Supporting Information

Mn^{2+}-Coordinated Glycyrrhizic Acid Self-Adjuvating Hydrogel for Sustained Codelivery of Antigen and Mn^{2+} for a Potent Immune Response

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Table S1. The cGAS-STING pathway related gene and their primers used in this study.

Primer	Forward (5'-3')	Reverse (5'-3')
GAPDH	AGGTCGGTGTGAACGGATTTG	TGTAGACCATGTAGTTGAGGTCA
TNF-α	CCCTCACACTCAGATCATCTTCT	GCTACGACGTGGGCTACAG
IL-6	CTTCTTGGGACTGATGCTGGT	AGACAGGTCTGTTGGGAGTGG
IRF3	GAGAGCCGAACGAGGTTCAG	CTTCCAGGTTGACACGTCCG
NF-kB	CCAAAGCTCCCGAAACCAATC	GAGTAGCCGCCGTAATAGGC
TBK1	ACTGGTGATCTCTATGCTGTCA	TTCTGGAAGTCCATACGCATTG
CCL-2	ATGCAGTTAACGCCCCACTC	CTGCTGCTGGTGATCCTCTT
CCL-3	CGGAAGATTCCACGCCAATTC	TCTTTGGAGTCAGCGCAGAT
CXCL-2	CCAGTGAACTGCGCTGTCAA	GGGCGTCACACTCAAGCTCT
CXCL-5	GAGCTGCGTTGTGTTTGCTT	GCTATGACTTCCACCGTAGGG

Table S2. The GC B related gene and their primers used in this study.

Primer	Forward (5'-3')	Reverse (5'-3')
GAPDH	AGGTCGGTGTGAACGGATTTG	TGTAGACCATGTAGTTGAGGTCA
Bcl-6	CGGAAGGGTCTGGTTAGT	CATTCTGATTGAGGCTGTTG
Bach2	CATTCTGATTGAGGCTGTTG	ACTGGAGTGTTCGGTTGGT

Fig. S1. Optical image of GAgel.

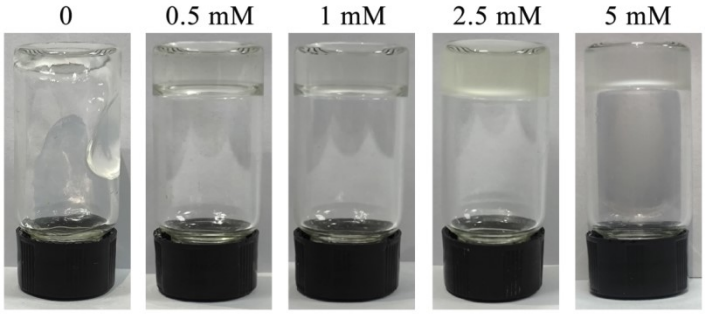

Fig. S2. Effect of different $MnCl_2$ concentrations on the formation of the GA@Mn hydrogel. The concentration of GA was 3% (w/v).

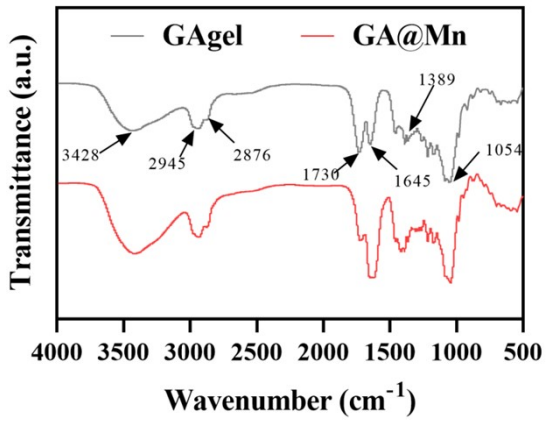

Fig. S3. The FTIR spectra of GAgel and GA@Mn.

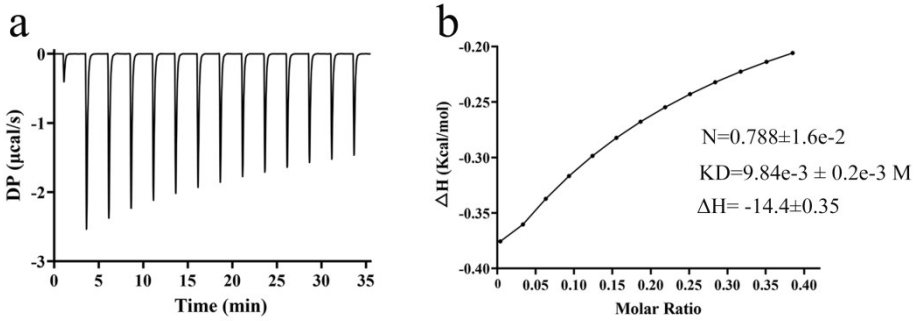

Fig. S4. Al^{3+} titration curve with GA and the derived thermodynamic parameters.

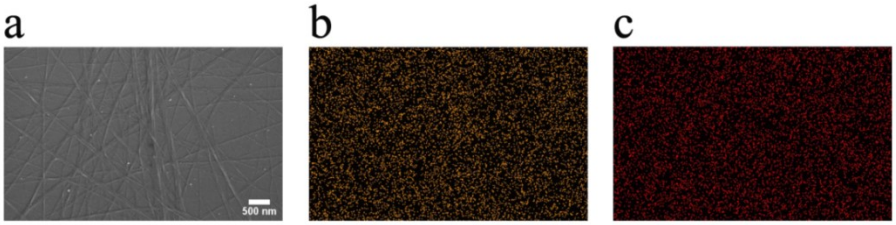

Fig. S5. (**a**) SEM image of the GA@Al microstructure and EDS elemental mapping of (**b**) carbon (C) and (**c**) aluminum (Al) in GA@Al.

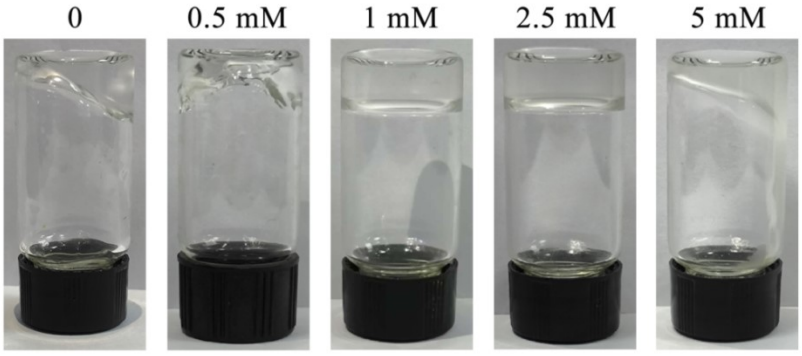

Fig. S6. Effect of different $AlCl_3$ concentrations on the formation of the GA@Al hydrogel. The concentration of GA was 3% (w/v).

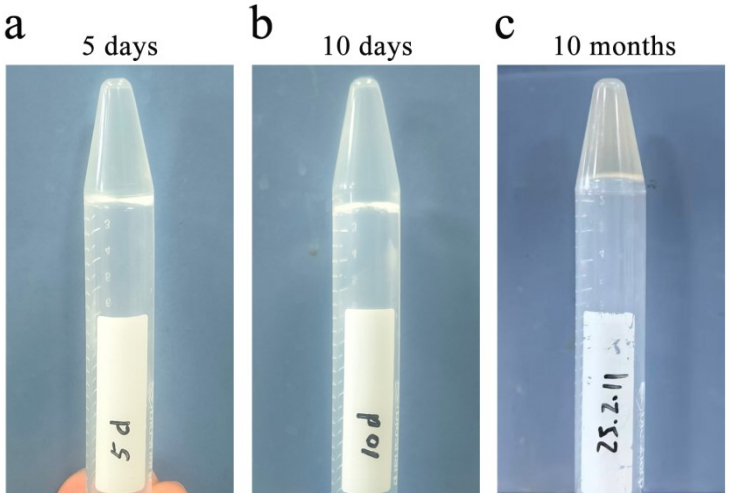

Fig. S7. State of the GA@Mn hydrogel after storage at room temperature for 5 days (**a**), 10 days (**b**), and 10 months (**c**).

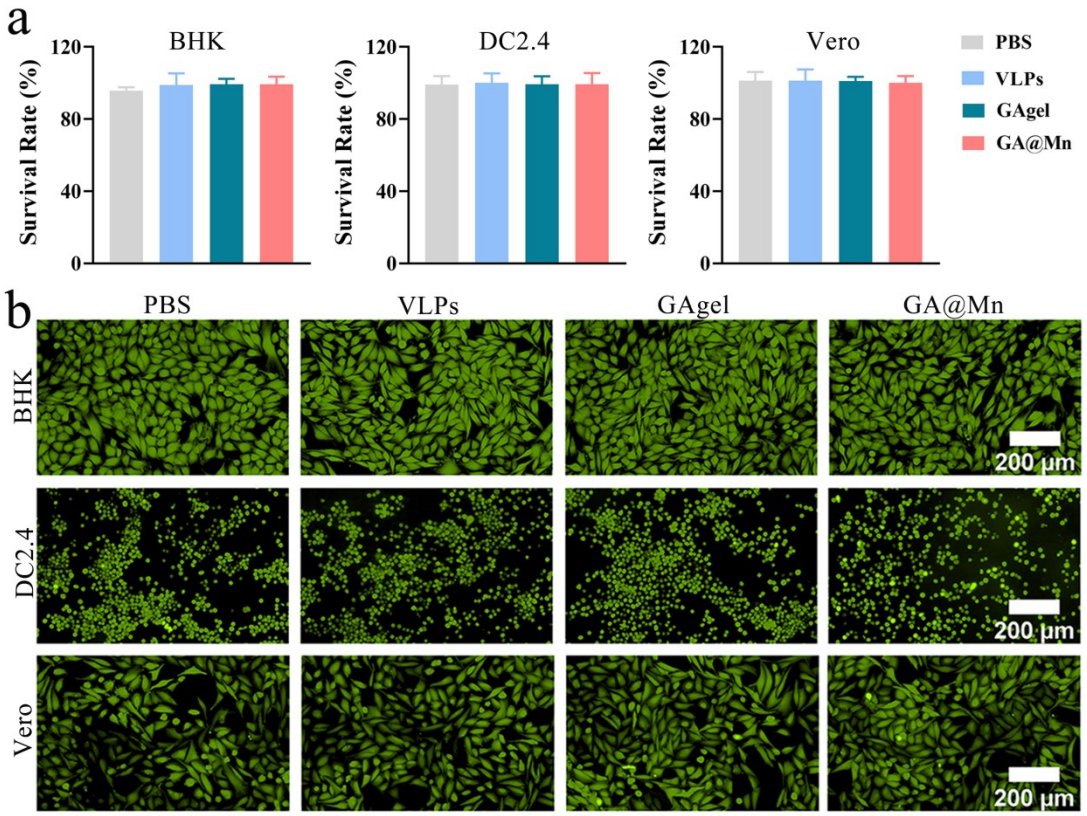

Fig. S8. Cytocompatibility of GA@Mn. (**a**) Viability of BHK, DC2.4 and Vero cells treated with PBS, VLPs, GAgel, and GA@Mn, assessed via MTS assay. (**b**) Live/dead staining images (Calcein-AM/PI) of BHK, DC2.4 and Vero cells after different treatment. Scale bar: 200 μm.

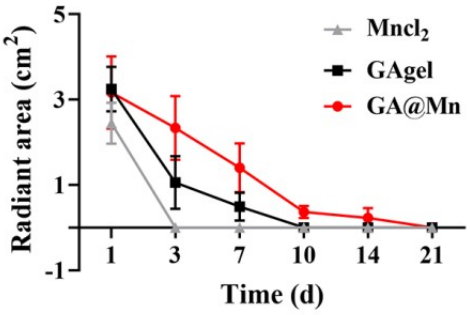

Fig. S9. *In vivo* radiant area of mice injected with $MnCl_2$, GAgel, and GA@Mn containing FITC-OVA.

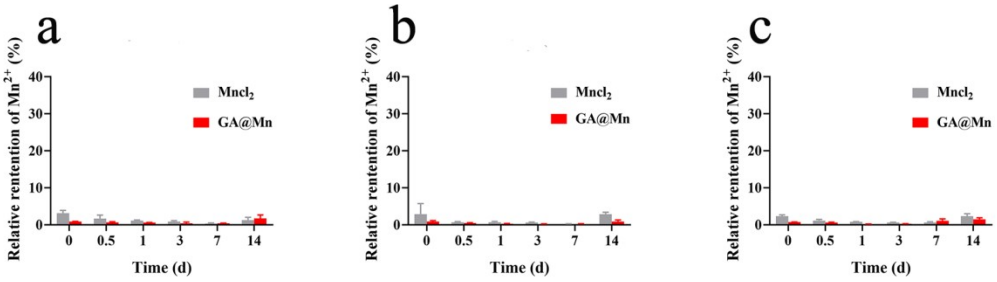

Fig. S10. Mn^{2+} content in heart (**a**), spleen (**b**), and lung (**c**) of mice injected with $MnCl_2$ or GA@Mn.

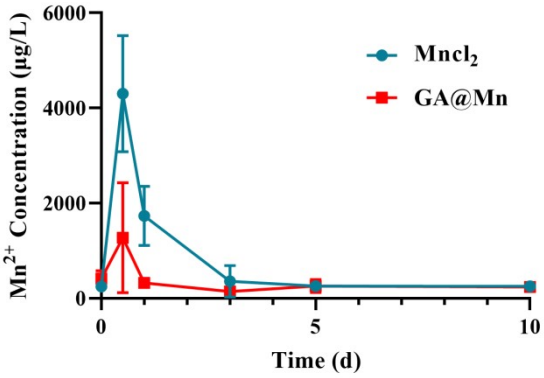

Fig. S11. Serum Mn^{2+} concentration. The concentration of Mn^{2+} in mouse serum was measured by ICP-MS at 0, 0.5, 1, 3, 5, and 10 days after injection of $MnCl_2$ or GA@Mn, respectively.

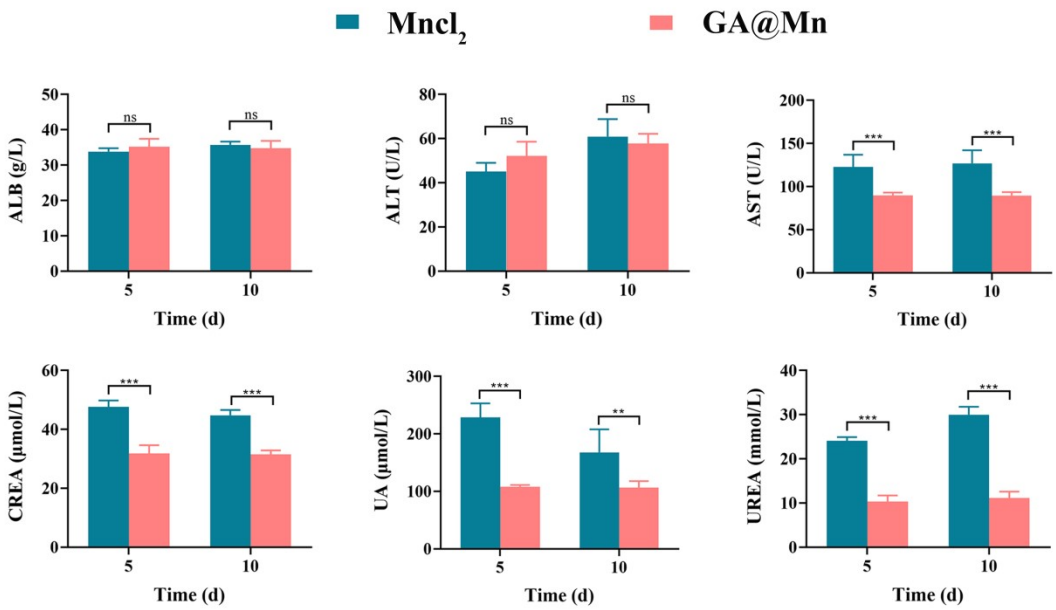

Fig. S12. Blood biochemical analysis. Blood biochemical analysis (ALB, ALT, AST, CREA, UA, UREA) on days 5 and 10 after injection of $MnCl_2$ or GA@Mn, respectively.

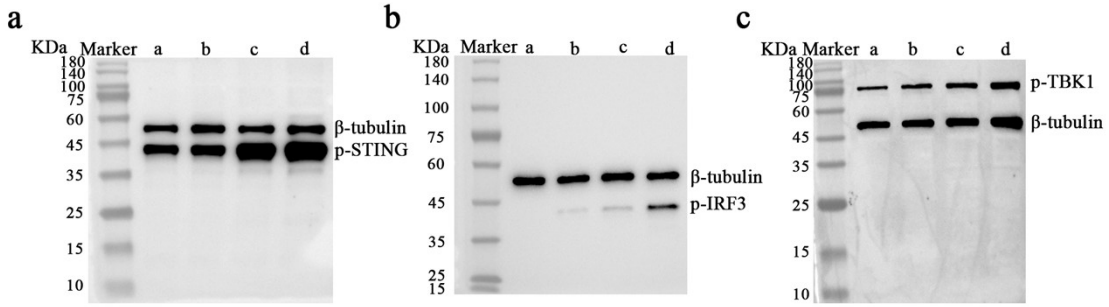

Fig. S13. The results of WB analysis of phosphorylation levels of cGAS-STING-related signaling proteins in DC 2.4.

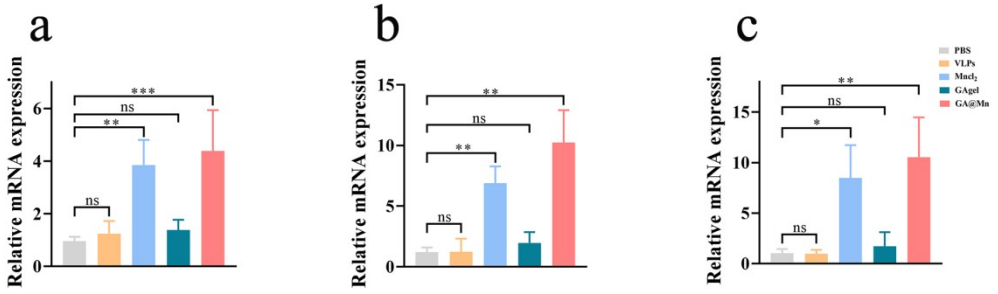

Fig. S14. RT-PCR analysis of IRF-3 (**a**), NF-κB (**b**), and TBK1 (**c**) mRNA levels at the injection site across treatment groups.

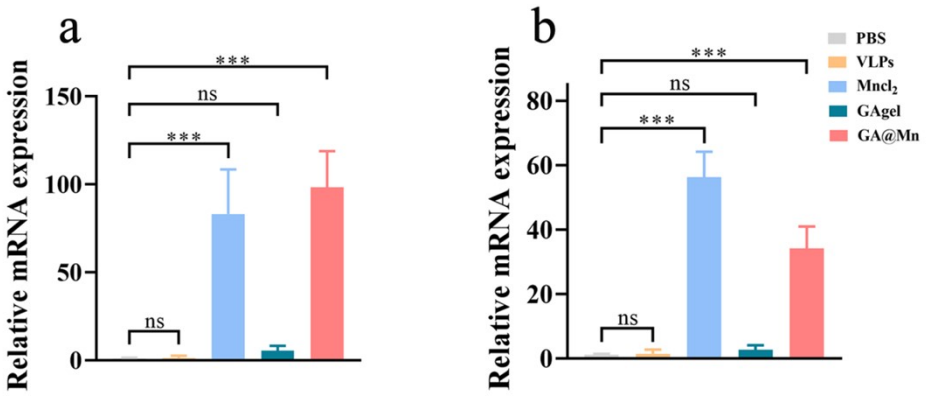

Fig. S15. RT-PCR analysis of TNF-α (**a**) and IL-6 (**b**) mRNA levels at the injection site across treatment groups.

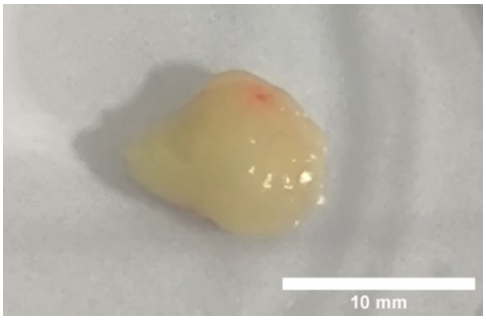

Fig. S16. Image of nodule formed up to 5 days after injection of GA@Mn *in vivo*.

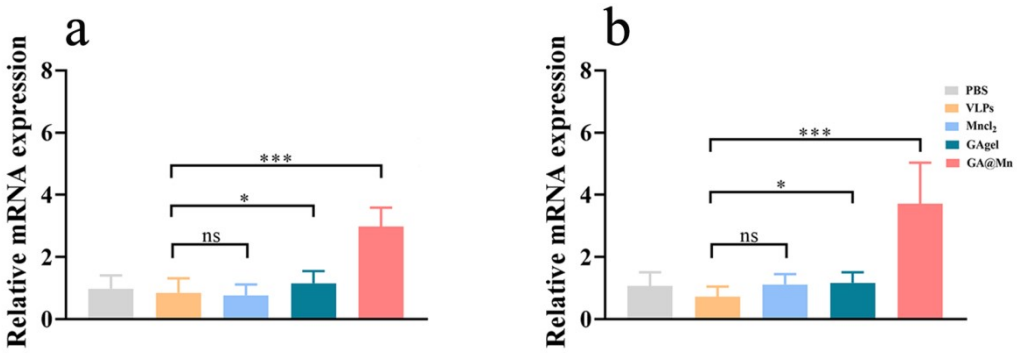

Fig. S17. RT-PCR analysis of Bcl6 (**a**) and Bach2 (**b**) mRNA levels in lymph node across treatment groups.

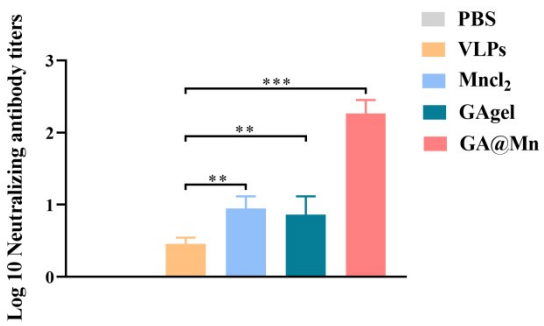

Fig. S18. Antigen-specific neutralizing antibody titers in serum on day 28 post-immunization.

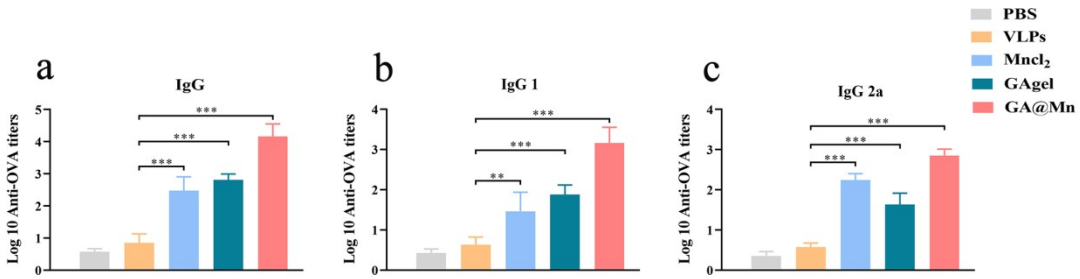

Fig. S19 ELISA analysis of OVA-specific IgG (**a**), IgG1 (**b**), and IgG2a (**c**) levels in serum on day 28 post-immunization.

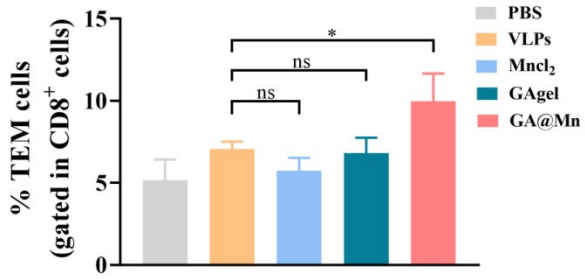

Fig. S20. Flow cytometry of TEM percentages among splenic $CD8^+$ T cells.

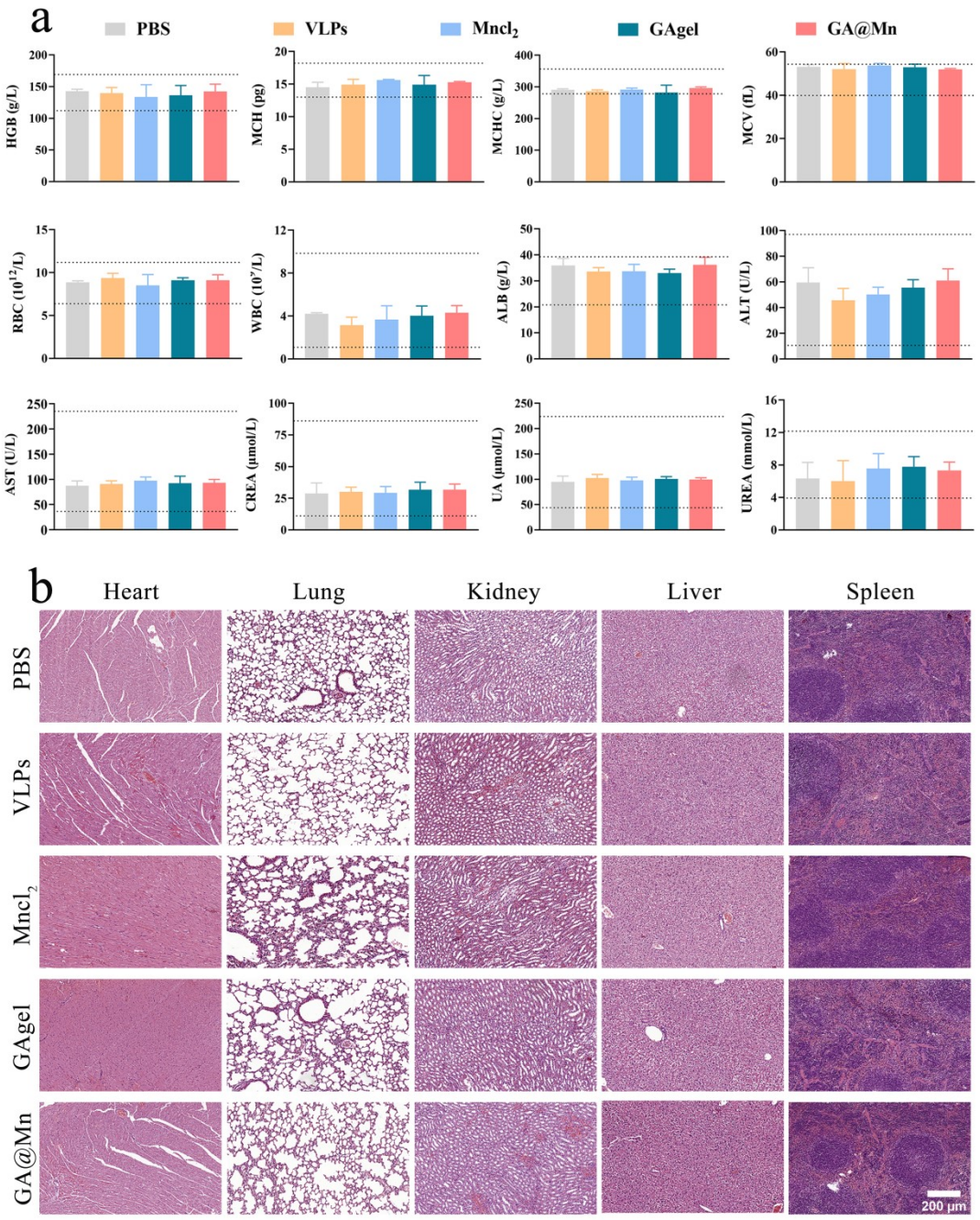

Fig. S21. *In vivo* biocompatibility. **(a)** Routine blood analysis (HGB, MCH, MCHC, MCV, RBC, WBC) and blood biochemistry analysis (ALB, ALT, AST, CREA, UA, UREA) from mice 56 days post-immunization. **(b)** Representative H&E staining of major organs from mice 56 days post-immunization. Scale bar: 200 μm.