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Evaluation of Gelatinase-responsive Copolymeric Nanoparticles Targeting PD-1 and TGF-β Pathways for Treating Immune-resistant Tumor

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Background

Immune checkpoint inhibitors have revolutionized cancer treatment but only a fraction of patients actually benefitted since the development of resistance. Simultaneously targeting PD-1 and TGFβ is prompted to be a favorable strategy to reverse the phenomenon but the hydrophobicity of TGFβ inhibitors and latent drug-related adverse events restrained the utility. To circumvent this hindrance, we construct an enzymatically responsive nanoscale drug delivery system on the basis of our previous work and loaded it with αPD-1 antibody and TGFβ inhibitor, referring to as GPNPs. Upon systemic administration, the loading would be particularly released at gelatinase-rich tumor environment with enhanced permeability and retention ability, followed by alterations in tumor heterogeneity.

Methods

Gelatinase-targeting polymeric nanoparticles were synthesized by ring-open polymerization and amidation reaction and verified by 1HNMR. Both hydrophilic αPD-1 antibody and hydrophobic TGF-β inhibitor were loaded onto the nanoparticles through double emulsion method. Sizes, morphology, stability and zeta potential were measured by dynamic light scattering (DLS), transmission electron microscope (TEM), high performance liquid chromatography (HPLC) and Bicinchoninic acid protein assay (BCA). Cell viability and *in vivo* distribution were measured by CCK8 and near-infrared imaging, respectively. We established subcutaneous Lewis lung carcinoma (LLC) murine model in an attempt to examine anti-tumor effect and long term immune-related memory of GPNPs by measuring tumor volume and monitoring the survival. Safety profile of GPNPs was evaluated by measuring weight loss, monitoring serum markers and observing HE staining of major organs. Subcutaneous A549 cell-derived xenograft was also established to mimic human tumor burden, to clarify the immune priming effect and to evaluate the potential of combining GPNPs with cytokine-induced killer cells (CIKs) adoptive cell transfer (ACT) therapy. The underlying mechanism was explored by mass cytometry (cyTOF), multi-color flow cytometry, single-cell RNA sequencing (scRNAseq), Masson trichrome staining and fate mapping.
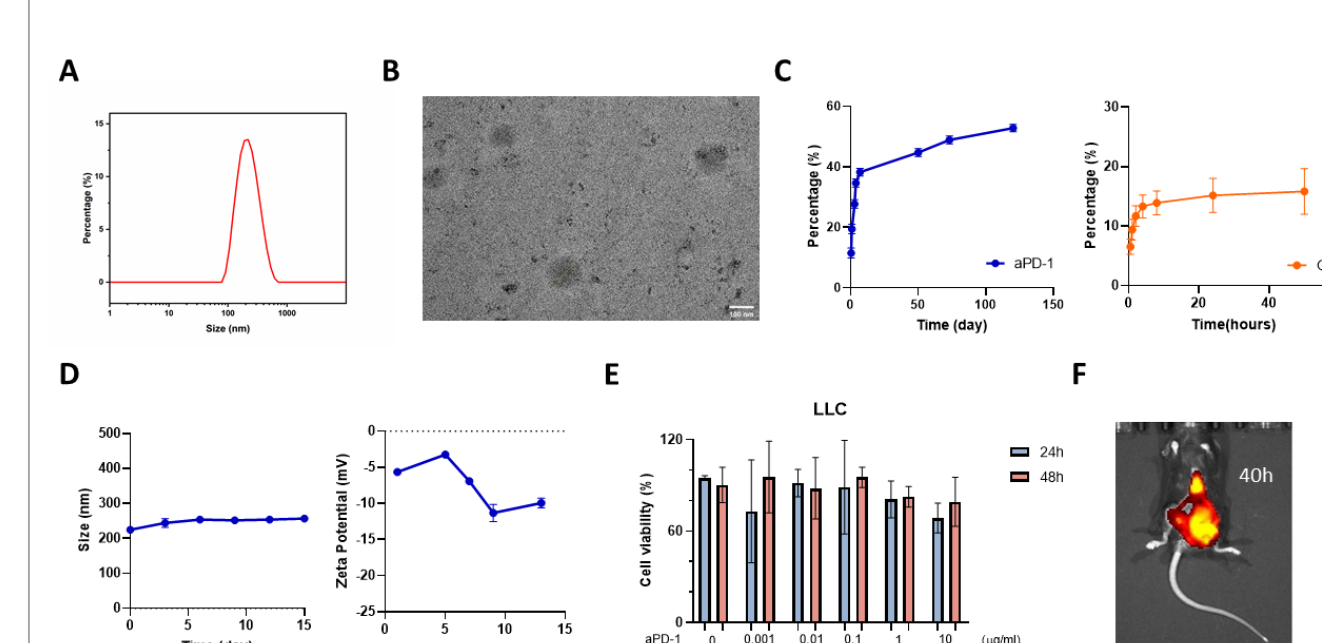
Results

Figure 1. The gelatinase targeting, round shape, stable GPNPs were successfully established without apparent toxicities.

Figure 2. Superior therapeutic efficacy of GPNP was validated in LLC model without obvious side effects
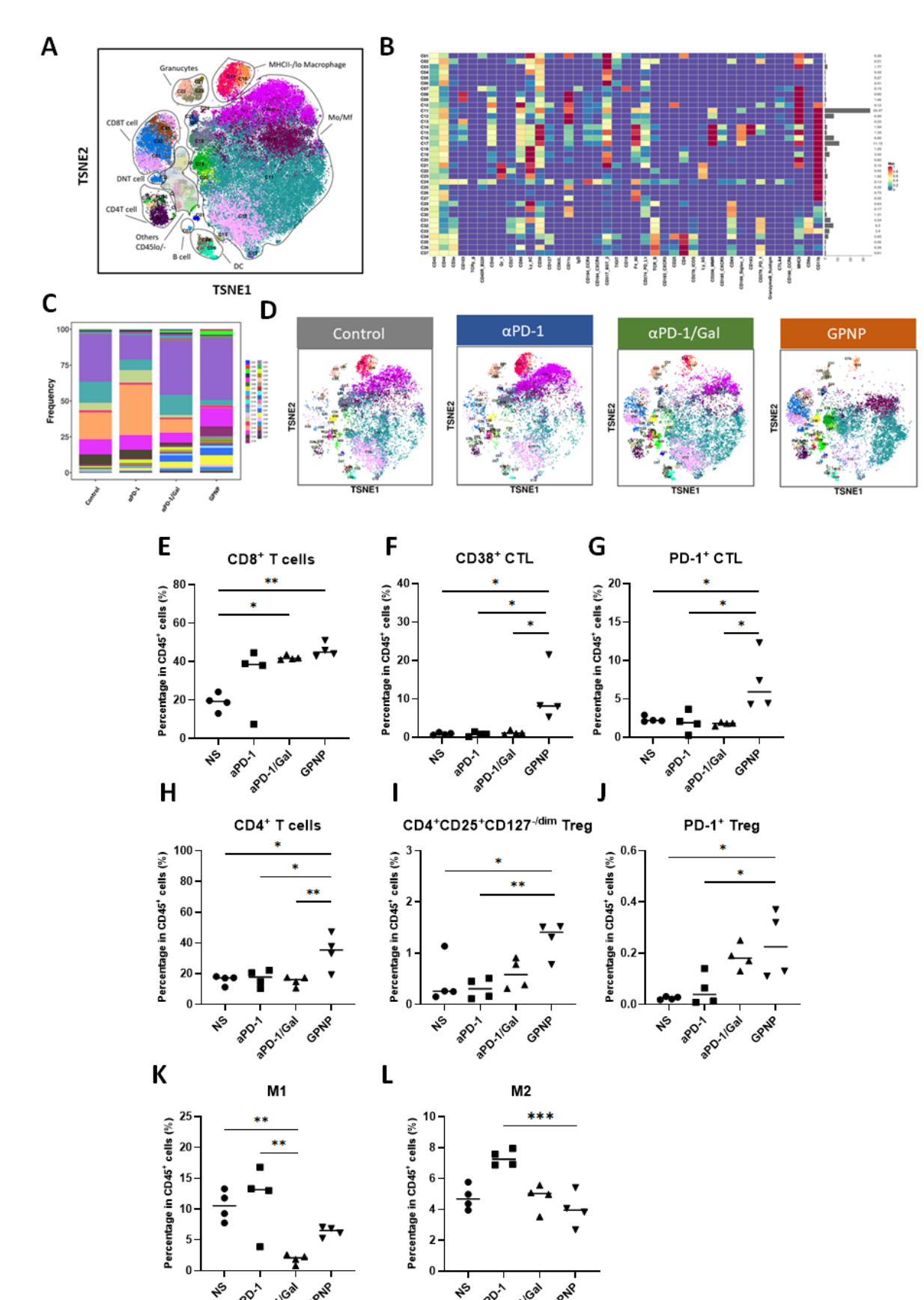

Figure 3. Immune profiling of each treatment revealed more infiltration of regulatory T cells and cytotoxic T cells as well as less M1 and M2 macrophages in tumors of GPNP-treated group (p<0.05).

NS   aPD-1

aPD-1/Gal   GPNP

Figure 4. Masson trichrome staining validated that tumors dissected from GPNP-treated group appeared to have less collagen disposition.

NS   GPNP

Figure 5. Immunohistochemistry validated that tumors dissected from GPNP-treated group appeared to have less αSMA expression.

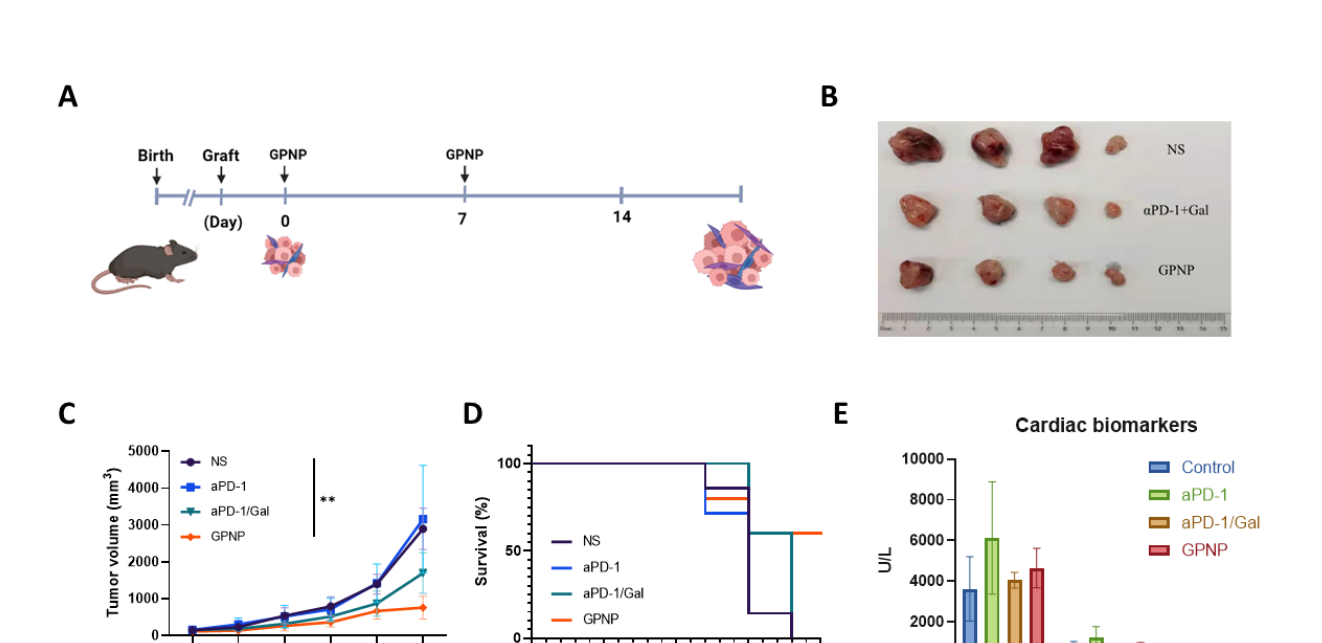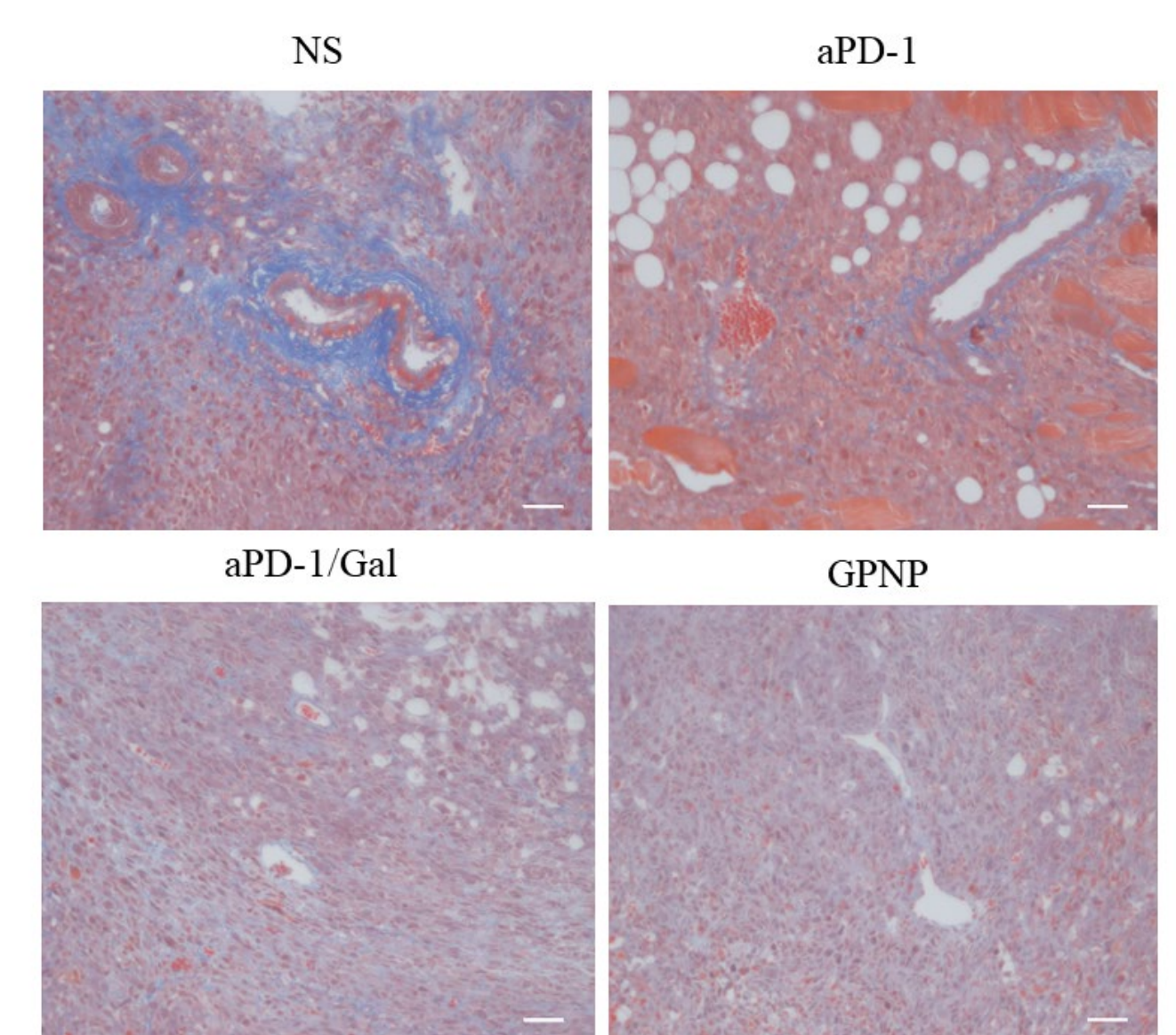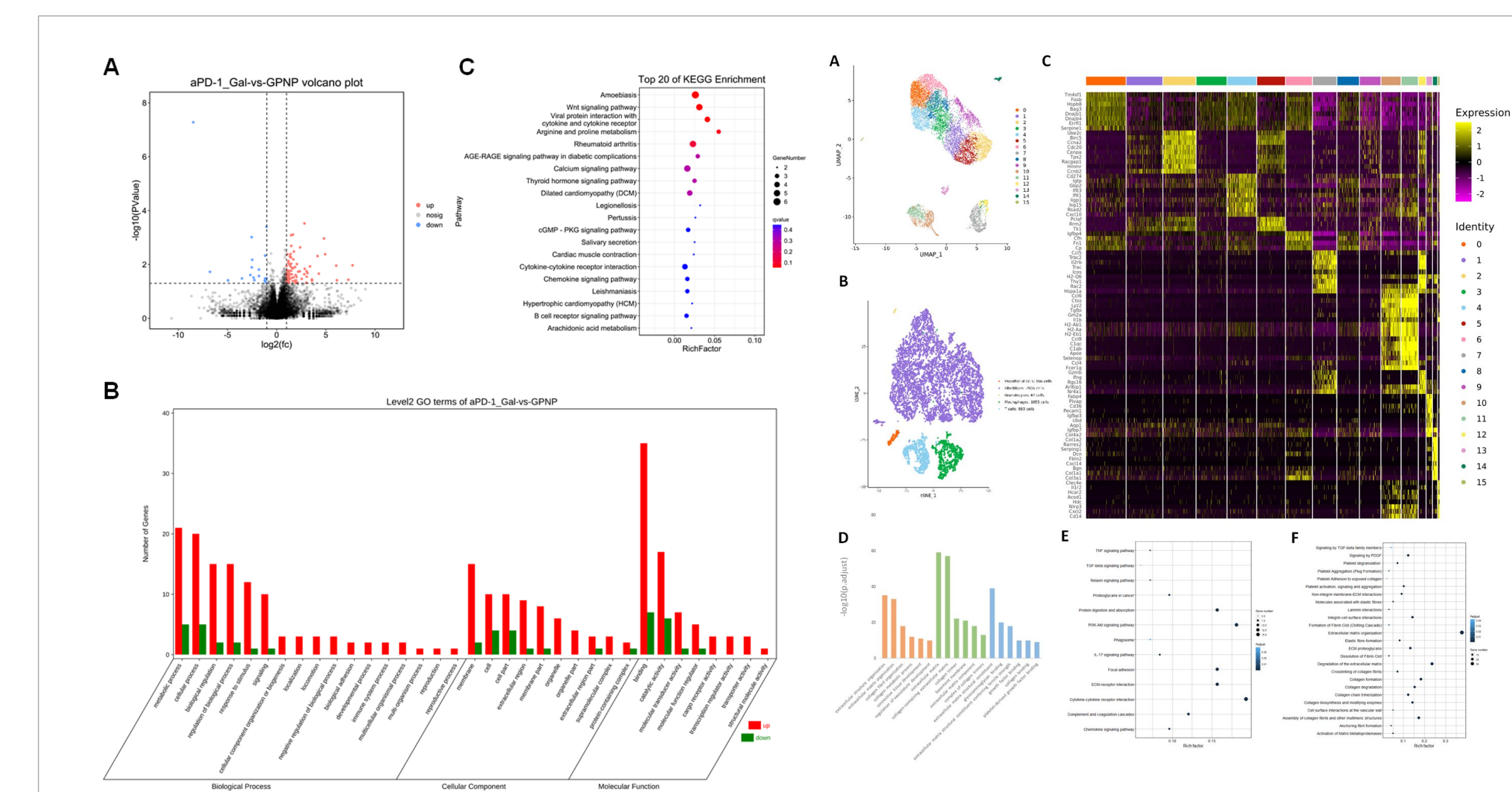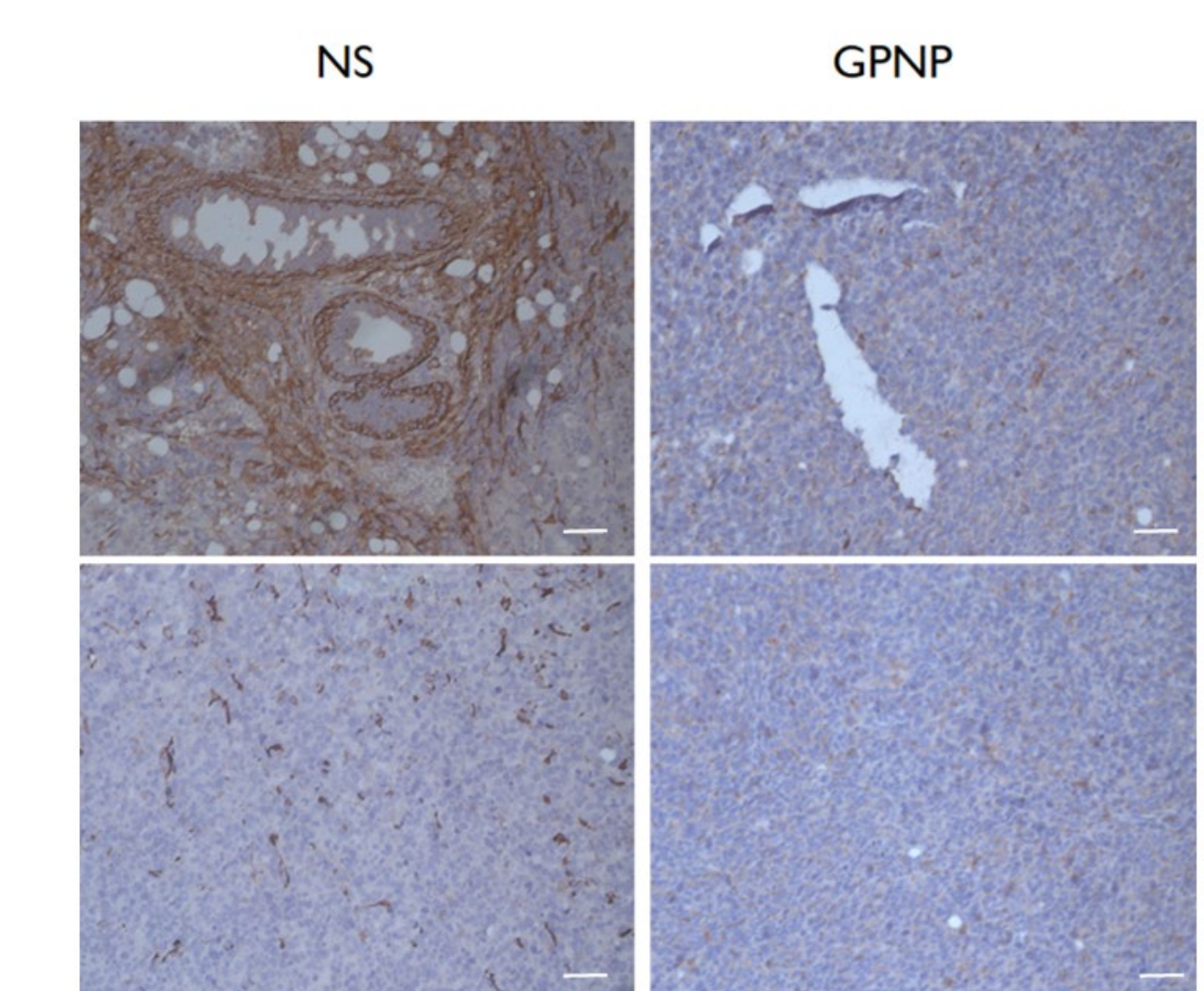Figure 6. RNA sequencing exhibited differential gene expression related with immune contexture and cardiac diseases.
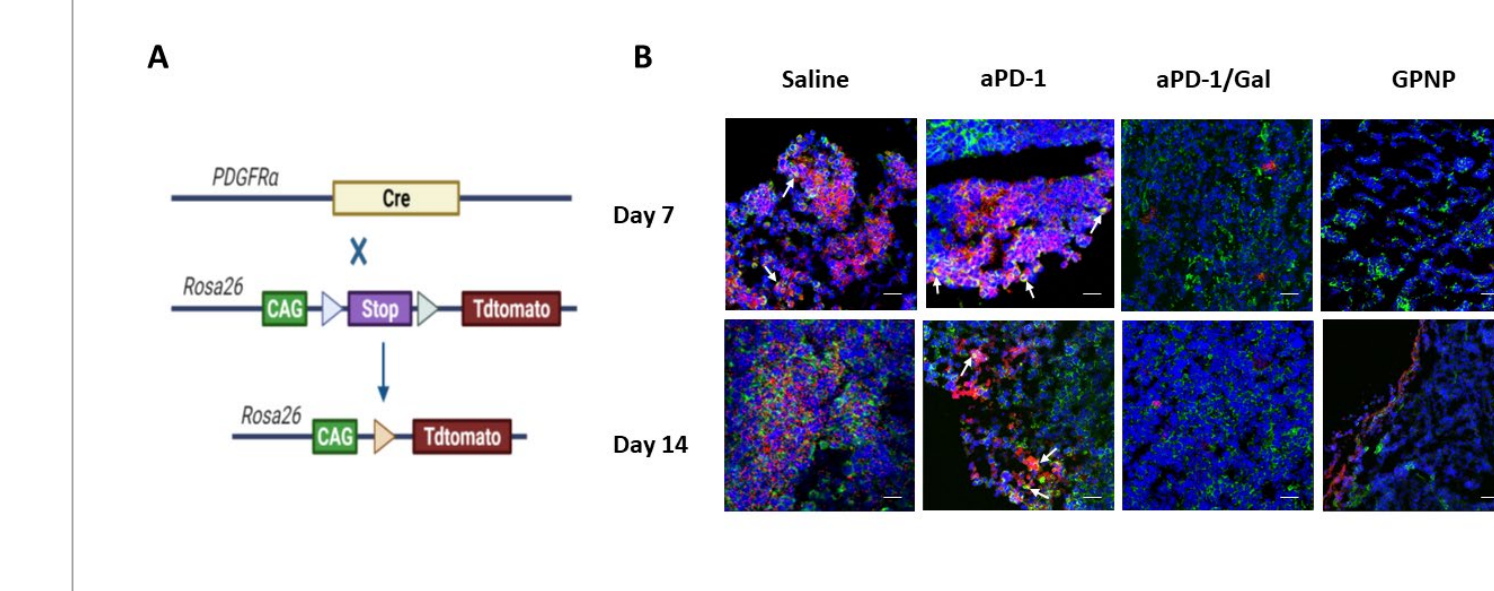

Figure7. A collagen-associated fibroblast cluster was discovered to be related to collagen, extracellular matrix organization, TGF-β signaling and PDGF signaling

Figure 7.Fate mapping showed more pdgfra+ fibroblasts underwent myofibroblastic activation in aPD-1-treated group but not GPNP group.

Conclusions

We developed a biocompatible gelatinase-responsive nanocarrier loaded with αPD-1 antibody and TGF-β inhibitor that was able to attenuate tumor growth without apparent toxicity. Enhanced antitumor efficacy, long term memory and safety profile were demonstrated in the murine model. Further, applying GPNPs in LLC treatment allowed modulation of TME, arousing specific anti-tumor immunity and creating an immune-permissive environment. Above, we comprehensively analyzed TME by multi-modal single-cell analysis in hope of providing a feasible strategy towards successful tumor immunotherapy.

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