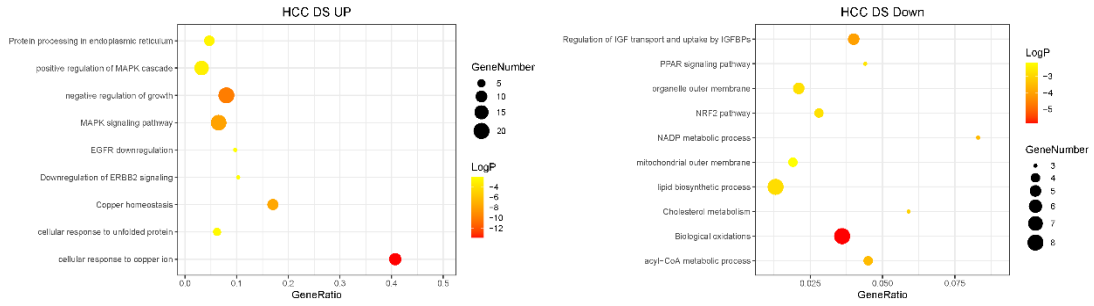


Figure S10. GO and KEGG enrichment analysis of DEGs in DSF group.

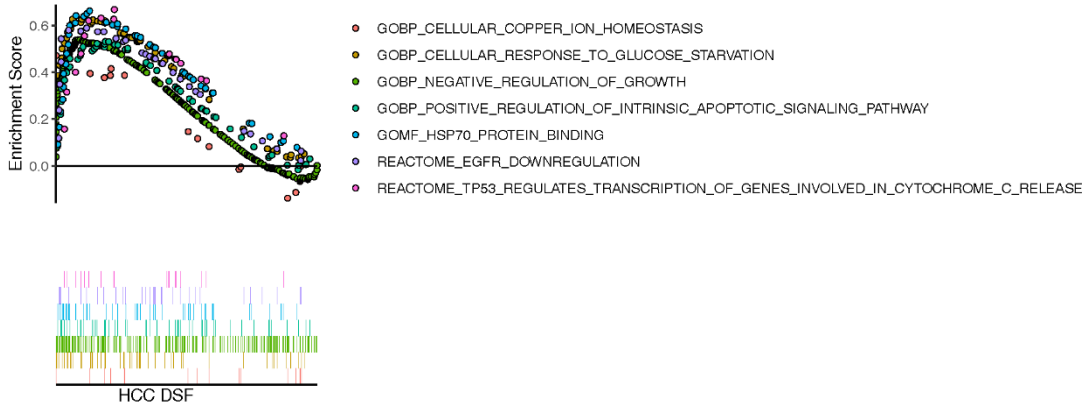


Figure S11. GSEA plots showing pathways upregulated and downregulated after DSF treatment.

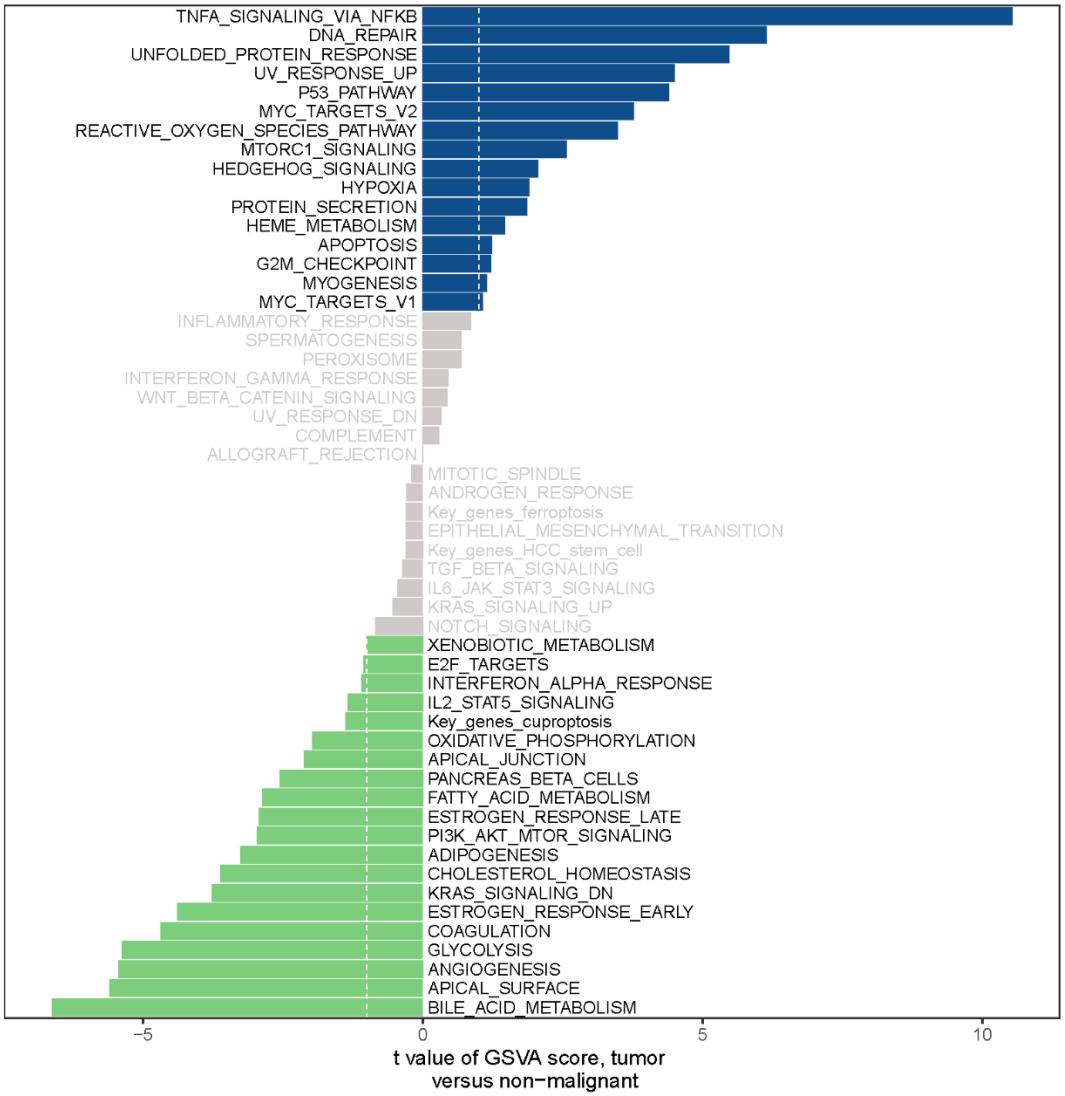


Figure S12. GSVA plots showing pathways upregulated and downregulated after DSF treatment

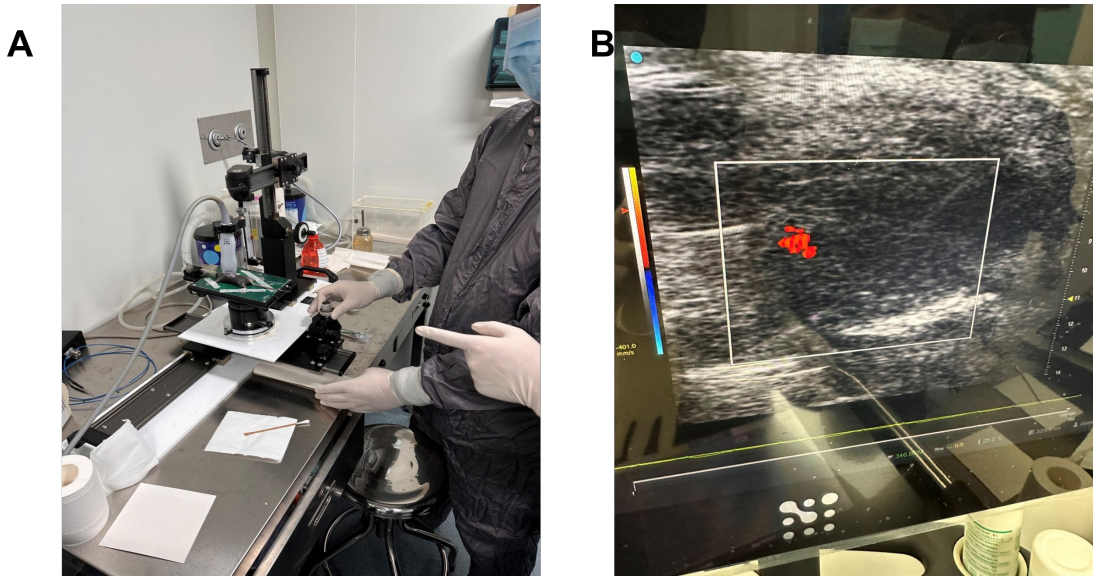


Figure S13. Under ultrasound guidance, the injectable hydrogel DSF@CuO-Gel is used for the treatment of liver orthotopic tumors.

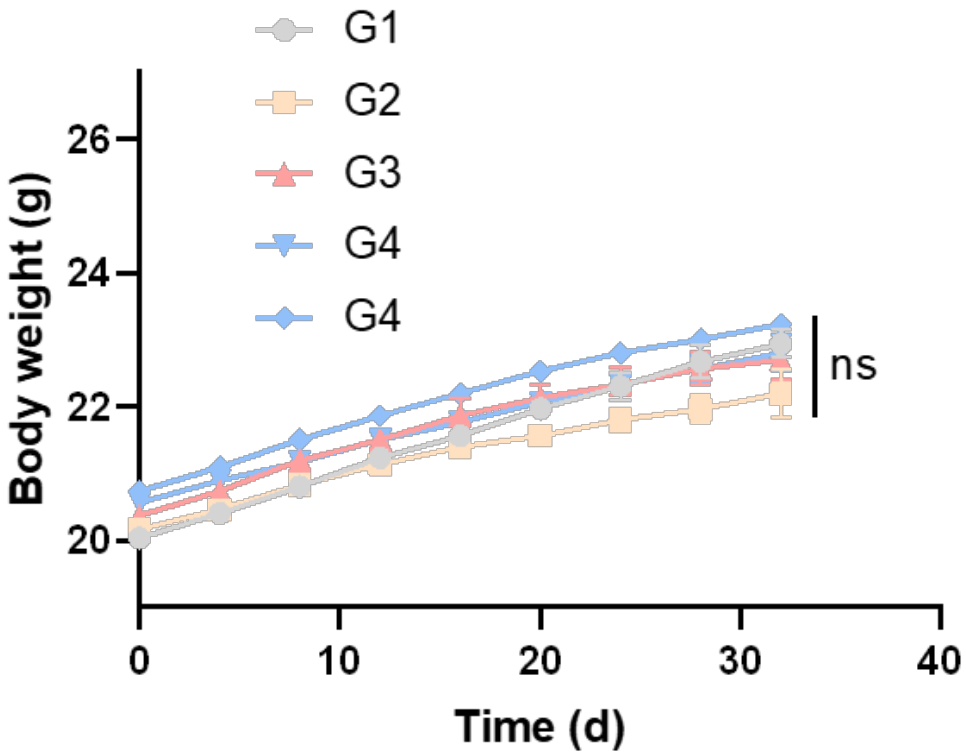


Figure S14. Graph of mice weight changes in different treatment groups. (G1: sodium alginate, G2: DSF, G3: CuO NPs, G4: DSF@CuO, and G5: DSF@CuO Gel). Data are presented as mean ± SD and are representative of three independent experiments. ns: no significance.

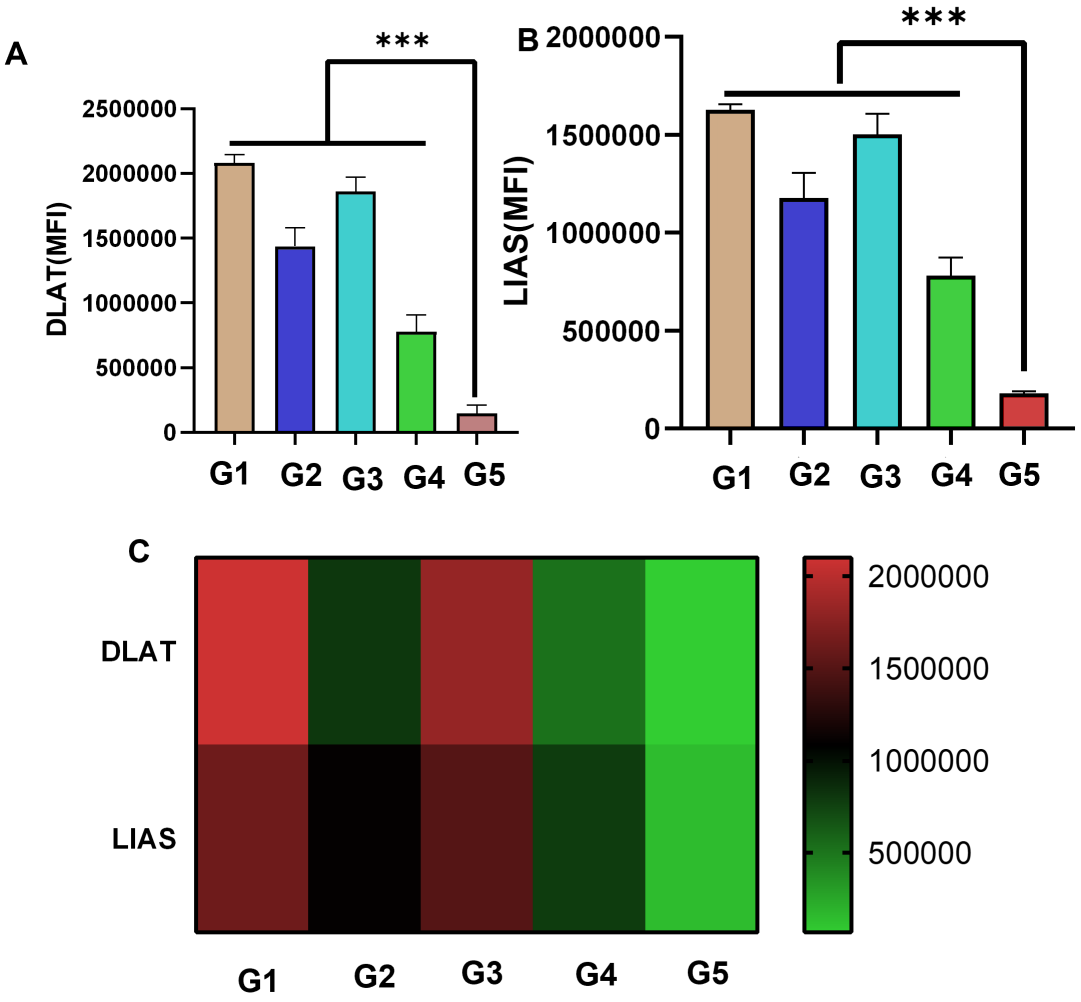


Figure S15. Statistical chart of DLAT and LIAS levels in tumor tissues after treatment in different groups. (G1: Sodium alginate, G2: DSF, G3: CuO NPs, G4: DSF@CuO, G5: DSF@CuO Gel). Data are presented as mean ± SD and are representative of three independent experiments. ***$p < 0.001$.

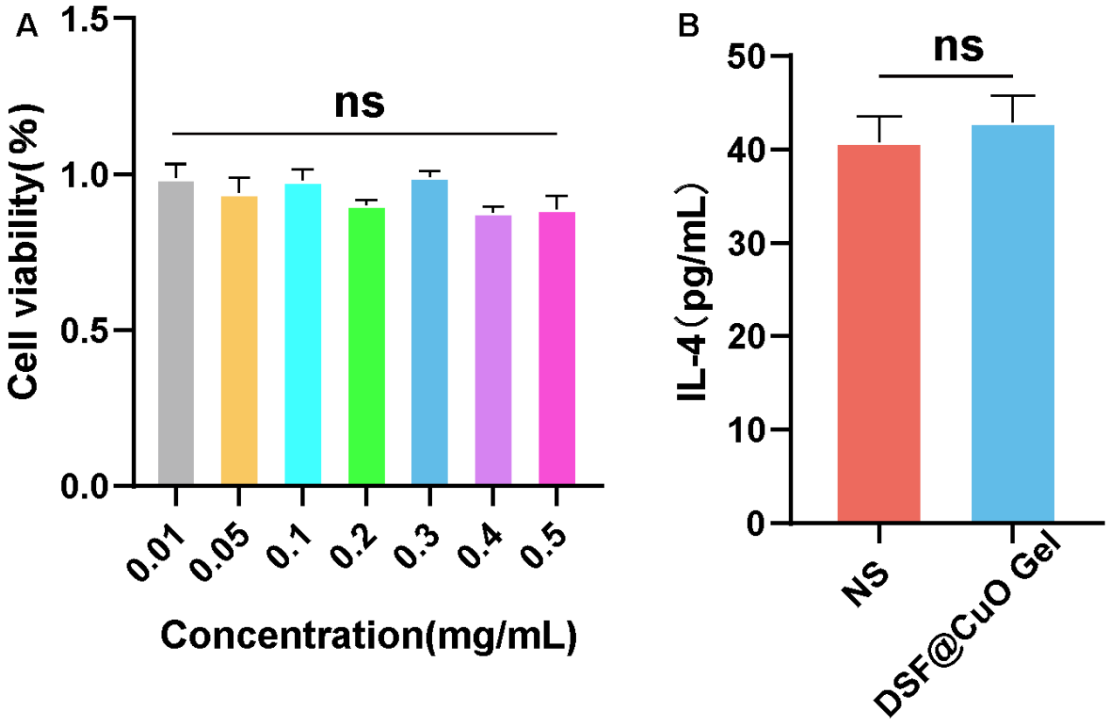


Figure S16. Foreign body assessment in different treatment groups. A) The effect of different treatment groups on the survival rate of HUVEs cells. B) ELASA detection of IL-4 levels in mice tumor tissues after different treatment. Data are presented as mean ± SD and are representative of three independent experiments. ns, not significant.

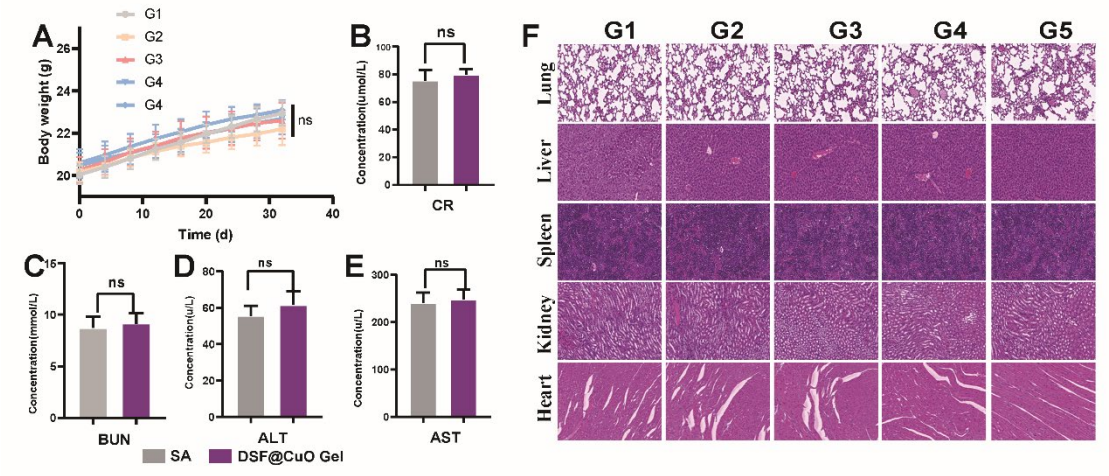


Figure S17. Biological safety assessment of DSF@CuO Gel. A) Changes in body weight of mice across different treatment groups (n = 3). B-E) Biochemical analysis of the serum of mice with various treatments (n = 3). F) H&E staining images of important organs from mice after completion of treatment across different treatment groups. Data are presented as mean ± SD and are representative of three independent experiments. ns, not significant.