

Article

Ganoderma lucidum Polysaccharide Potentiates mRNA-LNP Efficacy: Synergizing Oxidative Stress Mitigation with Innate Immune Modulation

Ling-Ling Tao, Zhe Zhai, Nan-Yu Chen, Yong-Xian Cheng  and Xu-Han Liu 

Guangdong Provincial Key Laboratory of Chinese Medicine Ingredients and Gut Microbiomics, Institute for Inheritance-Based Innovation of Chinese Medicine, Marshall Laboratory of Biomedical Engineering, School of Pharmacy, Shenzhen University Medical School, Shenzhen University, Shenzhen 518055, China

* Correspondence: yxcheng@szu.edu.cn (Y.-X.C.); liuxuhan891130@163.com (X.-H.L.);

Tel.: +86-(0755)-8671-3994 (Y.-X.C.)

Abstract

Background/Objectives: As a primary mRNA delivery platform, lipid nanoparticles (LNPs) often induce oxidative stress that compromises mRNA translation efficiency. Natural polysaccharides are known for their antioxidant properties. **Methods:** To lower LNP toxicity and boost mRNA delivery, we conducted a preliminary pro-proliferation screen of 34 natural polysaccharides using a CCK-8 cytotoxicity assay in murine macrophage RAW264.7 cells, serving as an initial filter for bioactivity. Subsequently, their ability to improve LNP-mediated transfection efficiency was validated in HEK293T cells—a standard model for quantifying protein expression. After that, *Ganoderma lucidum* polysaccharide (GLP) was selected as a lead candidate for potential adjuvant. **Results:** Formulated into mRNA-LNPs by first preparing the LNPs via a one-step nano-precipitation process, followed by direct incorporation of GLP through mixing, the resulting GLP-LNP formulation significantly alleviated intracellular oxidative stress by elevating glutathione and superoxide dismutase while reducing malondialdehyde, indicating restored redox homeostasis. This modulation correlated with markedly enhanced transfection efficiency, achieving significantly higher protein expression both in vitro (3.2-fold) and in vivo (2.1-fold) compared to LNP alone. Mechanism studies implicated the activation of the nuclear factor erythroid 2-Related Factor 2 (Nrf2) pathway in this protective effect. **Conclusions:** We conclude that GLP represents a novel adjuvant paradigm that concurrently enhances mRNA transfection and mitigates oxidative toxicity, demonstrating significant potential for advanced vaccinology.

Keywords: *Ganoderma lucidum* polysaccharides (GLP); adjuvant; mRNA-LNP; oxidative stress



Academic Editor: Duncan Craig

Received: 31 December 2025

Revised: 4 February 2026

Accepted: 16 February 2026

Published: 19 February 2026

Copyright: © 2026 by the authors.

Licensee MDPI, Basel, Switzerland.

This article is an open access article distributed under the terms and conditions of the [Creative Commons Attribution \(CC BY\)](https://creativecommons.org/licenses/by/4.0/) license.

1. Introduction

As the representative third-generation vaccine technology, mRNA vaccines have rapidly emerged as a pivotal focus in contemporary vaccinology research, primarily attributed to their advantageous characteristics: rapid design capacity, abbreviated production cycles, cost-effective manufacturing, and high antigenic specificity [1,2]. However, the inherent instability, enzymatic degradability, and potent immunogenicity of mRNA necessitate the development of advanced delivery systems to realize its full therapeutic potential. Lipid nanoparticles (LNPs) have been extensively used for mRNA delivery. Assembly of

ionizable lipids, phospholipids, cholesterol, and polyethylene glycol derivatives in specific ratios generates stable core-shell LNPs, which encapsulate mRNA through charge interactions [3], enhancing stability while limiting degradation. However, the potential toxicity of LNPs has been demonstrated to induce abnormal levels of reactive oxygen species (ROS) in vivo, leading to oxidative stress [4,5]. This oxidative damage impairs cellular integrity and decreases mRNA transfection efficiency. This impairment not only compromises the stable expression of mRNA therapeutics but also limits the safety profile and widespread clinical application of vaccines [6]. Consequently, developing advanced delivery systems that mitigate LNP-associated toxicity or introducing novel mRNA vaccine adjuvants has emerged as a critical research imperative to enhance mRNA transfection efficiency.

To mitigate LNP-associated toxicity, current research employs biodegradable ionizable lipids by incorporating hydrolysable bonds such as ester or acetal groups into the backbone, which accelerates in vivo clearance and reduces tissue accumulation and inflammatory responses [7,8]. Additionally, precise regulation of helper lipid ratios like cholesterol and phospholipids optimizes surface hydrophobic/hydrophilic balance and pKa to alleviate cellular and complement activation [9,10]. Furthermore, improvements in PEG layers through degradable PEG derivatives or alternative hydrophilic polymers aim to reduce anti-PEG antibody risks and complement-associated pseudoallergic reactions (CARPA) [11]. Although these innovations enhance safety profiles while preserving delivery efficiency, they inevitably introduce additional synthetic steps and stringent quality control requirements, thereby increasing process complexity and manufacturing costs. Adjuvant co-delivery represents a complementary strategy to carrier modification, aimed at enhancing mRNA transfection efficiency and immunogenicity while alleviating LNP toxicity. Current adjuvant systems primarily comprise self-adjuvanting RNA constructs or exogenous immunostimulants [12], yet exhibit significant functional limitations. For example, TLR agonist adjuvants (e.g., monophosphoryl lipid A, MPLA) effectively activate dendritic cells to initiate antigen presentation [13], but their propensity to hyperactivate pattern recognition receptors (PRRs) triggers pathological inflammation that disrupts immune tolerance homeostasis [14].

Current adjuvant research remains trapped in a ‘unifunctional limitation’ dilemma: synthetic carrier engineering requires sophisticated manufacturing protocols, while exogenous immunostimulants may induce pathological inflammation. Polysaccharides—naturally ubiquitous across botanical, zoological, and microbial sources—demonstrate potent antioxidant capacity, precise immunomodulatory function, and significant anti-inflammatory efficacy. Their intrinsic low toxicity coupled with multifunctional versatility establishes these biomolecules as premier adjuvant candidates for next-generation vaccine platforms [15].

We first screened 34 natural polysaccharides for bioactive properties in macrophages, identifying candidates with proliferative effects. This systematic evaluation identified *Ganoderma lucidum* polysaccharide (GLP) as a prime candidate, aligning with its millennia-long recognition in Asian pharmacopeia as ‘the mushroom of immortality’ [16]. Modern studies substantiate GLP’s multifunctional capacities, including potent antioxidant, immunomodulatory, and antitumor activities [17–22], establishing it as a premier biological response modifier (BRM) with significant adjuvant potential [23]. Subsequent phases characterized the GLP’s physicochemical profile and assessed its capacity to enhance mRNA transfection efficiency in vitro while modulating oxidative responses. Parallel investigations compared formulation-driven variations in cellular stress markers and quantified in vivo protein expression dynamics. Our work transcends current adjuvants’ constraints by demonstrating natural GLPs’ capacity as a dual-action adjuvant that simultaneously mitigates toxicity and

enhancing efficacy, thus establishing a nature-derived adjuvant for next-generation mRNA vaccines (Figure 1).

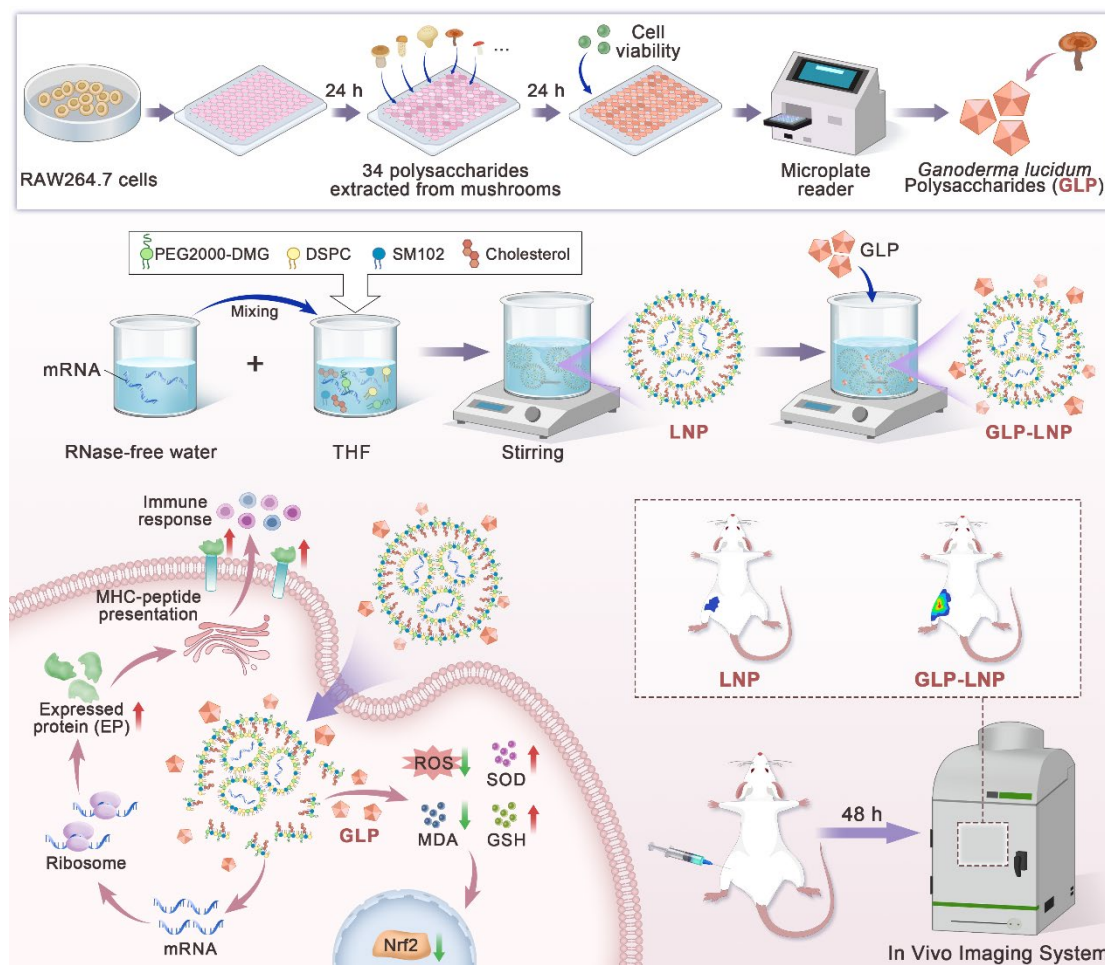


Figure 1. Schematic graph of the intracellular transport of GLP-LNP and mechanism of enhanced mRNA transfection through decreased oxidative stress by GLP. Arrows denote the subsequent step, the addition of specific substances, upward or downward trends, the required duration.

2. Materials and Methods

2.1. Experimental Materials

Ionizable amino lipid SM-102, 1,2-distearoyl-sn-glycero-3-phosphate (DSPC), and cholesterol (Rhawn, Shanghai, China). Polyethylene glycol di-myristate glycerol (PEG2000-DMG) (Shanghai McLean Biochemical Technology Co., Ltd., Shanghai, China). Tetrahydrofuran (THF) (Sigma-Aldrich, St. Louis, MO, USA). Fetal bovine serum (FBS) and 1% penicillin/streptomycin (Gibco, Carlsbad, CA, USA). EZ CapTM firefly luciferase mRNA (Apexbio, Houston, TX, USA). The luciferase reporter gene assay kit (Shanghai Yisen Company, Shanghai, China). Phosphate-buffered saline (PBS) and Dulbecco's Modified Eagle's Medium (DMEM medium) (Hyclone Laboratories, Logan, UT, USA). RNase-free water and TPCK-trypsin (Thermo Fisher Scientific, Loughborough, UK). CCK-8 reagent (Dalian Meilun Biotechnology Co., Ltd., Dalian, China). The enzyme-linked immunosorbent assay reader (Bio Tek Company, Winooski, VT, USA). The PAGE gel rapid preparation kit (Shanghai Epizyme Biomedical Technology Co., Ltd., Shanghai, China). 2',7'-Dichlorodihydrofluorescein diacetate (H2DCFDA), DID and Hoechst 33342 (MedChemExpress, Monmouth Junction, NJ, USA). Nrf2 Antibody (Antibody Affinity Biosciences, Cincinnati, OH, USA). HO-1 Recombinant antibody, NQ01 Polyclonal antibody,

and Lamin B1 Polyclonal antibody (Proteintech Group, Inc., Rosemont, IL, USA). Goat Anti-Rabbit IgG (H + L) HRP (Affinity Biosciences, Cincinnati, OH, USA). Protein-free rapid blocking solution (1×) (Shanghai Epizyme Biomedical Technology Co., Ltd., Shanghai, China). Polyvinylidene difluoride (PVDF) membrane (Zhongshan Jinqiao Company, Beijing, China). A total superoxide dismutase (T-SOD) colorimetric assay kit (Hydroxylamine Method) (Elabscience Biotechnology Co., Ltd., Wuhan, China). The Human Reduced Glutathione (GSH) ELISA Research Kit and Human Malondialdehyde (MDA) ELISA Research Kit (Jiangsu Meimian Industrial Co., Ltd., Yancheng, China). The Cell Nucleus Protein and Cytoplasmic Protein Extraction Kit (Beyotime Biotechnology, Shanghai, China). *Ganoderma lucidum*, *Craterellus lutescens*, *Lepista nuda*, *Lignosus rhinocerus* and *Lactifluus aff. Tropicosinicus* (Yunnan Peak Mushroom Industry Wild Mushroom Wholesale, Kunming, China). *Volvariella volvacea*, *Agaricus subrufescens*, *Lyophyllum decastes*, *Morchella sextelata*, *Butyriboletus roseoflavus*, *Coprinus comatus*, *Grifola frondosa*, *Agrocybe chaxingu*, *Tremella fuciformis*, *Auricularia heimuer*, and *Lentinula edodes* (Zhejiang Pinjun Food Co., Ltd., Lishui, China). *Tricholoma matsutake*, *Hypsizygus marmoreus*, *Pleurotus citrinopileatus*, *Tuber indicum*, *Russula paludosa*, *Cordyceps militaris*, and *Phallus rubrovolvatus* (Yunnan Haigushun Biotechnology Co., Ltd., Kunming, China). *Phlebopus portentosus*, *Termitomyces intermedius*, *Boletus bainiugan*, *Russula rosea*, *Sarcodon imbricatus*, *Auricularia delicata*, *Russula variata*, *Thelephora ganbajun*, *Tuber huidongense*, and *Boletus shiyong* (Kunming Yunzhen Mushroom Industry Co, Ltd., Kunming, China). *Sparassis latifolia* and *Cordyceps militaris* (Yunnan Lanye Food Co., Ltd., Qujing, China).

2.2. Extraction of Polysaccharides

Dried *G. lucidum* fruiting bodies were pulverized and sieved (40 mesh). The powder was defatted with 95% ethanol for 5 h to remove lipids and pigments. The residue was extracted twice with distilled water (1:10 *w/v*) at 80 °C for 3 h. The combined filtrates were concentrated and precipitated with ethanol (final concentration of 80% *v/v*) at 4 °C overnight. The precipitate was collected by centrifugation (4500 rpm, 15 min), redissolved in water, and deproteinized three times using the Sevag reagent (chloroform:n-butanol = 4:1 *v/v*). The purified GLP was dried. The other 33 polysaccharides were obtained using the same protocol as above. More details about the polysaccharide extraction process are provided in the Supporting Information.

2.3. Cell Lines

Human embryonic kidney HEK293T cells and mouse mononuclear macrophage leukemia RAW 264.7 cells (Wuhan Pricella Biotechnology Co., Ltd., Wuhan, China). They were cultured in high-glucose DMEM medium supplemented with 10% FBS and 1% (*v/v*) penicillin/streptomycin at 37 °C in a cell culture incubator containing 5% CO₂.

2.4. Animals

Female BALB/c mice aged 9–12 weeks were purchased from the Guangdong Provincial Center for Medical Laboratory Animals. The mice were maintained under specific pathogen-free conditions in the animal facility at Shenzhen University. All animal experiments were conducted in full compliance with the Regulations for the Care and Use of Laboratory Animals and the Guideline for Ethical Review of Animals (China, GB/T 35892-2018) [24].

2.5. Polysaccharide-Mediated Regulation of Cell Proliferation

Polysaccharides were dissolved in sterile, enzyme-free water respectively to prepare a stock solution of 10 mg mL^{−1}. RAW267.4 cells were seeded at a density of 4 × 10⁴ cells/well in a 96-well plate and cultured for 24 h until cell confluence reached

60~80%. After culturing RAW 264.7 cells for 24 h until 60~80% confluency, the original medium was removed. Then, 198 μL of serum-free DMEM and 2 μL of natural polysaccharide stock solution was added to each well, achieving a final concentration of 100 $\mu\text{g mL}^{-1}$. The cells were incubated for 24 h. The medium was replaced with 100 μL of CCK-8 working solution (prepared by mixing serum-free medium and CCK-8 reagent at a 9:1 volume ratio), and incubated at 37 °C for 40 min. The optical density (OD) was measured at 450 nm using a Bio-Rad Multimode Microplate Reader (Bio-Rad Laboratories, Inc., Hercules, CA, USA), and the cell viability was calculated.

2.6. Preparation and Characterization of Polysaccharide-Modified LNP Formulations

The molecular weight distribution and composition of GLP were analyzed by Agilent PL-GLPC50 gel permeation chromatography (GPC) (Santa Clara, CA, USA), while its functional group composition was characterized using Nicolet iS50 FTIR spectroscopy (Waltham, MA, USA). LNP was prepared by a simple one-step nano-precipitation and solvent evaporation method. The organic phase was prepared by dissolving SM-102, DSPC, cholesterol, and PEG2000-DMG in a THF/methanol co-solvent (6:1, *v/v*) at molar ratios of 3.5:2.7:7:1. This solution was then mixed with the aqueous phase—comprising sterile RNase-free water and luciferase-encoding mRNA—at a 1:3 volumetric ratio (organic:aqueous), maintaining an SM-102-to-mRNA N/P ratio of 5:1. The mixture was continuously stirred under a fume hood until the organic solvent was completely evaporated, thereby forming the mRNA-loaded LNP formulation. GLP was introduced directly into the pre-formed LNP suspension at a final concentration of 100 $\mu\text{g mL}^{-1}$ to create a *Ganoderma lucidum* polysaccharide–LNP mixture, generating the GLP–LNP complex. All polysaccharide–LNP complexes were prepared using identical methods. The particle size distribution and zeta potential of each sample was determined by a Nano Particle Size and Zeta Potential Analyzer (Malvern Zetasizer Nano ZS90, Malvern, UK), and the morphological characteristics of different LNPs were observed using Transmission Electron Microscopy (TEM) (Hitachi High-Tech Corporation, Tokyo, Japan).

2.7. In Vitro Transfection Assay

HEK293T cells were seeded at a density of 3×10^4 cells per well in a 96-well plate for 48 h until confluence reached 60~80%. Then, the growth medium was removed and replaced with serum-free DMEM containing LNP or polysaccharide-modified LNPs, all standardized to 1 $\mu\text{g mL}^{-1}$ mRNA content. Following a 4 h period of co-incubation, the transfection medium was replaced with complete growth medium supplemented with 10% FBS and 1% (*v/v*) penicillin–streptomycin, followed by an additional 24 h incubation period. Luciferase activity was quantified using a GloMax[®] Microplate Reader (Promega, Madison, WI, USA). For detection, 50 μL of luciferase substrate was combined with 50 μL of culture medium per well, initiating a luminescent reaction proportional to transfection efficiency. The resulting signal was measured in Relative Light Units (RLU).

2.8. Detection of Intracellular Reactive Oxygen Species

First, 1×10^5 HEK293T cells were seeded in 20 mm confocal culture dishes and cultured for 48 h. Subsequently, cells were treated with PBS, LNP, or GLP–LNP (containing 0.5 $\mu\text{g mL}^{-1}$ mRNA dissolved in serum-free medium) for 4 h. Subsequently, a mixture of cell membrane stain DID, nuclear stain Hoechst 33342, and ROS probe H2DCFDA was diluted 1:1000 in serum-free DMEM medium and used for staining at 37 °C for 20 min. The cells were then washed three times with serum-free DMEM medium for 5 min each, followed by the addition of PBS and observation under a ZEISS LSM 880 Super-resolution Confocal Laser Scanning Microscope (Carl Zeiss Microscopy GmbH, Jena, Germany). Intracellular ROS levels were quantified based on the mean green fluorescence intensity

of H2DCFDA. All confocal images were acquired under identical parameters. Image analysis was performed using ImageJ software (version 1.53k), where cellular regions were uniformly identified by applying a consistent grayscale threshold, and the mean fluorescence intensity within these regions was calculated.

2.9. Intracellular Oxidative Stress

HEK293T cells (3×10^6 cells/well) were cultured in 6-well plates for 48 h to 60–80% confluency, then treated with serum-free medium containing LNP or GLP-LNP ($0.5 \mu\text{g mL}^{-1}$ mRNA) or PBS control for 8 h. Cells were washed with PBS, trypsinized, resuspended in 500 μL ice-cold PBS, and homogenized by ultrasonication. The homogenate was centrifuged at $10,000 \times g$ (4°C , 10 min); supernatants were collected on ice for: (1) protein quantification via BCA assay (absorbance measured at 550 nm using UV spectrophotometry, Hangzhou Haiyouke Instrument Co., Ltd., Hangzhou, China); (2) SOD activity determination by the hydroxylamine method based on the superoxide anion ($\text{O}_2^{\cdot-}$) scavenging capacity; (3) MDA and GSH analysis via ELISA ($\text{OD}_{450 \text{ nm}}$).

2.10. In Vivo Bioluminescence Imaging

The mice were placed into groups of $n = 4$ and housed in an acclimatized environment with free access to food and water for at least 7 days prior to the experiment. Each group of mice received intramuscular injections of LNP, VC-LNP (LNP modified with Vitamin C, VC = 50 μmmol , M), or GLP-LNP (LNP/GLP = 100 $\mu\text{g mL}^{-1}$, w/v) formulation at 2 μg of fLuc mRNA per leg. After 48 h, the mice were intraperitoneally injected with D-fluorescein substrate (150 mg kg^{-1}) and allowed to rest for 15 min. The mice were anesthetized in the induction chamber with isoflurane at a flow rate of 3 L min^{-1} using the XGI-8 gas anesthesia system. After anesthetizing the mice with isoflurane, administered at a flow rate of 1 L min^{-1} , in vivo imaging was performed using the IVIS[®] spectral imaging system. Signal intensity was expressed as RLU (relative light units), and signal quantification at each injection site was performed using molecular imaging software (version 4.5). At the end of the experiment, mice were euthanized by intraperitoneal injection of sodium pentobarbital at a dose of 300 mg kg^{-1} .

2.11. Oxidative Stress-Related Nrf2 Signaling Proteins

First HEK293T cells (3×10^6) were seeded into 6 cm dishes and cultured for 48 h, followed by treatment with LNP or GLP-LNP ($0.5 \mu\text{g mL}^{-1}$ in serum-free medium) for 12 h. The control group was treated with PBS for 12 h. After thorough PBS washing, nucleoproteins were extracted using a Nuclear and Cytoplasmic Protein Extraction Kit. Protein samples were denatured at 90°C for 10–15 min, subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE), and transferred to a polyvinylidene difluoride (PVDF) membranes, which were blocked with Fast Blocking Western and washed three times with TBST (5 min per wash). Membranes were incubated with primary antibodies (1:1500) against Lamin B1, Nrf2, HO-1, and NOQ1 at 4°C for 16h, followed by a species-specific secondary antibody (1:5000), Goat Anti-Rabbit IgG (H + L) HR, at room temperature for 1 h. After three 5 min TBST washes, protein bands were visualized using enhanced chemiluminescence (ECL) detection kits, with chemiluminescent signal acquisition and quantitative analysis performed on an Invitrogen iBright[™] CL1000 Imaging System (Waltham, MA, USA).

2.12. Statistical Analysis

Statistical analysis was used in the data of this study. All the data are shown as mean $\pm$ SD and were analyzed using one-way ANOVA statistical analysis for inter/intra-group comparisons. Graphical representations, including bar charts, line graphs, and Kaplan–

Meier survival curves, were generated using GraphPad Prism 9 (GraphPad Software, San Diego, CA, USA).

3. Results and Discussion

3.1. Polysaccharide-Mediated Regulation of Cell Proliferation and In Vitro Transfection

RAW264.7 macrophages were selected as the screening model due to their highly sensitive immune response characteristics [25], representative antigen-presenting cell (APC) functionality [2,26], and stable in vitro growth kinetics with low basal activation, providing a high-signal-background platform for adjuvant evaluation. Moreover, the proliferation and metabolic activity of RAW264.7 cells are not only indicators of cell viability but also closely related to phagocytosis, antigen processing and subsequent immune activation. Because LNP itself has a certain degree of toxicity, it will lead to deterioration of the cell state, thereby reducing the ability of mRNA-encoding antigen expression. Polysaccharides that promote macrophage viability under normal conditions suggest that they lay a more robust cellular foundation for subsequent nanoparticle uptake, such as LNP internalization, and antigen expression challenges. Crucially, their proliferative capacity serves as a dual-parameter indicator: it directly reflects cellular metabolic vitality and immunocompetence [27], while also predicting APC-enhanced vaccine immunogenicity through optimized LNP uptake and antigen presentation efficiency [28]. Therefore, we prioritized candidates that positively influenced macrophage vitality.

Using CCK-8 assays, we evaluated 34 natural polysaccharides for proliferative effects on RAW 264.7 macrophages. At $100\ \mu\text{g mL}^{-1}$, five candidates exhibited significant bioactivity: *Ganoderma lucidum* polysaccharide (GLP), *Tuber huidongense* polysaccharide (THP), *Hypsizygus marmoreus* polysaccharide (HMP), *Coprinus comatus* polysaccharide (CCP), and *Lactifluus aff. tropicosinicus* polysaccharide (LATP). GLP demonstrated the most potent enhancement (**** $p < 0.0001$), whereas THP, CCP, and LATP showed robust but comparatively lower activity (*** $p < 0.001$). HMP exhibited the lowest activity (** $p < 0.01$), as quantified in Figure 2A–H. A concentration of $100\ \mu\text{g mL}^{-1}$ was selected for the primary polysaccharide screening based on a scientifically grounded and strategically balanced rationale. This concentration is well-established in the literature on polysaccharide bioactivity [29,30] and aligns with the unique pharmacological properties of high-molecular-weight polysaccharides. Due to their limited passive diffusion and receptor-mediated uptake (e.g., via TLRs or Dectin-1), polysaccharides typically exhibit a broad therapeutic window, wherein bioactivity often plateaus after a saturation threshold is reached. As such, moderate concentration variations within the active range generally yield consistent effects, making a single well-chosen concentration appropriate for initial comparative screening.

The proliferative capacity of polysaccharides on RAW 264.7 macrophages serves as a direct indicator of cellular metabolic vitality and immunocompetence [27]. Given that LNP-induced oxidative stress often impairs cellular viability, and considering that active polysaccharides can enhance both cell survival and proliferative potential, we hypothesized that their mechanism may involve the alleviation of oxidative stress, thereby improving mRNA transfection efficiency and ultimately contributing to their adjuvant efficacy. Based on proliferative screening results, five candidates—GLP, THP, HMP, CCP, and LATP—were evaluated as adjuvants using HEK293T cells for in vitro transfection efficiency alongside the antioxidant VC control group. HEK293T cells were used for in vitro transfection due to their well-established suitability, including unique membrane properties, high permissiveness to exogenous nucleic acids, and strong protein expression capacity. Their high sensitivity enables accurate quantification of transfection efficiency and protein expression levels with minimal interference from complex innate immune defense mechanisms, thereby minimizing potential immune-related variables that might otherwise mask or

confound the specific impact of the polysaccharides. This solidifies the utility of HEK293T cells as the gold-standard model for evaluating transfection efficiency [31]. Luciferase reporter assays revealed that GLP-LNP and THP-LNP significantly enhanced transfection efficiency in HEK293T cells compared to other formulations, while VC-LNP showed no statistically meaningful improvement (Figure 2I). This critical divergence demonstrates that merely scavenging reactive oxygen species [32,33], as achieved by VC, is insufficient to substantially enhance mRNA-LNP delivery efficiency. Beyond oxidative stress mitigation, GLP's adjuvant efficacy may involve immune receptor-mediated activation, particularly through TLR/RLR signaling cascades to amplify antigen processing efficiency [34].

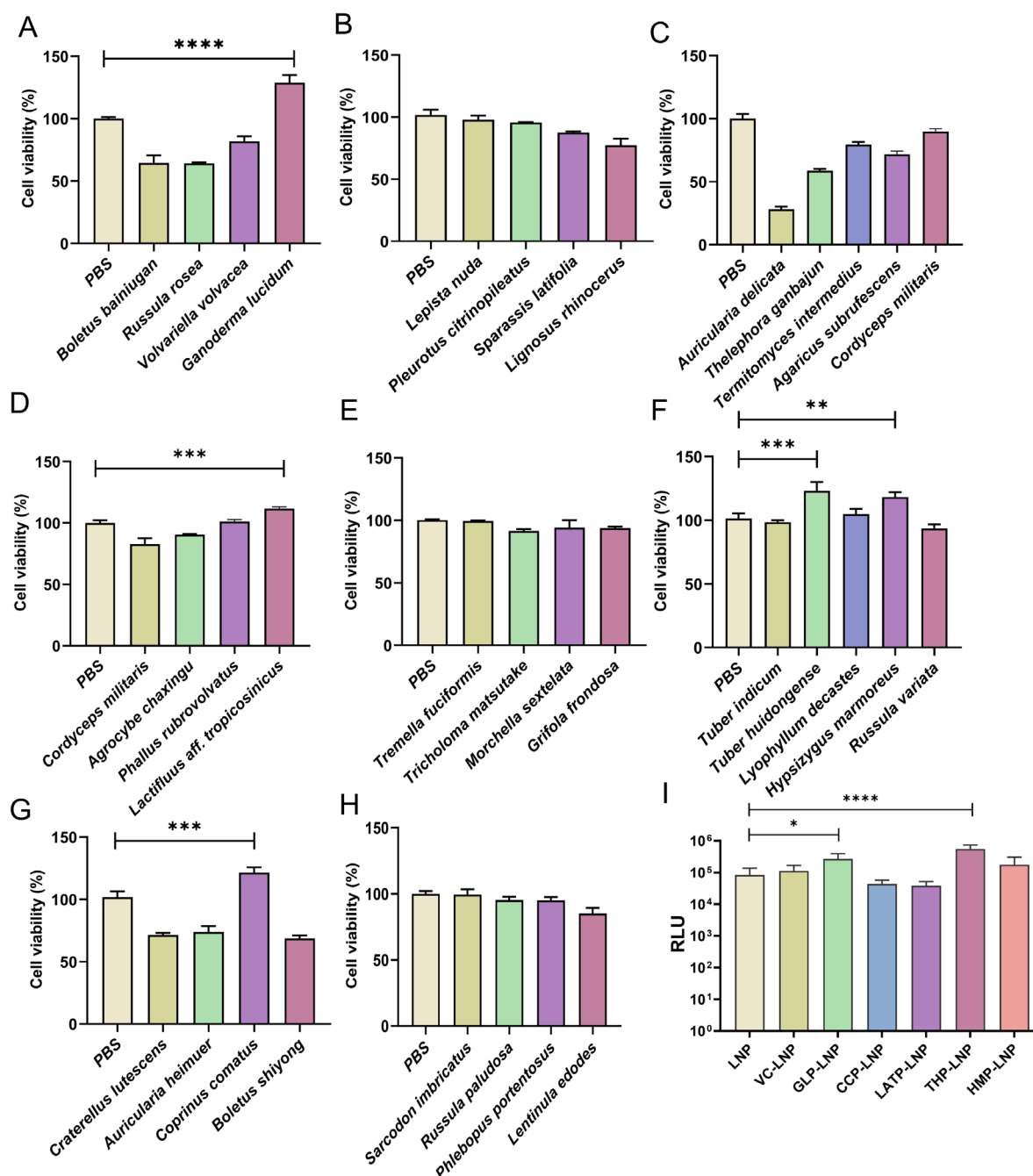


Figure 2. (A–H) Proliferation effect of 34 natural polysaccharides on RAW264.7 cells, (n = 3). (I) In vitro transfection efficiency of various polysaccharide-modified LNPs, (n = 5). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

Consequently, we focused on GLP as the core adjuvant candidate due to its superior ability to enhance proliferative capacity in macrophages and improve LNP transfection efficiency. Although THP yielded comparable results, its origin from common edible fungi limits its medicinal value. Furthermore, the scarcity of existing research on THP hinders subsequent studies and practical application. GLP is derived from traditional *Ganoderma lucidum*, a ‘herb of immortality’ highly esteemed in classical pharmacopoeias with millennia of documented use. Extensive modern research further substantiates *Ganoderma*’s anti-tumor, immunomodulatory, and antioxidant properties. These intrinsic bioactivities strongly support its potential as a vaccine adjuvant capable of regulating cellular and oxidative stress responses. Moreover, standardized cultivation of *Ganoderma lucidum* ensures stable, traceable, and consistent raw material quality. These characteristics—high efficiency, low toxicity, industrial maturity—make GLP an optimal candidate for in-depth mechanism and translational research.

3.2. Preparation and Characterization of Polysaccharide-Modified LNP Formulations

GPC analysis revealed GLP’s unimodal molecular weight distribution, with the dominant peak at a retention time of 10.85 min, corresponding to weight-average ($M_w = 10,316 \text{ g}\cdot\text{mol}^{-1}$) and number-average ($M_n = 7809 \text{ g}\cdot\text{mol}^{-1}$) molecular weights. The narrow polydispersity index ($PDI = 1.32$) confirms high molecular homogeneity, indicating that the molecular weight distribution of GLPs was relatively uniform (Figure 3A,B). The FTIR results identify signature functional groups: a broad O-H stretch at 3370 cm^{-1} ; C-H ring vibrations at 1649 cm^{-1} ; characteristic furanose absorptions at 1032 , 1077 , and 1152 cm^{-1} ; and a definitive β -glycosidic bond signal at 899 cm^{-1} , establishing GLP’s β -pyranose structural framework (Figure 3C).

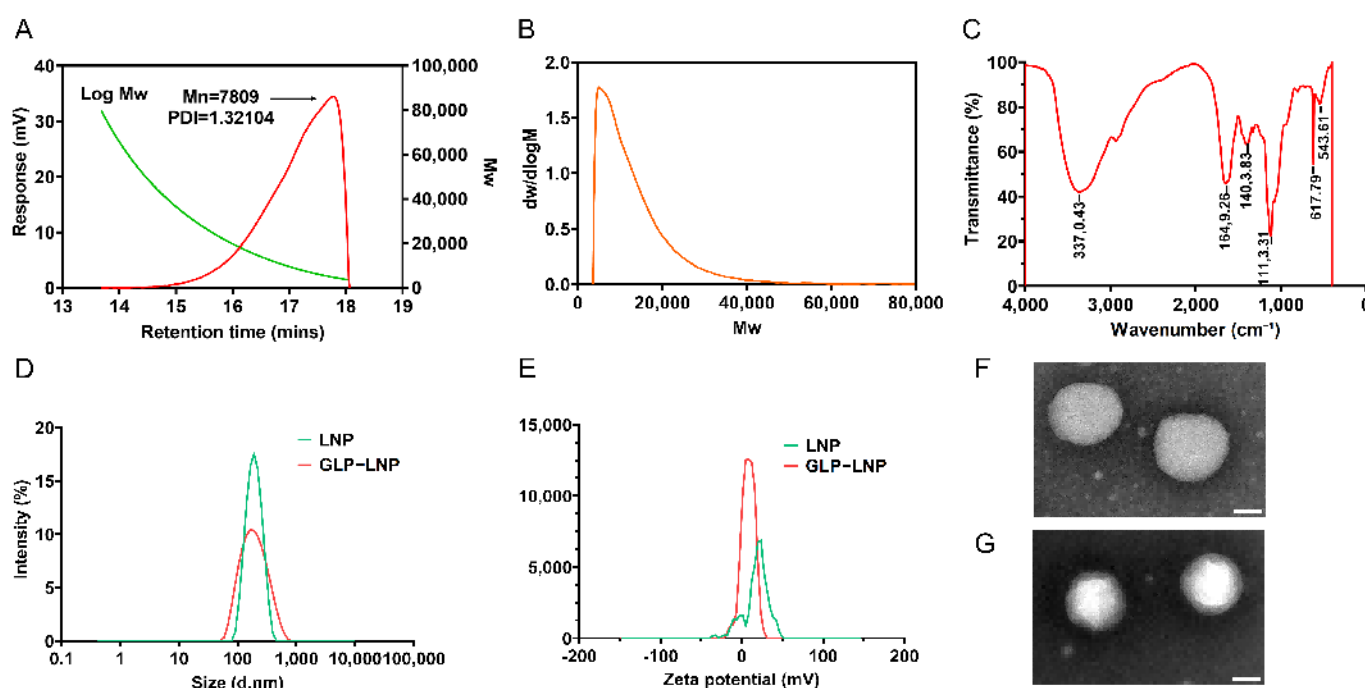


Figure 3. (A) Retention time and (B) molecular weight distribution of GLP determined by GPC. (C) FTIR spectrum of GLP. (D,E) Size distribution and zeta potentials of LNP and GLP-LNP formulations. TEM images of (F) LNP and (G) GLP-LNP. Bar = 50 nm.

Dynamic light scattering (DLS) demonstrated that GLP functionalization preserved core nanoparticle properties: unmodified LNP exhibited $164.5 \pm 8.4 \text{ nm}$ a hydrodynamic diameter ($PDI = 0.16$) versus GLP-LNP’s $161.7 \pm 6.3 \text{ nm}$ ($PDI = 0.21$). Zeta potential analysis

showed marginal charge modulation (LNP: $+18 \pm 0.6$ mV; GLP-LNP: $+7.7 \pm 0.9$ mV), confirming surface modification without colloidal destabilization (Figure 3D,E) (Table 1). TEM revealed unmodified LNPs as monodisperse spherical nanostructures with well-defined peripheries and smooth surfaces (Figure 3F). Following GLP functionalization, GLP-LNPs retained structural integrity with preserved core-shell architecture, but exhibited a distinct low-electron-density coating uniformly encapsulating the nanoparticle surface (Figure 3G). This continuous polysaccharide layer confirms GLP's localization on the particle exterior modification. Collectively, these physicochemical analyses confirm successful GLP functionalization through non-disruptive surface adsorption. The preserved nanoarchitecture provides the structural foundation for mRNA delivery in subsequent biological evaluations. Our one-pot method produced GLP-LNP and LNP samples with batch-to-batch size stability, PDI < 0.3, and three replicates are described in the Supporting Information (Table S1).

Table 1. Size distribution and zeta potentials of LNP and GLP-LNP formulations.

Sample	Size (d. nm)	Zeta Potential (mV)	PDI
LNP	164.5 ± 8.4	18.0 ± 0.6	0.16 ± 0.05
GLP-LNP	161.7 ± 6.3	7.7 ± 0.9	0.21 ± 0.04

3.3. Detection of Intracellular Reactive Oxygen Species

To verify the mechanism of GLP in alleviating LNP-induced oxidative stress—a key pathway for enhancing cell viability and transfection efficiency—we employed the H2DCFDA probe for quantitative reactive oxygen species (ROS) assessment. As can be seen from the confocal images in Figure 4A, the PBS control group exhibited weak green fluorescence signals, staying in a state of equilibrium, whereas the LNP group displayed strong green fluorescence signals, indicating the occurrence of certain oxidative damage within the cells; conversely, the green fluorescence intensity of the GLP-LNP co-treatment group decreased to a level close to that of the PBS group, visually confirming the scavenging of partial ROS and the recovery of metabolism. Analysis in Figure 4B revealed significant differences in mean fluorescence intensity (MFI): PBS-treated cells maintained redox equilibrium, whereas cells exposed to LNPs exhibited severe oxidative damage with a significant increase in MFI (**** $p < 0.0001$); crucially, GLP-LNP co-treatment significantly reduced ROS levels, showing a significantly lower MFI compared to the LNP group (*** $p < 0.0001$). These quantitative MFI data are completely consistent with the confocal image observations.

3.4. Intracellular Oxidative Stress

Superoxide dismutase (SOD), the primary enzymatic scavenger of superoxide anions ($O_2^{\bullet-}$), constitutes the frontline defense against oxidative stress [35], where its activity serves as a critical biomarker: diminished levels indicate compromised antioxidant capacity leading to macromolecular damage, while restoration signifies enhanced redox resilience [36]. Our assays revealed that LNP exposure induced SOD suppression but no significant difference, confirming nanoparticle-triggered impairment of endogenous antioxidant defenses; crucially, GLP-LNP co-treatment rescued SOD activity to near-baseline levels ($\approx 92\%$ recovery), demonstrating effective mitigation of enzymatic inhibition (Figure 5A).

Glutathione (GSH), the predominant non-enzymatic antioxidant in cellular systems, directly scavenges free radicals while serving as an essential co-substrate for glutathione peroxidase (GPx) during peroxide degradation [37]. Diminished GSH levels signify impaired reducing capacity, precipitating oxidative modifications that disrupt protein folding and function; conversely, GSH restoration facilitates reconstruction of the cellular reduc-

ing environment, thereby supporting physiological protein synthesis and translational fidelity. ELISA quantification demonstrated concordance with SOD trends. The LNP exposure induced severe depletion ($*** p < 0.001$ vs. PBS), indicating oxidative defense compromise. However, GLP-LNP intervention achieved near-complete recovery ($\approx 85\%$ of PBS, $** p < 0.01$ vs. LNP), validating GLP's role in rescuing cellular antioxidant capacity (Figure 5B).

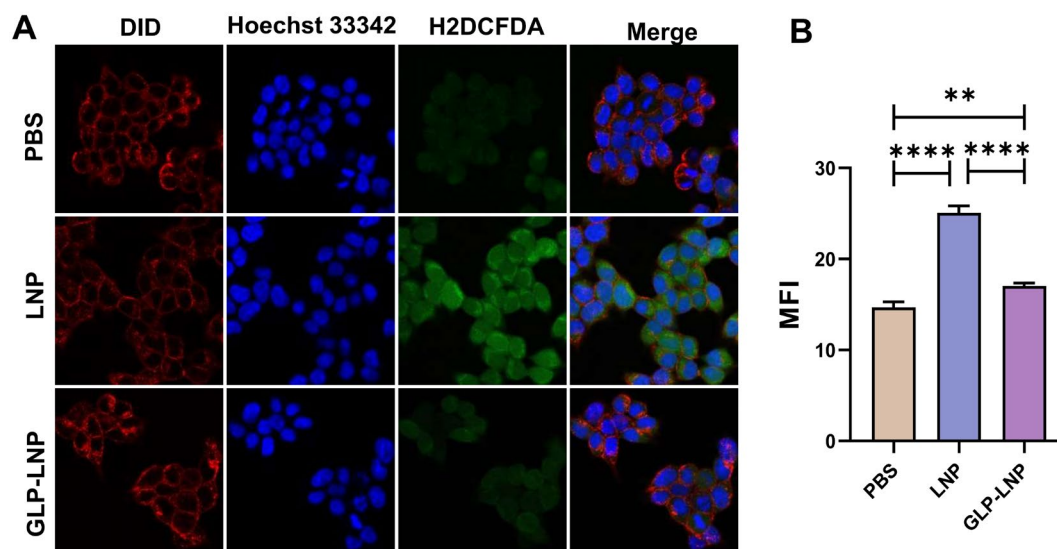


Figure 4. (A) Visualization of intracellular ROS in HEK293T cells after treatment with freshly prepared PBS, LNP and GLP-LNP for 4 h. (B) Quantitative intracellular ROS analysis of HEK293T cells after incubation with LNP or GLP-LNP formulation (at $1 \mu\text{g mL}^{-1}$ mRNA) for 4 h. Intracellular fluorescence microscopy images of HEK293T cells after incubation, ($n = 3$). $** p < 0.01$, $**** p < 0.0001$.

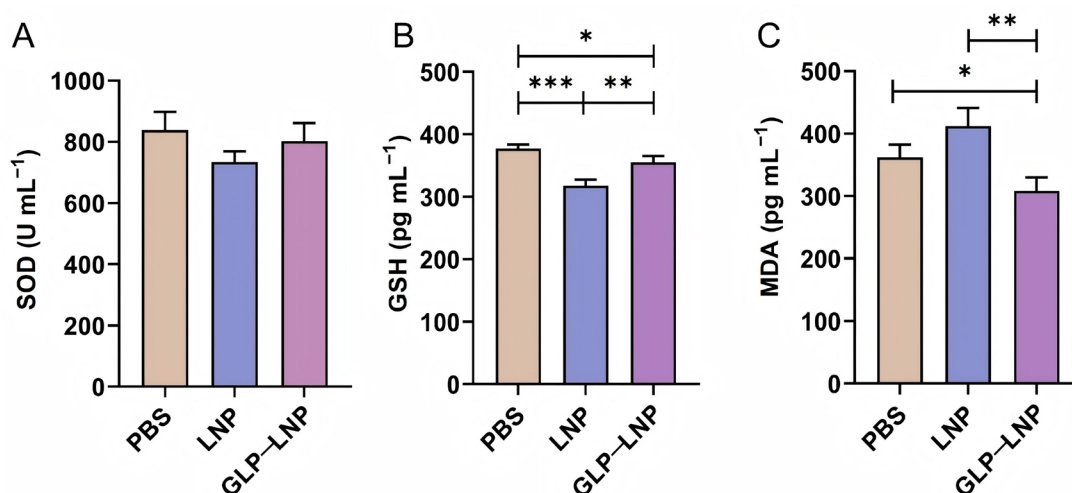


Figure 5. Intracellular oxidative stress evaluation. (A–C) The results of SOD, GSH, and MDA levels in HEK293T cells treated with three different formulations (PBS, LNP, and GLP-LNP) for 8 h. $* p < 0.05$, $** p < 0.01$, $*** p < 0.001$, ($n = 3$).

Malondialdehyde (MDA) is one of the end products of lipid peroxidation and is commonly used as a marker of membrane lipid oxidative damage. MDA generated when cell membrane lipids are attacked by free radicals can undergo addition reactions with proteins and DNA, disrupting membrane integrity, altering membrane protein function, and affecting cellular signal transduction [38]. Elevated MDA levels indicate oxidative damage to the cell membrane, impaired permeability, and disruption of membrane-related processes;

a decrease in MDA indicates that lipid peroxidation is inhibited and membrane function is restored. For LNP-mediated mRNA delivery, the integrity of membrane lipids and endosome/lysosome membranes, membrane proteins, and the membrane microenvironment directly affect nanoparticle uptake, endosomal escape, and mRNA release. Therefore, a decrease in MDA is typically associated with a healthier membrane microenvironment, which in turn facilitates effective mRNA transport and expression. MDA, as a lipid peroxidation end product, reflects the extent of oxidative damage in cells. Experimental results show (Figure 5C) that MDA levels in the LNP group were significantly higher than those in the PBS group, indicating that LNP-induced oxidative stress led to elevated lipid peroxidation levels. In contrast, MDA levels in the GLP-LNP group decreased significantly (** $p < 0.01$ vs. LNP; * $p < 0.05$ vs. PBS), with the lowest levels observed. This suggests that GLP-modified LNP can effectively alleviate lipid peroxidation damage caused by oxidative stress.

3.5. *In Vivo* Bioluminescence Imaging

To evaluate *in vivo* mRNA delivery efficacy, we assessed protein expression in mice using an IVIS[®] Spectrum *In Vivo* Imaging System (PerkinElmer, Waltham, MA, USA). Strikingly, GLP-LNP exhibited superior *in vivo* luminescent intensity in bilateral hindlimbs (>2.1-fold vs. LNP, ** $p < 0.01$), significantly outperforming both LNP and VC-LNP (Figure 6A,B), validating its enhanced transfection efficiency that aligns with *in vitro* results. Crucially, VC-LNP (identical preparation but with antioxidant vitamin C) showed similar efficacy to LNP, underscoring that mere oxidative stress mitigation cannot recapitulate GLP's adjuvant properties. The results indicate that beyond antioxidant capacity, GLP may enhance mRNA delivery efficiency through coordinated immune regulation [39,40], including phagocyte/dendritic cell activation, MHC/co-stimulatory molecule upregulation [41,42], and pattern recognition receptor (e.g., TLRs) stimulation [34].

3.6. *Oxidative Stress-Related Nrf2 Signaling Proteins*

Nuclear factor erythroid 2-related factor 2 (Nrf2), a pivotal transcription factor that translocates to the nucleus and binds the antioxidant response element (ARE) [43–45], orchestrates detoxification gene transcription to mitigate oxidative stress. Its dynamic expression serves as a sensitive indicator of intracellular oxidative burden: initial stress triggers Nrf2 upregulation as a cytoprotective response, while stress resolution prompts its downregulation, reflecting diminished demand for antioxidant defenses. To elucidate GLP's oxidative stress alleviation mechanism, we quantified Nrf2 pathway proteins (Nrf2, HO-1, NQO1) using Lamin B1 as a nuclear loading control. Western blot analysis revealed there was no significant differences in Nrf2, HO-1, or NQO1 expression between PBS and LNP groups. GLP-LNP treatment induced marked suppression of nuclear Nrf2, with concordant suppression of its canonical downstream effectors HO-1 and NQO1 (Figure 6C). We propose that this coordinated attenuation is not the cause but rather a consequence of GLP's potent antioxidant activity: by efficiently scavenging ROS and mitigating lipid peroxidation, GLP alleviates the oxidative burden, thereby reducing the cellular demand for Nrf2-mediated adaptive activation. This mechanism highlights GLP's capacity to restore redox homeostasis fundamentally [46]. Furthermore, this Nrf2 pathway downregulation aligns with the significantly reduced intracellular oxidative signals, mechanistically validating GLP's efficacy in mitigating LNP-induced toxicity. Based on these findings, we hypothesize that the enhancement of transfection efficiency by GLP may extend beyond oxidative stress mitigation. Its established immunomodulatory properties could potentially induce mild activation of antigen-presenting cells (APCs), thereby synergistically enhancing antigen processing and immune responses. This prospective mechanism warrants further investigation to fully elucidate the multifaceted adjuvant potential of GLP.

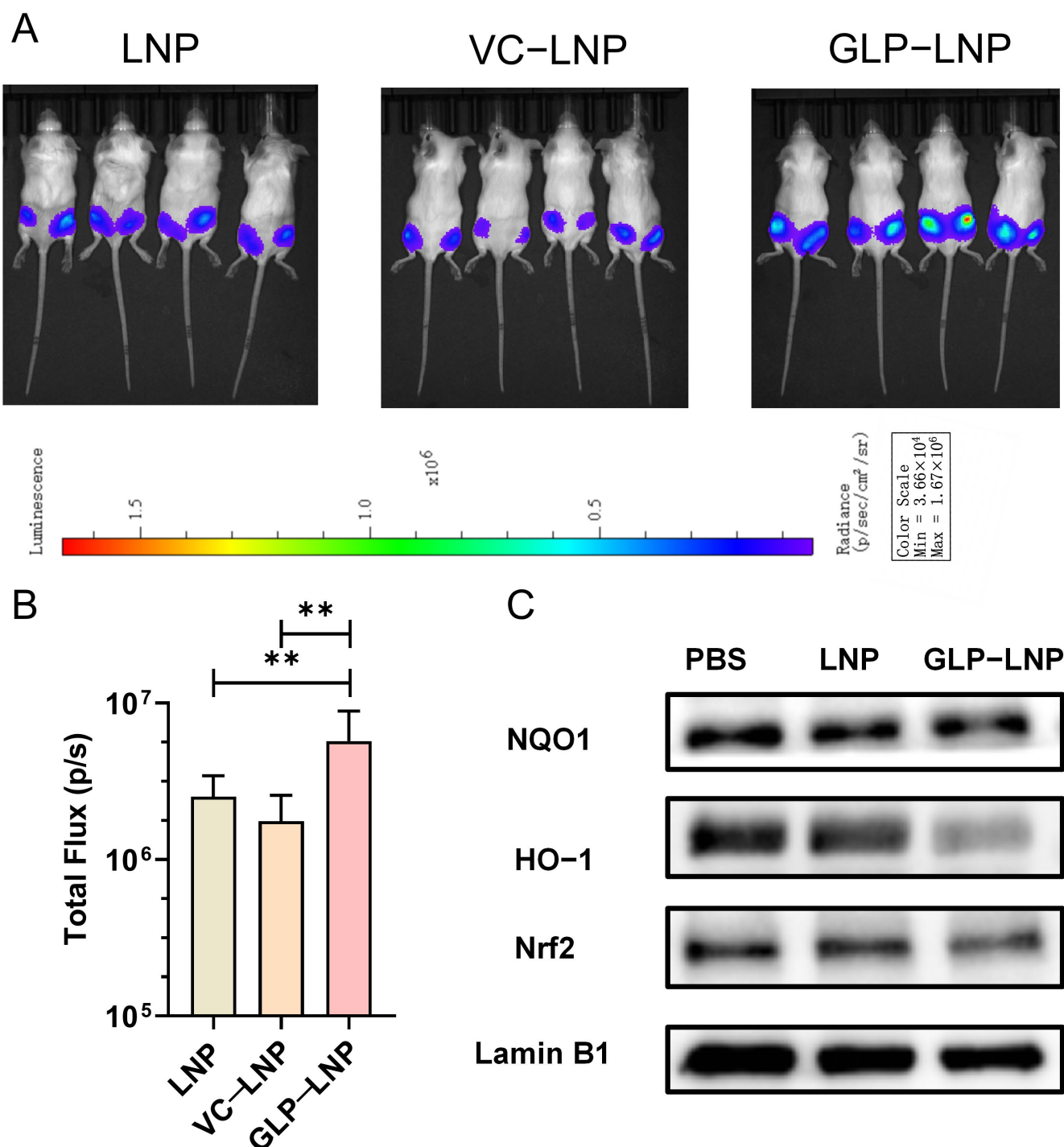


Figure 6. (A) In vivo luminescence images and (B) quantitative analysis of fLuc expression in Balb/C mice after intramuscular injection of various formulations at 2 μ g of fLuc mRNA per leg for 48 h, ($n = 8$). (C) Western blotting analysis of Nrf2 in the nuclei of HEK293T cells after incubation with PBS, LNP, and GLP-LNP for 8 h. ** $p < 0.01$.

4. Conclusions

This study establishes *Ganoderma lucidum* polysaccharide (GLP) as a novel dual-functional adjuvant for mRNA-LNP vaccines, effectively resolving the longstanding challenge of balancing efficacy with safety in nucleic acid delivery. Rather than merely offering incremental improvement, our work introduces a paradigm shift in adjuvant design by leveraging the innate bioactivity of natural polysaccharides to simultaneously modulate oxidative stress and enhance immunogenicity. We demonstrate that GLP-LNP robustly

restores redox homeostasis, as evidenced by Nrf2 pathway downregulation, and enhances mRNA transfection efficiency both in vitro and in vivo, outperforming conventional LNP and antioxidant-only controls. The superior performance of GLP over conventional antioxidants underscores a fundamental principle: effective mRNA vaccine augmentation requires coordinated multifunctionality rather than singular biological activity. This positions GLP not as a simple additive but as a biologically active mediator that reshapes the cellular microenvironment for improved mRNA translation and expression. Critically, this study illuminates the transformative potential of natural macromolecules in advancing vaccine technology. GLP's favorable safety profile, biocompatibility, and dual functional potency suggest strong clinical translation potential, particularly for next-generation mRNA vaccines requiring enhanced stability and reduced reactogenicity. We frame the present findings as a foundational step that establishes a promising platform. The investigation of specific antigen-encoded vaccines, including the evaluation of humoral and cellular immune responses, as well as in vivo protective efficacy, constitutes the direct and active focus of our subsequent research. We propose GLP as a reference material for developing nature-inspired vaccine adjuvants, offering a sustainable and pharmacologically sound alternative to synthetic counterparts. By integrating traditional pharmacological knowledge with contemporary vaccine technology, this work opens new avenues for harnessing natural products in advanced drug delivery systems.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/pharmaceutics18020259/s1>, Table S1: Size distribution of LNP and GLP-LNP with different batches.

Author Contributions: Conceptualization: X.-H.L. Data curation: L.-L.T. and X.-H.L. Investigation: L.-L.T., Z.Z., and N.-Y.C. Project administration: L.-L.T., Z.Z., and N.-Y.C. Funding acquisition: X.-H.L. Supervision: Y.-X.C. and X.-H.L. Writing—original draft: L.-L.T. Writing—review and editing: X.-H.L., L.-L.T., Z.Z., N.-Y.C., and Y.-X.C. All authors have read and agreed to the published version of the manuscript.

Funding: This research received financial support from the National Natural Science Foundation of China (32500829), the Guangdong Basic and Applied Basic Research Foundation (2023A1515110428, 2025A1515012065), the Shenzhen Medical Research Fund (D2401001), the Shenzhen Science and Technology Program (JCYJ20240813142059020) and the Shenzhen High-level Talent Scientific Research Start-up Foundation (827-000743).

Institutional Review Board Statement: The animal study protocol was approved by the Institutional Animal Care and Use Committee of Shenzhen University Medical School (protocol code IACUC-202400129, approved on 18 September 2024).

Data Availability Statement: All data generated or analyzed in this study are included in the article and its supplementary information files. Source data can be made available upon request to the corresponding authors.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Igyártó, B.Z.; Qin, Z. The mRNA-LNP vaccines—The good, the bad and the ugly? *Front. Immunol.* **2024**, *15*, 1336906.
2. Hou, X.; Zaks, T.; Langer, R.; Dong, Y. Lipid nanoparticles for mRNA delivery. *Nat. Rev. Mater.* **2021**, *6*, 1078–1094. [[CrossRef](#)]
3. Huang, X.; Ma, Y.; Ma, G.; Xia, Y. Unlocking the therapeutic applicability of LNP-mRNA: Chemistry, formulation, and clinical strategies. *Research* **2024**, *7*, 0370. [[CrossRef](#)]
4. Ndeupen, S.; Qin, Z.; Jacobsen, S.; Bouteau, A.; Estanbouli, H.; Igyártó, B.Z. The mRNA-LNP platform's lipid nanoparticle component used in preclinical vaccine studies is highly inflammatory. *iScience* **2021**, *24*, 103479. [[CrossRef](#)]
5. Dokka, S.; Toledo, D.; Shi, X.; Castranova, V.; Rojanasakul, Y. Oxygen radical-mediated pulmonary toxicity induced by some cationic liposomes. *Pharm. Res.* **2000**, *17*, 521–525. [[CrossRef](#)]

6. Schoenmaker, L.; Witzigmann, D.; Kulkarni, J.A.; Verbeke, R.; Kersten, G.; Jiskoot, W.; Crommelin, D.J. mRNA-lipid nanoparticle COVID-19 vaccines: Structure and stability. *Int. J. Pharm.* **2021**, *601*, 120586. [\[CrossRef\]](#) [\[PubMed\]](#)
7. Liu, Z.M.; Yang, M.H.; Yu, K.; Lian, Z.X.; Deng, S.L. Toll-like receptor (TLRs) agonists and antagonists for COVID-19 treatments. *Front. Pharmacol.* **2022**, *13*, 989664. [\[CrossRef\]](#)
8. Wang, W.; Chen, K.; Jiang, T.; Wu, Y.; Wu, Z.; Ying, H.; Yu, H.; Lu, J.; Lin, J.; Ouyang, D. Artificial intelligence-driven rational design of ionizable lipids for mRNA delivery. *Nat. Commun.* **2024**, *15*, 10804. [\[CrossRef\]](#) [\[PubMed\]](#)
9. Hald Albertsen, C.; Kulkarni, J.A.; Witzigmann, D.; Lind, M.; Petersson, K.; Simonsen, J.B. The role of lipid components in lipid nanoparticles for vaccines and gene therapy. *Adv. Drug Deliv. Rev.* **2022**, *188*, 114416. [\[CrossRef\]](#)
10. Tilstra, G.; Couture-Sen  cal, J.; Lau, Y.M.A.; Manning, A.M.; Wong, D.S.M.; Janaeska, W.W.; Wuraola, T.A.; Pang, J.; Khan, O.F. Iterative design of ionizable lipids for intramuscular mRNA delivery. *J. Am. Chem. Soc.* **2023**, *145*, 2294–2304. [\[CrossRef\]](#) [\[PubMed\]](#)
11. Tenchov, R.; Sasso, J.M.; Zhou, Q.A. PEGylated lipid nanoparticle formulations: Immunological safety and efficiency perspective. *Bioconjugate Chem.* **2023**, *34*, 941–960. [\[CrossRef\]](#)
12. Xie, C.; Yao, R.; Xia, X. The advances of adjuvants in mRNA vaccines. *npj Vaccines* **2023**, *8*, 162. [\[CrossRef\]](#)
13. Romerio, A.; Gotri, N.; Franco, A.R.; Artusa, V.; Shaik, M.M.; Pasco, S.T.; Atxabal, U.; Matamoros-Recio, A.; M  n  ez-Toral, M.; Zalamea, J.D.; et al. New glucosamine-based TLR4 agonists: Design, synthesis, mechanism of action, and in vivo activity as vaccine adjuvants. *J. Med. Chem.* **2023**, *66*, 3010–3029. [\[CrossRef\]](#) [\[PubMed\]](#)
14. Wang, L.L.-W.; Gao, Y.; Feng, Z.; Mooney, D.J.; Mitragotri, S. Designing drug delivery systems for cell therapy. *Nat. Rev. Bioeng.* **2024**, *2*, 944–959. [\[CrossRef\]](#)
15. Wu, Y.; Liang, X.; Mao, C.; Jiang, Y. The distinct properties of polysaccharide nanoparticles tune immune responses against mRNA antigen via stimulator of interferon genes-mediated autophagy and inflammasome. *ACS Nano* **2023**, *17*, 21782–21798. [\[CrossRef\]](#)
16. Wachtel-Galor, S.; Yuen, J.; Buswell, J.A.; Benzie, I.F.F. *Ganoderma lucidum* (Lingzhi or Reishi): A Medicinal Mushroom. In *Herbal Medicine: Biomolecular and Clinical Aspects*, 2nd ed.; CRC Press: Boca Raton, FL, USA; Taylor & Francis: Hoboken, NJ, USA, 2011.
17. Lin, Z.B. Cellular and molecular mechanisms of immuno-modulation by *Ganoderma lucidum*. *J. Pharmacol. Sci.* **2005**, *99*, 144–153. [\[CrossRef\]](#)
18. Wang, S.Y.; Hsu, M.L.; Hsu, H.C.; Lee, S.S.; Shiao, M.S.; Ho, C.K. The anti-tumor effect of *Ganoderma lucidum* is mediated by cytokines released from activated macrophages and T lymphocytes. *Int. J. Cancer* **1997**, *70*, 699–705. [\[CrossRef\]](#)
19. Sohretoglu, D.; Huang, S. *Ganoderma lucidum* Polysaccharides as An Anti-cancer Agent. *Anticancer Agents Med. Chem.* **2018**, *18*, 667–674. [\[CrossRef\]](#)
20. Suarez-Arroyo, I.J.; Rosario-Acevedo, R.; Aguilar-Perez, A.; Clemente, P.L.; Cubano, L.A.; Serrano, J.; Schneider, R.J.; Mart  nez-Montemayor, M.M. Anti-tumor effects of *Ganoderma lucidum* (reishi) in inflammatory breast cancer in in vivo and in vitro models. *PLoS ONE* **2013**, *8*, e57431. [\[CrossRef\]](#) [\[PubMed\]](#)
21. Chen, Y.S.; Chen, Q.Z.; Wang, Z.J.; Hua, C. Anti-Inflammatory and Hepatoprotective Effects of *Ganoderma lucidum* Polysaccharides against Carbon Tetrachloride-Induced Liver Injury in Kunming Mice. *Pharmacology* **2019**, *103*, 143–150. [\[CrossRef\]](#)
22. Luo, H.J.; Zhang, Y.K.; Wang, S.Z.; Lin, S.Q.; Wang, L.F.; Lin, Z.X.; Lu, G.D.; Lin, D.M. Structural characterization and anti-oxidative activity for a glycopeptide from *Ganoderma lucidum* fruiting body. *Int. J. Biol. Macromol.* **2024**, *261*, 129793. [\[CrossRef\]](#)
23. Xu, F.; Li, X.; Xiao, X.; Liu, L.F.; Zhang, L.; Lin, P.P.; Zhang, S.L.; Li, Q.S. Effects of *Ganoderma lucidum* polysaccharides against doxorubicin-induced cardiotoxicity. *Biomed. Pharmacother.* **2017**, *95*, 504–512. [\[CrossRef\]](#)
24. GB/T 35892-2018; Guidelines for the Ethical Review of Laboratory Animal Welfare. Standardization Administration of China: Beijing, China, 2018.
25. Ou, Y.; Zhu, L.; Xu, S.; Wei, Q. Activation of RAW264.7 macrophage by Exopolysaccharide from *Aphanothece halaphytica* (EPSAH) and the underlying mechanisms. *Fundam. Clin. Pharmacol.* **2020**, *34*, 591–602. [\[CrossRef\]](#)
26. Manicassamy, S.; Pulendran, B. Modulation of adaptive immunity with Toll-like receptors. *Semin. Immunol.* **2009**, *21*, 185–193. [\[CrossRef\]](#)
27. Ross, E.A.; Devitt, A.; Johnson, J.R. Macrophages: The good, the bad, and the gluttony. *Front. Immunol.* **2021**, *12*, 708186. [\[CrossRef\]](#) [\[PubMed\]](#)
28. Lemdani, K.; Marlin, R.; Mayet, C.; Perkov, V.; Pascal, Q.; Ripoll, M.; Relouzat, F.; Dhooze, N.; Bossevot, L.; Dereuddre-Bosquet, N.; et al. Distinct dynamics of mRNA LNPs in mice and nonhuman primates revealed by in vivo imaging. *npj Vaccines* **2024**, *9*, 113. [\[CrossRef\]](#)
29. Huang, L.; Zhao, J.; Wei, Y.; Yu, G.; Li, F.; Li, Q. Structural characterization and mechanisms of macrophage immunomodulatory activity of a pectic polysaccharide from *Cucurbita moschata* Duch. *Carbohydr. Polym.* **2021**, *269*, 118288. [\[CrossRef\]](#) [\[PubMed\]](#)
30. Liu, Y.; Yang, J.; Guo, Z.; Li, Q.; Zhang, L.; Zhao, L.; Zhou, X. Immunomodulatory effect of *Cordyceps militaris* polysaccharide on RAW 264.7 macrophages by regulating MAPK signaling pathways. *Molecules* **2024**, *29*, 3408. [\[CrossRef\]](#) [\[PubMed\]](#)
31. Dumont, J.; Euwart, D.; Mei, B.; Estes, S.; Kshirsagar, R. Human cell lines for biopharmaceutical manufacturing: History, status, and future perspectives. *Crit. Rev. Biotechnol.* **2016**, *36*, 1110–1122. [\[CrossRef\]](#)

32. Kaźmierczak-Barańska, J.; Boguszevska, K.; Adamus-Grabicka, A.; Karwowski, B.T. Two Faces of Vitamin C—Antioxidative and Pro-Oxidative Agent. *Nutrients* **2020**, *12*, 1501. [[CrossRef](#)]
33. Duarte, T.L.; Lunec, J. Review: When is an antioxidant not an antioxidant? a review of novel actions and reactions of vitamin C. *Free Radic. Res.* **2005**, *39*, 671–686. [[CrossRef](#)]
34. Seweryn, E.; Ziła, A.; Gamian, A. Health-Promoting of Polysaccharides Extracted from *Ganoderma lucidum*. *Nutrients* **2021**, *13*, 2725. [[CrossRef](#)]
35. Rosa, A.C.; Corsi, D.; Cavi, N.; Bruni, N.; Dosio, F. Superoxide Dismutase Administration: A Review of Proposed Human Uses. *Molecules* **2021**, *26*, 1844. [[CrossRef](#)]
36. Alscher, R.G.; Erturk, N.; Heath, L.S. Role of superoxide dismutases (SODs) in controlling oxidative stress in plants. *J. Exp. Bot.* **2002**, *53*, 1331–1341. [[CrossRef](#)]
37. Jomova, K.; Alomar, S.Y.; Alwasel, S.H.; Nepovimova, E.; Kuca, K.; Valko, M. Several lines of antioxidant defense against oxidative stress: Antioxidant enzymes, nanomaterials with multiple enzyme-mimicking activities, and low-molecular-weight antioxidants. *Arch. Toxicol.* **2024**, *98*, 1323–1367. [[CrossRef](#)] [[PubMed](#)]
38. Zheng, Y.; Sun, J.; Luo, Z.; Li, Y.; Huang, Y. Emerging mechanisms of lipid peroxidation in regulated cell death and its physiological implications. *Cell Death Dis.* **2024**, *15*, 859. [[CrossRef](#)]
39. Meng, J.; Hu, X.; Shan, F.; Hua, H.; Lu, C.; Wang, E.; Liang, Z. Analysis of maturation of murine dendritic cells (DCs) induced by purified *Ganoderma lucidum* polysaccharides (GLPs). *Int. J. Biol. Macromol.* **2011**, *49*, 693–699. [[CrossRef](#)] [[PubMed](#)]
40. Lin, G.; Da, F.; Wan, X.; Huang, Y.; Yang, S.; Jian, J.; Cai, S. Immune-enhancing effects of Astragalus polysaccharides and *Ganoderma lucidum* polysaccharides on *Vibrio harveyi* flgJ DNA vaccine in grouper. *J. Fish Dis.* **2023**, *46*, 147–156. [[CrossRef](#)] [[PubMed](#)]
41. Li, J.; Gu, F.; Cai, C.; Hu, M.; Fan, L.; Hao, J.; Yu, G. Purification, structural characterization, and immunomodulatory activity of the polysaccharides from *Ganoderma lucidum*. *Int. J. Biol. Macromol.* **2020**, *143*, 806–813. [[CrossRef](#)]
42. Zhang, S.; Pang, G.; Chen, C.; Qin, J.; Yu, H.; Liu, Y.; Zhang, X.; Song, Z.; Zhao, J.; Wang, F.; et al. Effective cancer immunotherapy by *Ganoderma lucidum* polysaccharide-gold nanocomposites through dendritic cell activation and memory T cell response. *Carbohydr. Polym.* **2019**, *205*, 192–202. [[CrossRef](#)]
43. Ulasov, A.V.; Rosenkranz, A.A.; Georgiev, G.P.; Sobolev, A.S. Nrf2/Keap1/ARE signaling: Towards specific regulation. *Life Sci.* **2022**, *291*, 120111. [[CrossRef](#)]
44. Dinkova-Kostova, A.T.; Copple, I.M. Advances and challenges in therapeutic targeting of NRF2. *Trends Pharmacol. Sci.* **2023**, *44*, 137–149. [[CrossRef](#)]
45. Ngo, V.; Duennwald, M.L. Nrf2 and Oxidative Stress: A General Overview of Mechanisms and Implications in Human Disease. *Antioxidants* **2022**, *11*, 2345. [[CrossRef](#)]
46. Liu, X.H.; Song, H.P.; Tao, L.L.; Zhai, Z.; Huang, J.X.; Cheng, Y.X. Trehalose-loaded LNPs enhance mRNA stability and bridge in vitro in vivo efficacy gap. *npj Vaccines* **2025**, *10*, 201. [[CrossRef](#)]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.