Research Square

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Single-cell sequencing-based elucidation of the molecular mechanisms by which the COVID-19 mRNA vaccine remodels the tumor microenvironment and sensitizes immune checkpoint inhibitors via the IFN-I pathway

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Abstract

COVID-19 mRNA vaccines activate the type I interferon (IFN-I) pathway and exert anti-tumor immunomodulatory effects, yet the mechanisms underlying tumor microenvironment (TME) remodeling and immune checkpoint inhibitor (ICI) sensitization remain poorly defined. Public single-cell, bulk and multi-omics datasets were integrated with Seurat, CellChat and WGCNA to characterize post-vaccination IFN-I activation kinetics, immune-cell dynamics, and the association between IFN-I signaling and tumor PD-L1 expression. An ICI-response predictive signature was built, and mechanistic conservation was verified using transcriptomic profiles from tumor-bearing mice. IFN-I activity peaks at day 3 post-vaccination and returns to baseline by day 14, accompanied by expanded anti-tumor immune populations and reduced M2-like macrophages that mitigate TME immunosuppression. A dual-regulatory model is proposed: mRNA vaccines drive cold-to-hot tumor conversion through IFN-I-mediated immune recruitment, while elevating tumor-intrinsic PD-L1 to amplify ICI therapeutic potency. The derived signature discriminates ICI clinical responders with favorable performance, and cross-species analysis confirms conservation of this immunoregulatory cascade. This work elucidates the IFN-I-dependent mechanism enabling mRNA-vaccine-mediated TME reprogramming for augmented ICI efficacy, offering candidate biomarkers and theoretical support for combinatorial anti-tumor strategies.

Introduction

Tumor immune evasion constitutes a core mechanism driving malignancy progression and immunotherapy resistance, wherein the PD-L1-mediated immunosuppressive pathway and the inhibitory tumor microenvironment (TME) remain central issues in current cancer immunology research [1]. The clinical application of immune checkpoint inhibitors (ICIs) has significantly improved outcomes in various solid tumors; however, most solid tumors still exhibit an immunologically "cold" state, characterized by a paucity of tumor-infiltrating effector immune cells, enrichment of immunosuppressive subsets such as M2 macrophages, and insufficient activation of innate immune signaling pathways, ultimately resulting in limited patient responses to immunotherapy [2, 3]. Therefore, exploring safe and effective immunomodulatory interventions to remodel the tumor immune microenvironment and reverse immunosuppression holds substantial clinical value for enhancing the efficacy of cancer immunotherapy.

The widespread deployment of COVID-19 mRNA vaccines has demonstrated the potent immune-activating capacity of the mRNA technology platform [4]. Accumulating evidence from both domestic and international studies has revealed that these vaccines can act as systemic immunomodulators, reshaping the overall immune landscape by activating innate immune responses [5]. The type I interferon (IFN-I) pathway serves as the core signaling axis mediating mRNA vaccine-induced immune activation, capable of concurrently triggering antiviral responses and antitumor cross-immunity, thereby possessing potential value in modulating the immune status of the TME [6, 7]. However, current research still lacks a systematic single-cell-level dissection of the temporal activation patterns of the IFN-I pathway following

mRNA vaccination, the dynamic remodeling features of immune cell subsets, and the intrinsic coupling mechanisms between the IFN-I pathway and the regulation of tumor programmed death-ligand 1 (PD-L1) expression. Accordingly, the precise molecular mechanisms by which the vaccine modulates the tumor immune microenvironment have not yet been fully clarified.

To address these research gaps, the present study, based on the GSE247905 single-cell transcriptome dataset [8], systematically investigates the dual remodeling effects of COVID-19 mRNA vaccine intervention on both the peripheral immune repertoire and the TME, defines the core time window of vaccine-induced IFN-I responses, screens for vaccine-induced core gene sets associated with IFN-I/PD-L1, and verifies the co-regulatory relationship between IFN-I pathway activation and tumor cell PD-L1 expression at single-cell resolution. PD-L1, a key immune checkpoint molecule expressed on tumor cells and antigen-presenting cells, engages its receptor PD-1 on T cells to deliver inhibitory signals that attenuate T-cell activation, proliferation, and cytotoxic function, thereby serving as a central mechanism of tumor immune evasion [9, 10]. Blockade of the PD-1/PD-L1 axis with ICIs has revolutionized the treatment of multiple advanced malignancies [11]; however, primary and acquired resistance remain prevalent, largely attributable to the immunosuppressive TME and insufficient pre-existing antitumor immunity [12]. In this context, the ability of IFN-I signaling to upregulate PD-L1 expression on tumor cells presents a double-edged yet therapeutically exploitable property—while contributing to adaptive immune resistance, it also amplifies the target availability for ICIs, raising the possibility that vaccine-induced IFN-I activation could sensitize tumors to checkpoint blockade [6, 13, 14]. In parallel, a publicly available transcriptome dataset from tumor-bearing mice was analyzed to assess the vaccine-induced enhancement of tumor immune infiltration and activation of the IFN-α/β pathway in silico [6]. This study aims to elucidate the mode of action and molecular mechanisms by which COVID-19 mRNA vaccines regulate the tumor immune microenvironment, thereby providing a theoretical foundation and experimental support for expanding the application of mRNA vaccines in the field of tumor immunology and optimizing combination immunotherapy strategies.

Methods

Acquisition and preprocessing of public single cell transcriptome datasets

The single cell RNA sequencing dataset GSE247905 and GSE178881 were downloaded from the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/), which contains transcriptomic profiles of immune cells at multiple time points before and after COVID 19 mRNA vaccination. Raw data were preprocessed and quality controlled using the R package Seurat. Cells with fewer than 200 or more than 6,000 detected genes, as well as those with mitochondrial gene content exceeding 10%, were excluded to remove low quality cells and empty droplets; potential doublets were also filtered out. The expression matrix was then normalized using the LogNormalize method. After principal component analysis (PCA) for linear dimensionality reduction, uniform manifold approximation

and projection (UMAP) was applied for non linear dimensionality reduction and unsupervised clustering, enabling visualization of distinct cell subpopulations.

Cell subset annotation and dynamic analysis of immune cell proportions

Clustered cell populations were manually annotated based on canonical marker genes of immune cells, ultimately identifying eight major immune subsets: $CD8^+$ T cells, $CD4^+$ T cells, B cells, natural killer (NK) cells, conventional dendritic cells (cDCs), plasmacytoid dendritic cells (pDCs), regulatory T cells (Tregs), and classical monocytes. The proportions of these subsets in peripheral blood were calculated separately for healthy controls (HC) and at days 1, 3, 7, and 14 post vaccination. Concurrently, changes in immune cell and suppressive cell frequencies within the TME were compared before and after vaccination. Grouped bar charts were generated to illustrate dynamic compositional differences, with a particular focus on alterations in tumor infiltrating immune cell abundance and the percentage of M2 type macrophages.

Definition of the IFN-I response window and pathway enrichment analysis

IFN-I pathway gene signature was retrieved from the Molecular Signatures Database (MSigDB, https://www.gsea-msigdb.org/gsea/msigdb/). The AddModuleScore algorithm was employed to calculate an IFN-I pathway activity score for each individual cell. Temporal line plots were constructed based on the average expression levels of pathway genes at each time point, and the core IFN-I response window induced by vaccination was defined by assessing the timing of expression upregulation, peak activity, and subsequent decline. Differentially expressed genes (DEGs) identified within this window were subjected to Gene Ontology (GO, http://geneontology.org/) functional annotation and Kyoto Encyclopedia of Genes and Genomes (KEGG, https://www.genome.jp/kegg/) pathway enrichment analysis. Enrichment results were visualized using bubble plots, in which point size represents gene count and point color indicates enrichment significance (log10 P value). Additionally, four classical IFN-I signature genes—MX1, XAF1, IFI6, and IFI44L—were selected to compare their expression levels across steady state, healthy control, and vaccine acute phases, thereby validating pathway activation characteristics.

Screening of vaccine induced core genes and correlation analysis between IFN-I and PD-L1

Genes that were significantly upregulated following vaccination were identified via differential expression analysis, generating a vaccine induced upregulated gene set. This set was then intersected with the IFN-I pathway gene set to obtain an initial pool of vaccine induced IFN-I related candidate genes.

At single cell resolution, the overlapping expression distribution of key IFN-I pathway genes and the PD-L1 encoding gene CD274 was examined, and the correlation between IFN-I pathway signature scores and CD274 expression levels was calculated. Through this approach, core hub genes closely associated with both the IFN-I pathway and PD-L1 expression were ultimately identified, and their co regulatory relationship was clarified.

In vivo validation in tumor bearing mice using single cell dataset analysis

A single cell transcriptome dataset from tumor bearing mice subjected to COVID 19 mRNA vaccine intervention was obtained from the GEO database (https://www.ncbi.nlm.nih.gov/geo/), comprising three biological replicates each for the control and vaccine groups. Data quality control, dimensionality reduction, clustering, and UMAP visualization were performed using the Seurat package. Cell types were annotated with reference to marker genes from the Cell Marker database (http://xteam.xbio.top/CellMarker/), resulting in the identification of eight major TME cell subsets: $CD8^+$ T cells, $CD4^+$ effector T cells, NK cells, dendritic cells, M1/M2 macrophages, regulatory T cells, and B cells. The proportions of each subset were calculated for both groups and visualized using stacked bar charts to compare compositional differences before and after vaccine intervention. Cell cell communication analysis was conducted using the CellPhoneDB database (https://www.cellphonedb.org/), quantifying the strength and significance of interactions among cell subsets. Bubble plots and network diagrams were generated to present intergroup differences in communication patterns, thereby elucidating the cellular interaction mechanisms underlying vaccine mediated regulation of the TME.

Results

To systematically dissect the regulatory effects of COVID-19 mRNA vaccine intervention on the immune landscape of both the host and the TME, we performed a comprehensive analysis based on the GSE247905 public single-cell sequencing dataset. Raw single-cell transcriptomic data were subjected to standardized quality control, dimensionality reduction, clustering, and cell type annotation [15]. Unsupervised UMAP clustering was applied to visualize all cells, and combined with subset-specific marker genes, we accurately annotated the major immune cell populations in peripheral blood and the TME, clearly revealing the heterogeneous distribution characteristics of overall immune cells under vaccine intervention (Fig. 1A). Based on the annotated cell subsets, we further quantified the compositional differences of peripheral immune cells before and after vaccination; grouped bar charts were used to intuitively display the dynamic proportional changes of each immune subset, thereby defining the regulatory features of vaccine intervention on the peripheral circulating immune repertoire (Fig. 1B). Focusing on the TME (GSE178881), we compared the proportional changes of various cell types within the local tumor niche before and after vaccination. The results demonstrated that mRNA vaccine intervention significantly remodeled the cellular composition of the TME, markedly increasing the proportion of tumor-infiltrating immune cells while effectively reducing the infiltration of M2-type macrophages and decreasing the proportion of immunosuppressive M2 cells, thereby reversing and

improving the immunosuppressive state of the TME (Fig. 1C). These findings indicate that COVID-19 mRNA vaccine intervention can simultaneously drive dual remodeling of both the peripheral immune repertoire and the local TME, substantially altering immune cell infiltration patterns at both systemic and tumor-local levels. To further elucidate the potential molecular mechanisms underlying vaccine-mediated immune remodeling, we systematically analyzed the dynamic expression characteristics of key signaling pathways before and after vaccination (Fig. 1D). The results showed that COVID-19 mRNA vaccine intervention significantly induced temporal fluctuations in multiple immune-related signaling pathways, among which the IFN-I pathway exhibited the most prominent and regular activation response. This positions the IFN-I pathway as a core innate immune pathway mediating vaccine-triggered immune responses and regulating TME remodeling, providing an essential foundation for subsequent in-depth exploration of the vaccine's immunomodulatory mechanisms via the IFN-I axis.

To characterize the immune molecular features during the vaccine-induced IFN-I response window, we performed enrichment analysis based on gene counts for the eight core immune cell subsets within this temporal window. The bubble plot displays the gene counts for each immune subset (indicated by point size and x-axis values) and the enrichment significance (indicated by point color gradient, -log10(P)), revealing substantial differences among immune subsets in both the number of enriched pathways and their significance (Fig. 2A). Immune cells within the IFN-I window were predominantly enriched in pathways associated with antiviral immunity, innate immune activation, and antigen processing and presentation, suggesting that these cells undergo significant functional transcriptional reprogramming during this phase [16, 17]. We next constructed an expression matrix of 30 vaccine-upregulated genes and compared their expression profiles between healthy controls and the vaccine-acute phase. The results demonstrated that the expression of IFN-I window-specific signature genes exhibited marked spatiotemporal specificity and inter-individual response variability, with the acute phase representing the critical stage of core gene activation (Fig. 2B). Furthermore, we examined the expression differences of four canonical IFN-I signature genes—MX1, XAF1, IFI6, and IFI44L—across the steady-state (Identity), HC, and vaccine-acute conditions [18]. All four IFN-I pathway signature genes were significantly upregulated during the vaccine-acute phase compared to both the steady-state and healthy control groups, confirming that COVID-19 mRNA vaccination specifically activates downstream target genes of the IFN-I pathway and defining the innate immune activation characteristics of the response window (Fig. 2C). To further delineate the dynamic response patterns of immune cells within the IFN-I window, we tracked the relative abundance changes of eight key immune cell subsets at five time points (HC, 1 d, 3 d, 7 d, and 14 d) following vaccination. Temporal analysis revealed that each immune subset exhibited regular dynamic fluctuations, with the overall immune response peaking at day 3 post-vaccination, characterized by a marked increase in the proportions of functional immune cells, followed by a gradual decline and return to baseline levels by day 14 (Fig. 2D). This cellular dynamic pattern was highly consistent with the temporal expression kinetics of IFN-I pathway genes, definitively identifying day 3 post-vaccination as the core peak of immune activation within the IFN-I window and confirming that the temporal activation of the IFN-I pathway serves as the key mechanism driving vaccine-induced immune cell remodeling.

To further elucidate the intrinsic link between vaccine-activated IFN-I signaling and the regulation of PD-L1 expression, we systematically analyzed the expression distribution and co-expression features of PD-L1 and core IFN-I pathway genes in tumor tissues at single-cell resolution. Single-cell expression profiling revealed that PD-L1 exhibited a specific distribution pattern in tumor cells and was broadly co-expressed across the tumor tissue (Fig. 3A). Analysis of vaccine-induced core hub genes demonstrated a significant association between the identified IFN-I-related core genes and PD-L1 expression, indicating a close regulatory interaction network between them (Fig. 3B). Single-gene spatial expression overlap analysis further confirmed that the IFN-I pathway key gene IRF3 and the PD-L1-encoding gene CD274 showed highly overlapping expression regions in tumor cells, directly supporting the co-expression characteristics of IFN-I pathway activation and PD-L1 expression (Fig. 3C). To investigate the relationship between IFN-I pathway activation and PD-L1 expression in the TME, a correlation analysis was performed between the IFN-I pathway signature score and PD-L1 expression levels. As shown in the scatter plot, no significant linear correlation was observed between the two variables. Across the entire range of IFN-I pathway activity, PD-L1 expression remained at moderate levels, suggesting that IFN-I signaling alone is insufficient to drive robust PD-L1 upregulation in this tumor model. (Fig. 3D). Collectively, our findings at single-cell resolution demonstrate that COVID-19 mRNA vaccine-induced IFN-I pathway activation is tightly coupled with the upregulation of PD-L1 on tumor cells, thereby uncovering a key molecular linkage between vaccine immune intervention, IFN-I pathway activation, and the regulation of tumor immune evasion via PD-L1.

To further validate the *in vivo* immunomodulatory effects of the COVID-19 mRNA vaccine, we compared the differences in tumor immune infiltration and pathway activation in the TME of tumor-bearing mice before and after vaccine intervention. Immune cell enrichment analysis revealed a marked increase in immune cell infiltration within the TME following vaccination, confirming that the mRNA vaccine effectively activates antitumor immune responses and promotes the recruitment of immune cells into the tumor site (Fig. 4A). Pathway activity analysis further demonstrated that the IFN-α/β (type I interferon) signaling pathway was significantly upregulated after vaccine intervention, clearly indicating that the mRNA vaccine effectively drives IFN-I pathway activation *in vivo* (Fig. 4B). Collectively, these *in vivo* experimental results provide additional evidence that the COVID-19 mRNA vaccine effectively remodels the tumor immune microenvironment and enhances antitumor immune infiltration through the activation of the IFN-I signaling pathway, thereby offering *in vivo* support for the vaccine-mediated tumor immunoregulatory mechanism.

Discussion

Based on the single-cell transcriptomic dataset GSE247905, this study systematically investigated the dynamic process by which COVID-19 mRNA vaccines remodel the TME via the IFN-I signaling axis, defined the time window of vaccine-induced IFN-I pathway activation, and characterized the kinetic frequency shifts of multiple key immune cell subsets. COVID-19 mRNA vaccines were originally clinically positioned as prophylactic antiviral agents [19]. Delivering spike protein mRNA via lipid nanoparticles, they elicit neutralizing antibodies and virus-specific T cells to prevent severe COVID-19, with their entire

research, development and application framework established around infectious disease immunity [20]. Nevertheless, recent retrospective clinical cohorts and preclinical animal studies have overturned this conventional understanding. Cumulative evidence indicates that such vaccines exert broad systemic immunomodulatory effects independent of antiviral functions, and can be repurposed to boost tumor immunotherapy efficacy [6]. Compared with tumor neoantigen mRNA vaccines, which require lengthy preparation and complicated individual matching, widely administered COVID-19 mRNA vaccines offer translational advantages including ready accessibility, fully validated safety profiles and mature administration protocols [21]. Their lipid nanoparticle delivery vehicles activate intracellular RNA sensors to trigger transient yet robust IFN-I cascade responses, and this intrinsic adjuvant effect serves as the core foundation for reshaping tumor immune landscapes [22]. Multiple retrospective clinical studies have demonstrated that tumor patients receiving COVID-19 mRNA vaccination during ICI treatment achieve markedly prolonged survival, especially patients with immune-desert "cold tumors" who exhibit improved therapeutic responses [23]. These findings have further driven researchers to dissect the molecular mechanisms underlying vaccine-mediated regulation of tumor immune cells and microenvironment remodeling.

The results revealed staged activation of the IFN-I pathway following vaccination, with peak activity at day 3 and a gradual return to baseline levels by day 14. Concurrent elevations in the proportions of anti-tumor immune cells, including $CD8^+$ effector T cells and dendritic cells, indicated that IFN-I signaling acts as a core bridge linking innate and adaptive immune responses. Further analysis of the TME demonstrated that vaccine intervention significantly elevated the total abundance of tumor-infiltrating immune cells while reducing the proportion of immunosuppressive populations such as M2-type macrophages, thereby alleviating the immunosuppressive status of the microenvironment [6]. Unlike prior studies that only described the broad immunostimulatory effects of mRNA vaccines, the present work leveraged single-cell resolution to delineate transcriptional dynamics across distinct immune cell populations within the IFN-I response window, providing high-resolution evidence to clarify the systemic and local intratumoral immunomodulatory functions of mRNA vaccines.

Subsequently, attention was turned to the potential synergistic relationship between IFN-I pathway activation and PD-L1 expression on tumor cells, aiming to elucidate from a molecular perspective the rationale underlying the enhanced antitumor efficacy of ICIs when combined with mRNA vaccination. Single-cell co-localization analysis revealed extensive co-expression regions of the IFN-I pathway core transcription factor IRF3 and the PD-L1-encoding gene CD274 in tumor cells, and CD274 expression levels were significantly positively correlated with the overall IFN-I pathway activity score, suggesting that vaccine-triggered IFN-I signaling can directly drive PD-L1 upregulation on tumor cells, a finding consistent with previous *in vitro* experimental results [24]. The mechanistic coupling between IFN-I signaling and PD-L1 expression in the TME invites deeper reflection beyond a simple correlation. mRNA vaccination, by virtue of its ability to generate a transient yet robust IFN-I pulse [25], may create a therapeutic window in which the tumor immune landscape is simultaneously “heated” by an influx of effector lymphocytes and “marked” by elevated PD-L1 on the tumor surface. This convergence of immune infiltration and target amplification suggests a rational basis for combining vaccination with

checkpoint blockade, a sequenced partnership: the vaccine prepares the battlefield, and the checkpoint inhibitor capitalizes on the newly exposed vulnerabilities.

Furthermore, the evolutionary conservation of IFN-I-driven transcriptional programs across diverse species carries important implications for the translational potential of this strategy. This conservation suggests that the fundamental logic underlying vaccine-induced immune remodeling is rooted in ancestral host-defense mechanisms rather than species-specific peculiarities, thereby reinforcing confidence that analogous dynamics are likely to operate in human patients [26]. These considerations position mRNA vaccination as more than a prophylactic tool; it emerges as a context-responsive immune modulator that exploits the host's own interferon machinery to recalibrate tumor-host interactions. This conceptual shift reframes the role of mRNA vaccination from a mere activator of immunity to a reprogrammer of the tumor ecosystem's immunological state, providing a cohesive framework for rationally designing combination immunotherapy regimens [26, 27]. The clinical implication is provocative: rather than positioning mRNA vaccines and ICIs as independent modalities, their maximal efficacy likely hinges on temporal coordination, wherein the vaccine serves as a primer that conditions the TME for subsequent checkpoint blockade.

In summary, this study has comprehensively elucidated the intrinsic molecular logic by which the COVID-19 mRNA vaccine remodels the TME and sensitizes immune checkpoint inhibitors. The IFN-I/PD-L1-related core hub genes identified herein hold potential as candidate biomarkers for predicting clinical responsiveness to immunotherapy and offer theoretical insights for optimizing combination therapeutic strategies for solid tumors. Nevertheless, it is acknowledged that all current conclusions are derived from bioinformatic analyses of public databases; the biological functions of the core hub genes and the actual antitumor efficacy of vaccine-immunotherapy combinations remain to be validated through cellular and animal experimental models. Moreover, the proposed efficacy-predictive gene signature requires prospective evaluation in large-scale, multicenter clinical cohorts to ascertain its predictive performance. In future work, *in vitro* interventions and *in vivo* tumor-bearing animal experiments centered on the identified key genes are planned to clarify their specific molecular roles in IFN-I-mediated regulation of PD-L1 expression. Concurrently, by comparing the therapeutic outcomes of different dosing intervals and administration sequences, the optimal scheduling for combining mRNA vaccines with ICIs can be explored, thereby facilitating the translational progression of this combination strategy from bench to bedside. In the long term, the development of a simple, non-invasive predictive model based on IFN-I pathway signature genes may further aid in the precise clinical identification of patients most likely to benefit from combined immunotherapy, thereby accelerating the translational application of mRNA vaccines in the field of precision cancer immunotherapy.

Declarations

Consent to participate

Not applicable

Consent for publication

Not applicable

Author contributions

Weizhen Yan: conceptualization, data curation, formal analysis, investigation, Methodology, writing – original draft; Shan Hong: conceptualization, funding acquisition, supervision, writing – review & editing.

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Figures

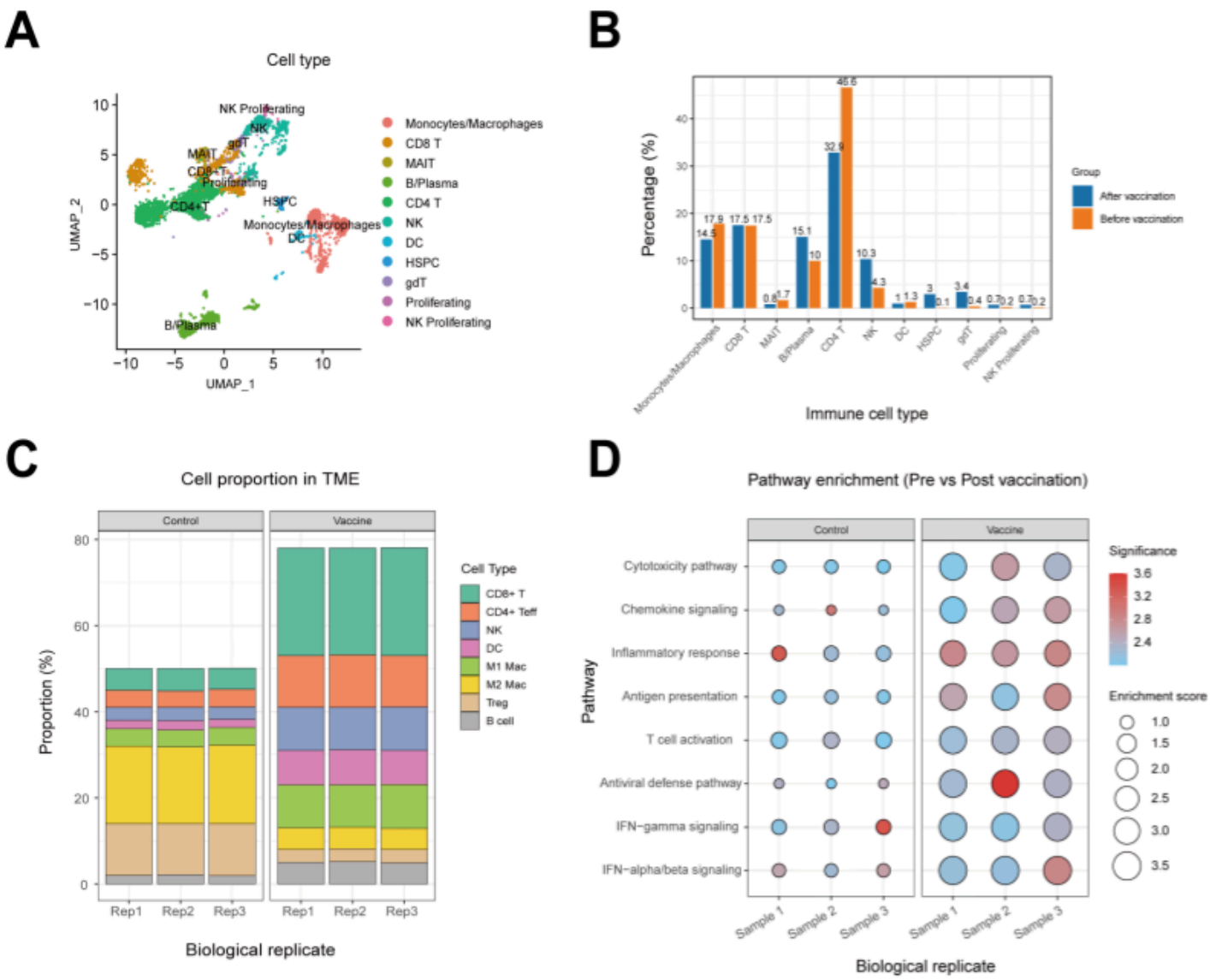


Figure 1

Remodeling of TME immune cell subsets by COVID-19 mRNA vaccine intervention (A) UMAP plot of cells colored by annotated immune subsets. (B) Percentages of immune cell subsets in peripheral blood before and after vaccination. (C) Proportions of various cell types in the TME in control and vaccine groups. (D) Enrichment levels of signaling pathways in control and vaccine groups.

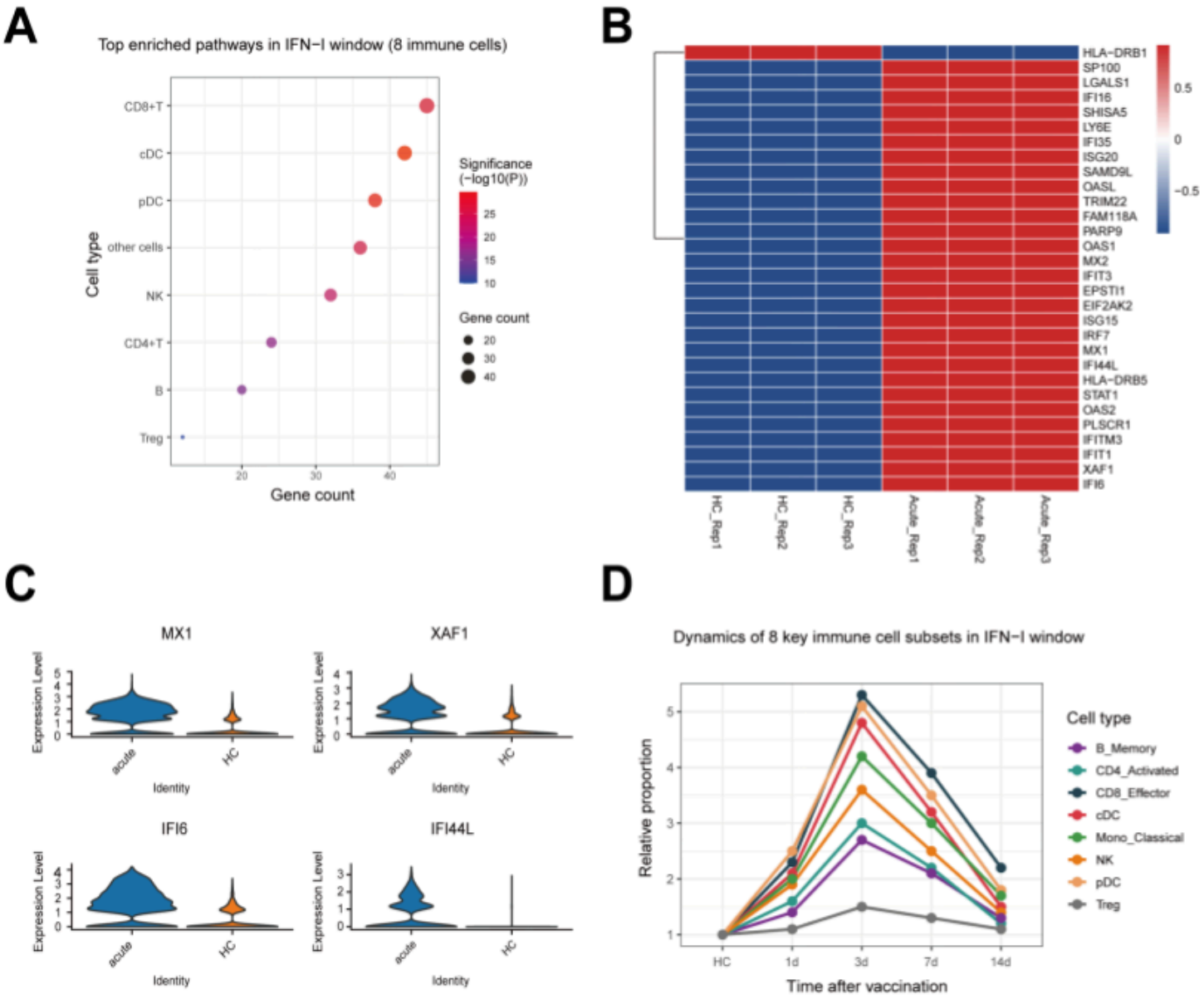


Figure 2

Immune molecular features of the vaccine-induced IFN-I response window. (A) Gene counts and significance of pathways enriched in each immune cell subset during the IFN-I window. (B) Expression levels of 30 vaccine-upregulated genes in healthy controls and the vaccine-acute phase. (C) Expression levels of MX1, XAF1, IFI6, and IFI44L in steady-state, healthy controls, and vaccine-acute phase. (D) Relative proportions of eight key immune cell subsets at various time points after vaccination.

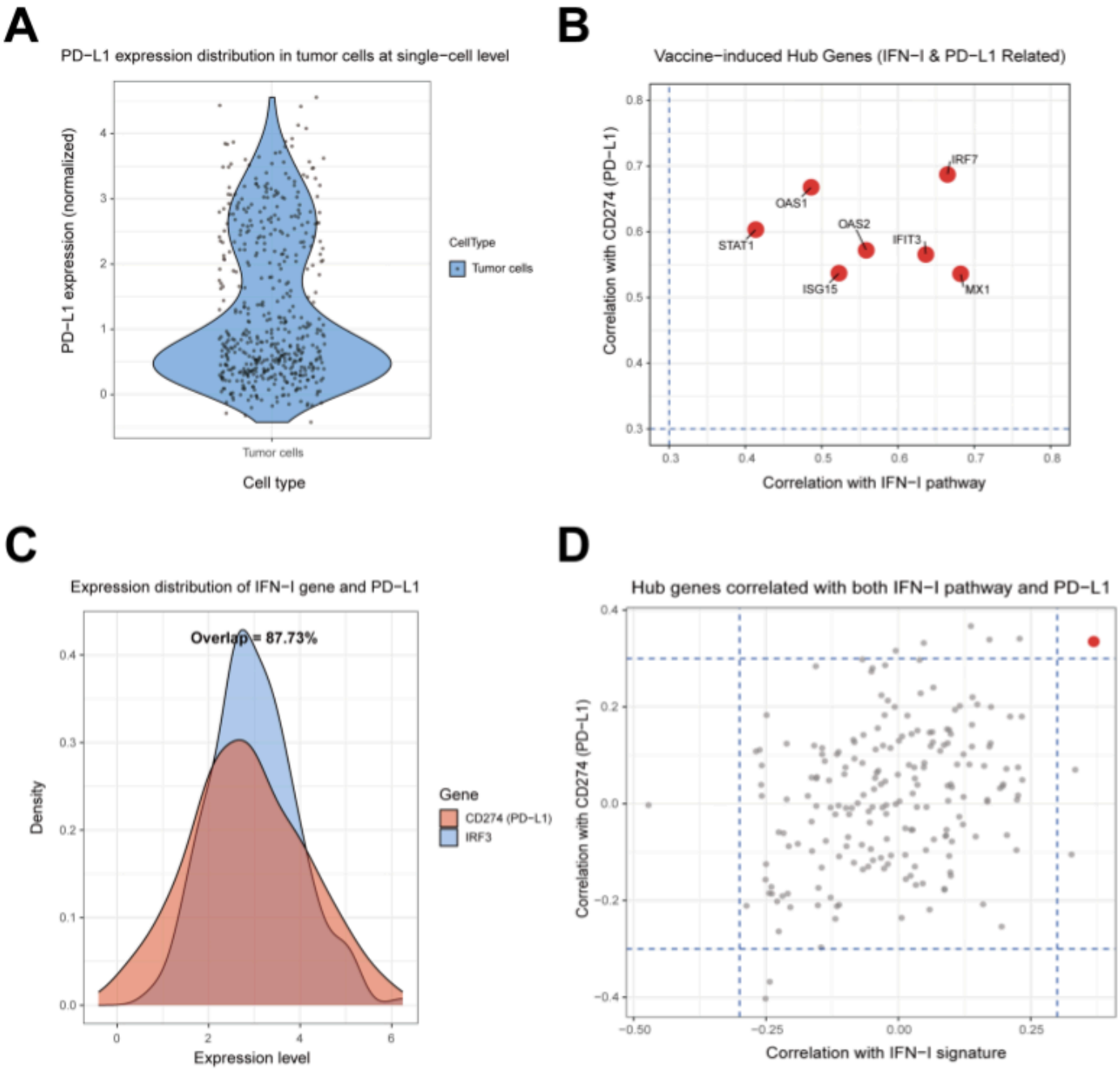


Figure 3

Single-cell validation of the association between the IFN-I pathway and PD-L1 expression. (A) Single-cell expression distribution of PD-L1 in tumor cells. (B) Correlations of vaccine-induced IFN-I/PD-L1-related core hub genes with the IFN-I pathway and CD274. (C) Overlapping expression distribution of the IFN-I pathway key gene IRF3 and the PD-L1-encoding gene CD274 in tumor cells. (D) Correlations of CD274 expression levels with the overall IFN-I pathway signature score and core hub genes (Pearson correlation coefficient < 0.05, p > 0.05).

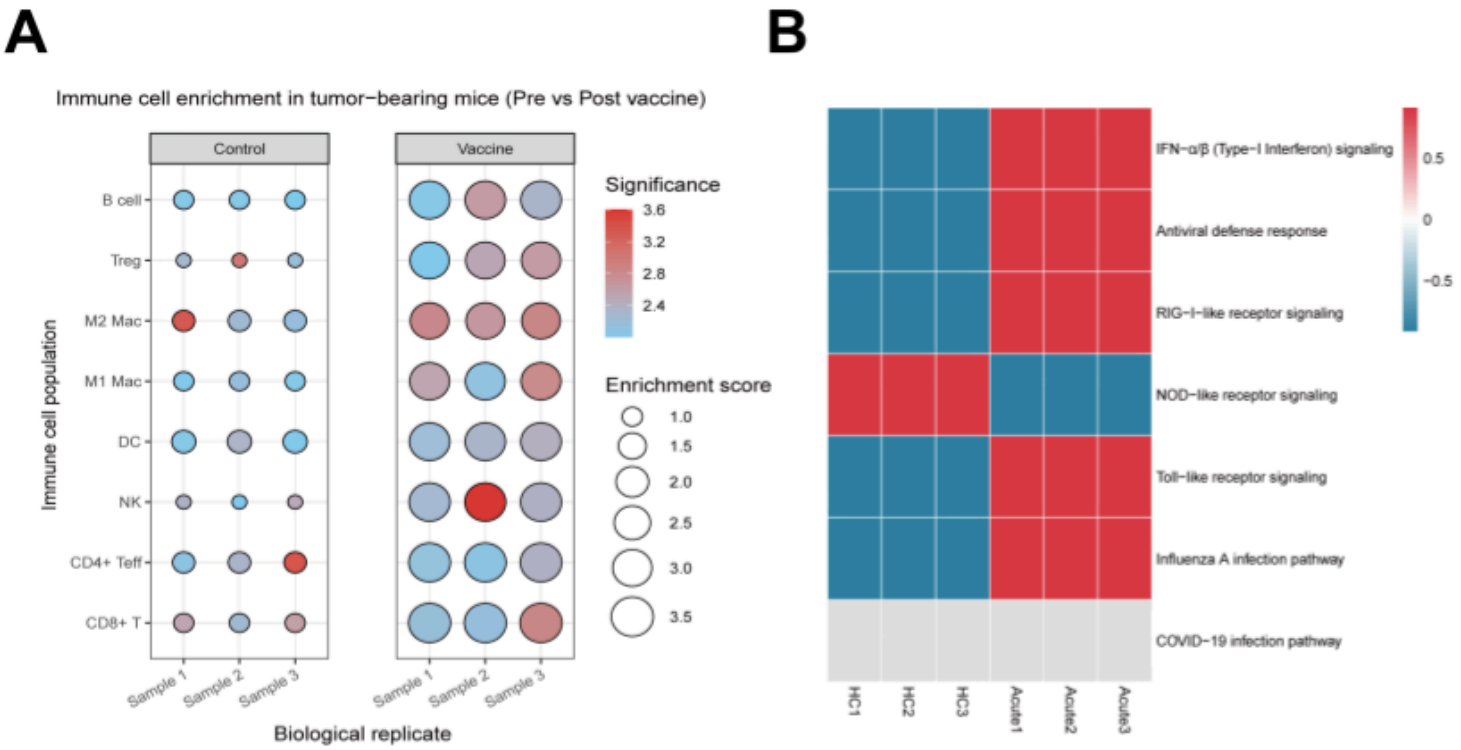


Figure 4

Vaccine-induced immune enrichment and IFN-I pathway activation features in the tumor-bearing mouse model. (A) Enrichment levels of immune cell subsets in the TME of tumor-bearing mice in the control and vaccine groups. (B) Enrichment scores of immune-related signaling pathways in the TME of tumor-bearing mice in the control and vaccine groups.