No.	K25-3146	
Project Title	Nanozymes for Tumor Catalytic Immunotherapy	
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(Form 2)

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Joint Research Report (Annual/Project Completion)

Annual Report

Report

During the first annual period of this project, we concentrated on clarifying how nanozyme catalysis regulates adaptive antitumor immunity in a vaccine setting. On the basis of the originally proposed nanozyme-assisted immunotherapy framework, we established a proton-driven nanozyme-integrated nanovaccine system and obtained a key mechanistic advance: nanozyme-generated reactive oxygen species (ROS) do not merely contribute to downstream immunogenic stress, but directly act on dendritic cells (DCs) as a catalytic adjuvant signal. This work shifted the project from a general ROS-assisted immunotherapy concept toward a more precise mechanism centered on DC activation, antigen cross-presentation, and CD8+ T-cell priming.

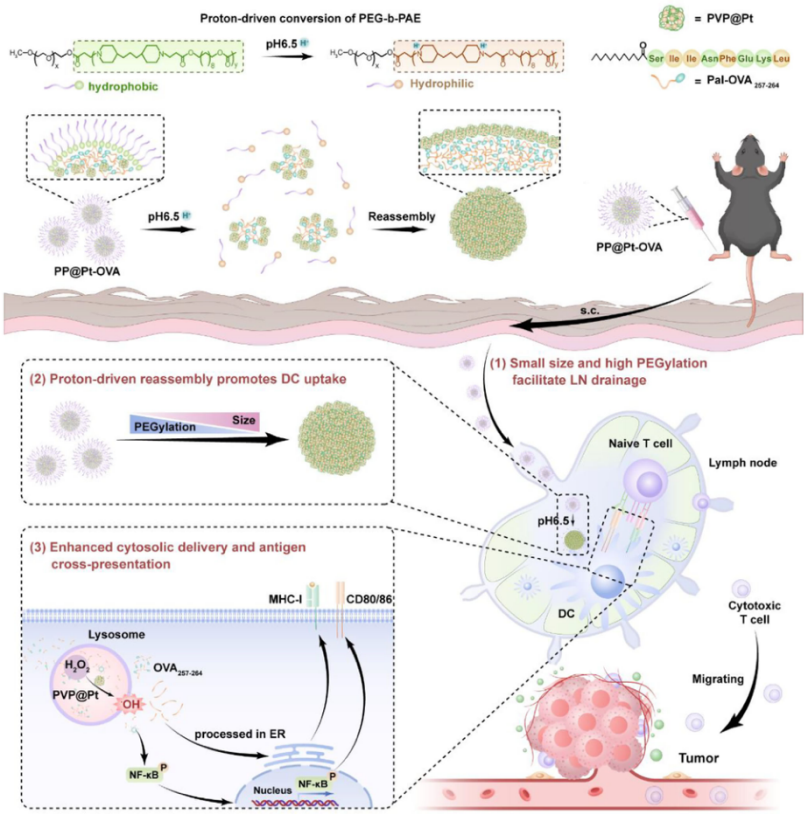

Figure 1. Schematic overview of the proton-driven nanozyme-integrated nanovaccine. The platform combines pH-responsive morphology transformation with nanozyme catalysis, enabling lymph-node targeting, dendritic-cell uptake, and catalytic adjuvant activity.

1. Construction of a proton-responsive nanozyme-integrated vaccine platform

To support mechanistic dissection in a biologically relevant vaccine system, we first constructed a proton-

driven nanovaccine (PP@Pt-OVA) composed of PVP@Pt nanozymes and OVA257-264 peptides encapsulated in PEG-b-PAE micelles. During the reporting period, we completed nanoparticle synthesis, structural characterization, catalytic evaluation, and pH-responsive behavior analysis. The nanozyme retained peroxidase-like catalytic activity after encapsulation, while the PEG-b-PAE carrier underwent proton-triggered disassembly under weakly acidic conditions, causing particle enlargement and reduced PEG shielding. This dynamic transformation was designed to reconcile two otherwise contradictory requirements in subunit vaccine delivery: efficient lymphatic drainage in the small-particle state, and enhanced lymph-node retention plus dendritic-cell uptake after acid-triggered reassembly.

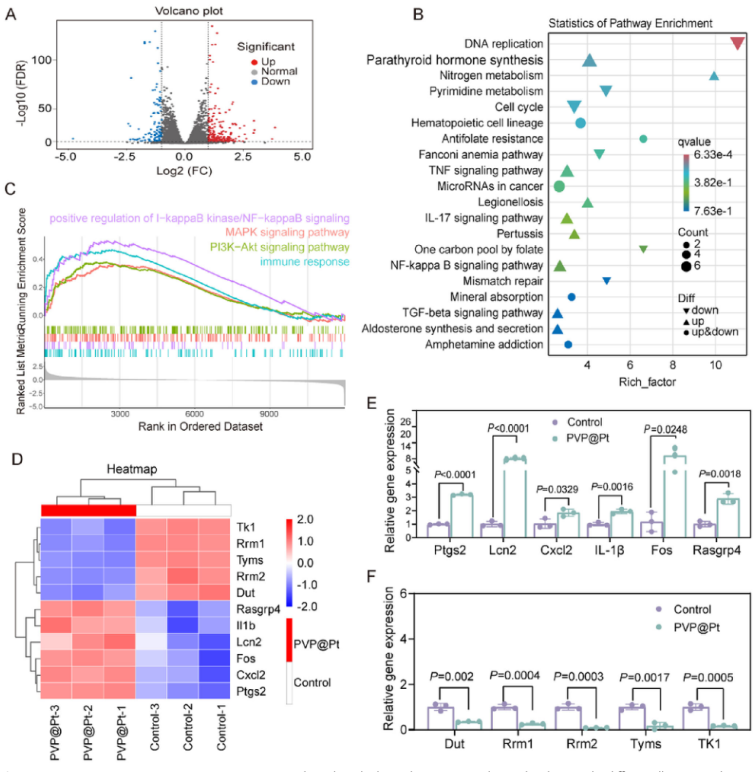

Figure 2. Representative evidence that nanozyme-generated ROS directly drives dendritic-cell maturation. Transcriptomic analyses and maturation-marker assays support activation of NF-kB-associated immune pathways after PVP@Pt treatment.

2. Nanozyme-derived ROS directly activates dendritic cells and drives maturation

The most important mechanistic finding obtained this year is that nanozyme-generated ROS can directly activate dendritic cells, independently of any secondary tumor-cell death process. Using transcriptomic analysis, pathway enrichment, RT-qPCR, and western blotting, we demonstrated that exposure of DC2.4 cells to PVP@Pt nanozymes induced robust oxidative stress and activated NF-kB-centered immune signaling. This was accompanied by clear upregulation of dendritic-cell maturation markers, including CD80, CD86, CD69, and H-2Kb. The ROS scavenger N-acetylcysteine markedly reduced these responses, demonstrating that ROS production is an upstream and necessary signal. These data establish a direct catalytic adjuvant mechanism in which nanozymes act not only as chemical catalysts but also as immune-regulatory materials capable of initiating innate immune activation at the antigen-presenting-cell level.

3. Catalytic ROS promotes lysosomal destabilization and antigen cross-presentation

A second major advance was the identification of the intracellular trafficking mechanism by which catalytic nanozymes enhance antigen processing. Confocal imaging showed that free peptide antigen remained largely confined within lysosomes, whereas PP@Pt-OVA treatment led to progressive loss of lysosomal colocalization and increased cytosolic redistribution of antigen over time. This result is consistent with a catalytic ROS-mediated lysosomal destabilization process, in which locally generated oxidative species disrupt endosomal and lysosomal membranes and facilitate endosomal escape. Functionally, this translated into enhanced H-2Kb antigen presentation, stronger DC activation, reduced phagocytic behavior associated with a mature DC phenotype, and markedly improved OT-1 CD8+ T-cell proliferation in co-culture assays. Together, these findings demonstrate that catalytic nanozymes promote cross-presentation not simply by carrying antigen, but by actively regulating the intracellular processing route required for efficient MHC-I loading.

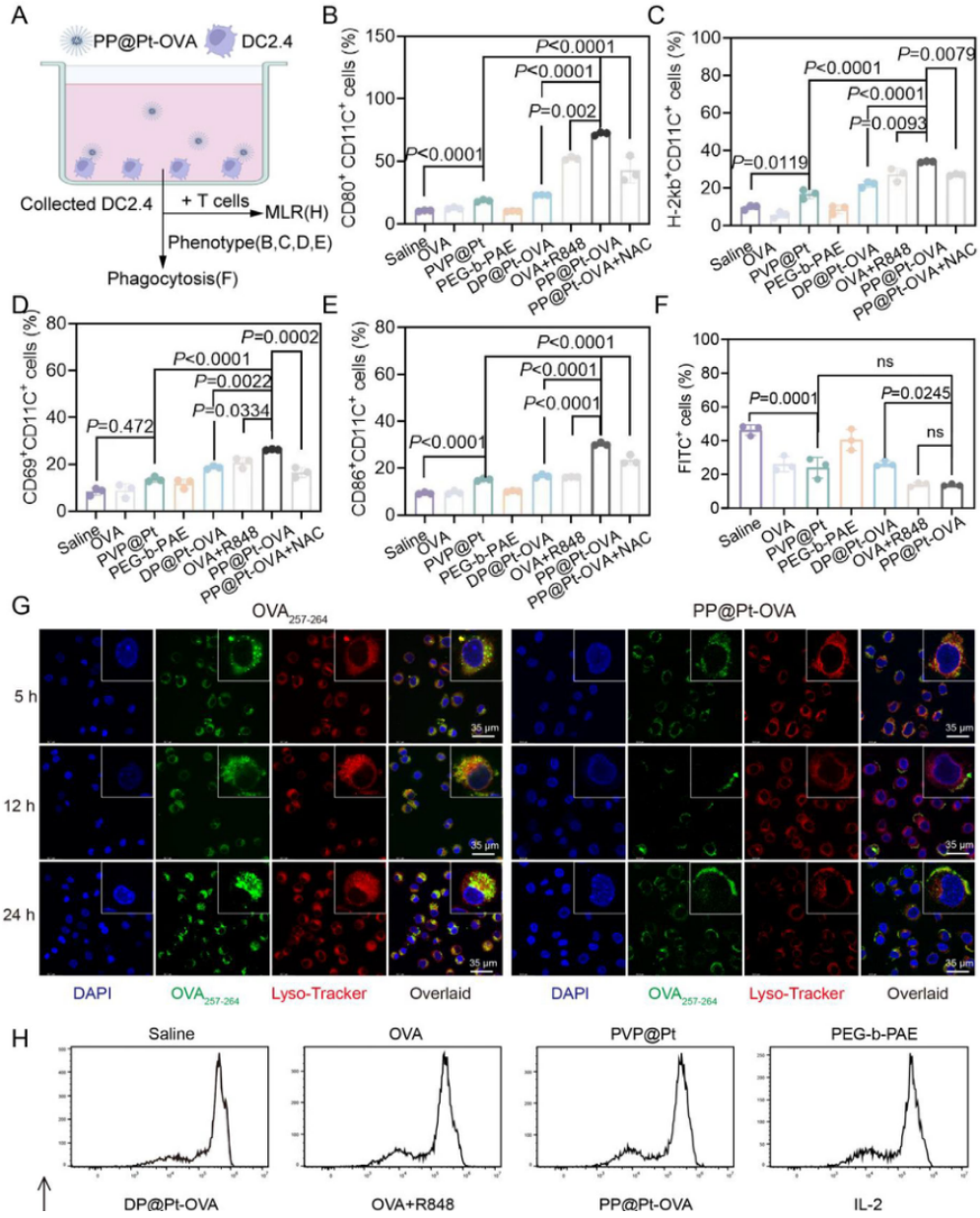

Figure 3. Nanozyme catalysis enhances antigen cross-presentation. ROS generation promotes lysosomal destabilization, antigen escape into the cytosol, increased MHC-I presentation, and stronger antigen-specific T-cell proliferation.

4. Catalytic adjuvant activity translates into potent CD8+ T-cell immunity and antitumor efficacy

The third key outcome of the year was the in vivo confirmation that the catalytic adjuvant mechanism substantially strengthens vaccine efficacy. In both prophylactic and therapeutic B16-OVA melanoma models, PP@Pt-OVA produced strong tumor inhibition without apparent systemic toxicity. In the prophylactic setting, tumor inhibition reached 92.3%, while therapeutic vaccination also achieved robust suppression of established tumors. Mechanistically, the vaccine promoted efficient lymph-node targeting and retention, improved dendritic-cell maturation in draining lymph nodes, increased intratumoral CD8+ T-cell infiltration, elevated antigen-specific CD8+ responses, and reduced immunosuppressive cell populations such as Tregs and MDSCs. These findings show that the nanozyme platform does not merely improve vaccine delivery; rather, it amplifies the entire antigen presentation-T-cell priming axis and converts catalytic ROS generation into functionally meaningful adaptive immunity.

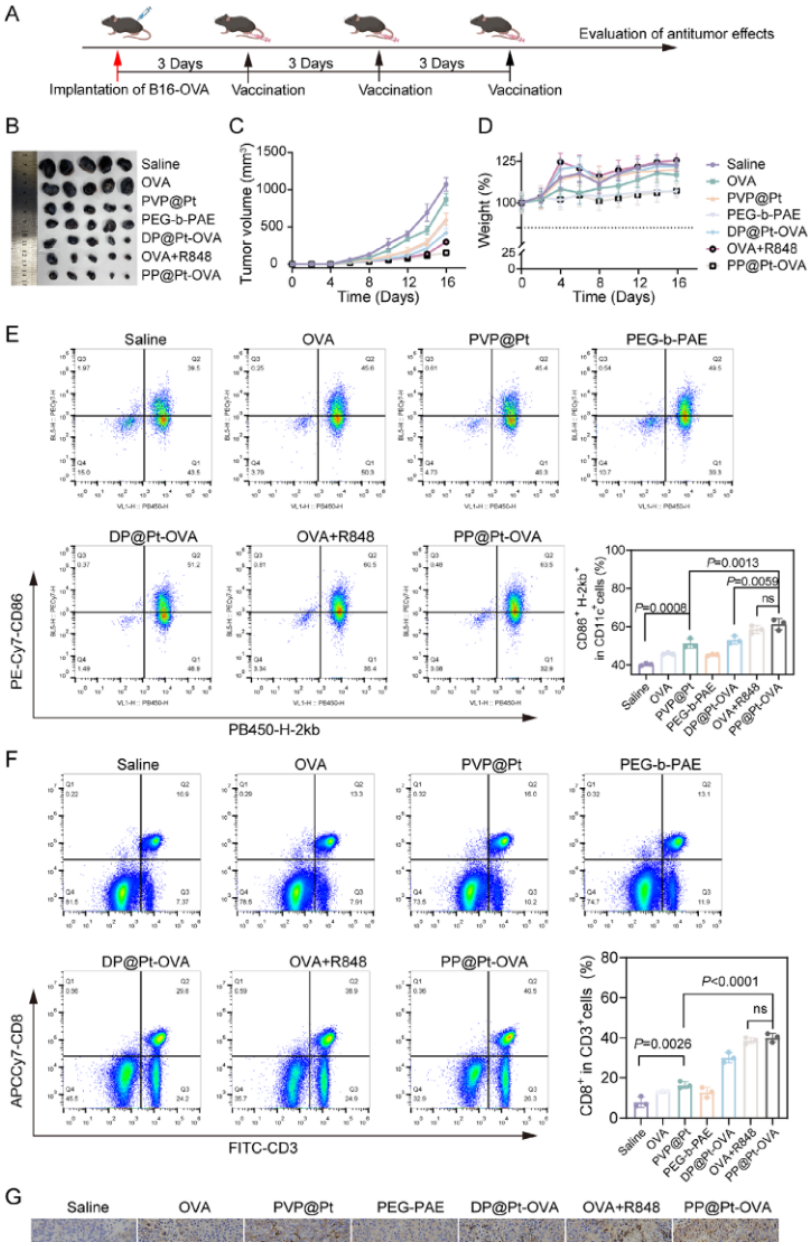

Figure 4. Representative therapeutic antitumor results of the nanozyme-integrated vaccine. The platform enhances dendritic-cell maturation, promotes CD8+ T-cell infiltration, and suppresses tumor growth in a therapeutic melanoma model.

5. Conceptual significance relative to the original research plan

This annual progress does not deviate from the original project goal; instead, it provides a substantial

mechanistic refinement. Our original plan emphasized nanozyme-catalyzed ROS as a means to promote immunogenic cell death and thereby improve antitumor immunity. The results obtained this year reveal a more direct and controllable pathway: nanozyme-derived ROS can function as a catalytic adjuvant that directly activates dendritic cells, promotes antigen cross-presentation, and strengthens cytotoxic T-cell immunity. This finding therefore represents a conceptual shift from indirect ICD-mediated immune activation toward direct nanozyme-enabled immune instruction, and it gives the project a clearer mechanistic center and a stronger translational direction for next-generation tumor subunit vaccines.

6. Outputs and next-stage objectives

The principal scientific output from the first annual period is the following publication:

An J., Yang Y., Feng Y., Wang S., Wang S., Zhang B., Yan X.*, Jiang B.* *Proton-Driven Deformability Enables Nanozyme-Integrated Vaccine for Enhanced Tumor Immunotherapy.* Advanced Materials (2026).

In the next stage of the project, we will further dissect how catalytic ROS regulates dendritic-cell signaling, define the upstream oxidative sensors and downstream cross-presentation machinery, and evaluate whether this catalytic adjuvant mechanism can be generalized to additional tumor-antigen systems and more translational subunit-vaccine settings.