



Article

Expression Profile of Metabotropic Glutamate Receptors in Lung Adenocarcinoma: GRM5 and Validation of Its Targeting Drug Cinchonine

Yajing Xue ^{1,2,3,†}, Wei Liu ^{1,2,3,†}, Yongfu Wang ^{1,2,3}, Pengzhuo Tao ^{1,2,3}, Yizhen Yuan ^{1,2,3}, Changmin Liu ^{1,2,3}, Shilin Chen ^{1,2,*} and Chi Song ^{1,2,3,*} 

¹ School of Pharmacy, Chengdu University of Traditional Chinese Medicine, Chengdu 611137, China; xueyajing1432@163.com (Y.X.); liuweili232021@126.com (W.L.); wyf@stu.cdutcm.edu.cn (Y.W.); pengzhuotao998@163.com (P.T.); yuanyizhen2025@163.com (Y.Y.); liuchangmin0908@163.com (C.L.)

² Institute of Herbgonomics, Chengdu University of Traditional Chinese Medicine, Chengdu 611137, China

³ Hubei Shizhen Laboratory, Wuhan 430061, China

* Correspondence: slchen@cdutcm.edu.cn (S.C.); songchi@cdutcm.edu.cn (C.S.)

† These authors contributed equally to this work.

Abstract

The incidence and mortality rates of lung adenocarcinoma (LUAD) continue to rise, highlighting an urgent need for novel therapeutic targets. In this study, bioinformatics analysis revealed that members of the metabotropic glutamate receptor (mGluR) family are significantly correlated with the expression profile, prognosis, genetic mutations, and tumor immune microenvironment of LUAD, with GRM5 being the most significantly associated member. Overexpression of GRM5 has been shown to inhibit LUAD proliferation and induce apoptosis, while cinchonine (CN) treatment further enhances these effects, suggesting that CN may act as a GRM5 agonist to synergistically exert antitumor activity. Transcriptome sequencing further identified four key downstream targets and their associated signaling pathways. In summary, this study confirms that GRM5 can serve as a potential prognostic biomarker and therapeutic target for LUAD, while the small-molecule compound CN shows promise as an antitumor candidate drug targeting GRM5.

Keywords: lung adenocarcinoma; mGluR family; GRM5; cinchonine

1. Introduction

Lung cancer, a malignant tumor with persistently high global incidence and mortality rates, has become a serious public health issue that threatens human health. From a pathological classification perspective, lung cancer can be divided into two major categories: small-cell lung cancer (SCLC) and non-small-cell lung cancer (NSCLC). NSCLC accounts for more than 80% of cases and is further subdivided into subtypes such as adenocarcinoma, squamous cell carcinoma, and large cell carcinoma [1,2]. Notably, lung adenocarcinoma (LUAD) represents the most prevalent histological subtype of NSCLC, characterized by a high incidence rate, marked molecular heterogeneity, and a propensity for early metastasis [3]. Clinically, LUAD is associated with dismal overall survival (OS) outcomes because the majority of patients are not diagnosed until the disease progresses to an advanced stage, which severely hinders effective therapeutic interventions and translates into chronically low five-year survival rates. Although recent advancements in surgical techniques, optimized chemotherapy regimens, and the emergence of targeted and immunotherapies have led to new breakthroughs



Academic Editor: Barbara Ruaro

Received: 20 December 2025

Revised: 3 February 2026

Accepted: 7 February 2026

Published: 13 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.

in the treatment of LUAD [4,5], the incidence and mortality rates of this disease continue to rise [6]. Therefore, in-depth exploration of the molecular mechanisms underlying the development and progression of LUAD, as well as the identification of novel and effective therapeutic targets, has become a key research priority in the field of oncology.

The family of metabotropic glutamate receptor (mGluR) belongs to the class C G protein-coupled receptors (GPCRs). They are widely distributed in the central nervous system and serve as key excitatory neurotransmitter receptors that regulate neural signal transmission. On the basis of differences in signal transduction pathways and pharmacological properties, the mGluR family can be divided into three major groups: Group I includes GRM1 and GRM5, Group II comprises GRM2 and GRM3, and Group III consists of GRM4, GRM6, GRM7, and GRM8 [7]. Studies have confirmed that members of the mGluR family are closely associated with the occurrence, development, metastasis, survival, and therapeutic resistance of human cancers. Their roles have been verified in various malignant tumors, including breast cancer [8,9], colorectal cancer [10,11], glioma [12,13], renal cell carcinoma [14], melanoma [15], oral cancer [16], osteosarcoma [17], pancreatic cancer [18], prostate cancer [19], and T-cell carcinoma [20]. However, the function and clinical significance of this family in lung cancer remain unclear, and systematic exploration is urgently needed.

Cinchonine ($C_{19}H_{22}N_2O$, CN), a monoterpenoid indole alkaloid derived from cinchona bark, was historically used in clinical malaria treatment alongside quinine, quinidine, and cinchonidine [21]. With the advancement of anticancer drug research, the antitumor activity of CN and its mechanism of action have gradually been elucidated. Currently, anti-tumor research on CN has been extensively conducted across multiple cancer models, including pancreatic cancer [22], breast cancer [23], lung cancer [24], and liver cancer [25]. Preliminary results indicate that CN exhibits broad-spectrum anti-tumor activity with relatively low toxicity and side effects. Systematically elucidating its molecular mechanisms of antitumor activity will provide crucial theoretical support for advancing the application of CN in precision treatment for malignant tumors.

Against this backdrop, identifying key regulatory targets in the progression of LUAD and developing specific targeted compounds holds significant importance for advancing precision medicine in clinical settings. This study integrates network pharmacology analysis, cellular functional assays, and transcriptomic sequencing to systematically investigate the biological functions of members of the mGluR family in LUAD. It specifically validates the feasibility of GRM5 as a potential therapeutic target and clarifies the application potential of CN targeting GRM5.

2. Results

2.1. Expression Profiles and Biological Functions of the mGluR Family in LUAD

To systematically characterize the expression patterns and potential biological functions of the mGluR family in LUAD, we first performed differential gene expression analysis on LUAD tissue samples and adjacent normal lung tissue samples. Tissue-based assays revealed that, compared with normal lung tissues, GRM2, GRM4, and GRM5 were significantly upregulated in LUAD lesions ($p < 0.05$), whereas GRM3 and GRM6 were markedly downregulated ($p < 0.05$) (Figure 1A). Further functional enrichment analysis using the CancerSEA database demonstrated that in LUAD, the expression of GRM1–GRM7 was strongly negatively correlated with key biological processes associated with tumor malignant phenotypes, including cell invasion, migration, and DNA repair. By contrast, GRM8 expression showed no obvious correlation with these processes (Figure 1B). These findings not only confirm the heterogeneous expression of mGluR family mRNAs in LUAD but also

suggest that the dysregulated expression of these genes may serve as a critical molecular event driving LUAD tumorigenesis and progression.

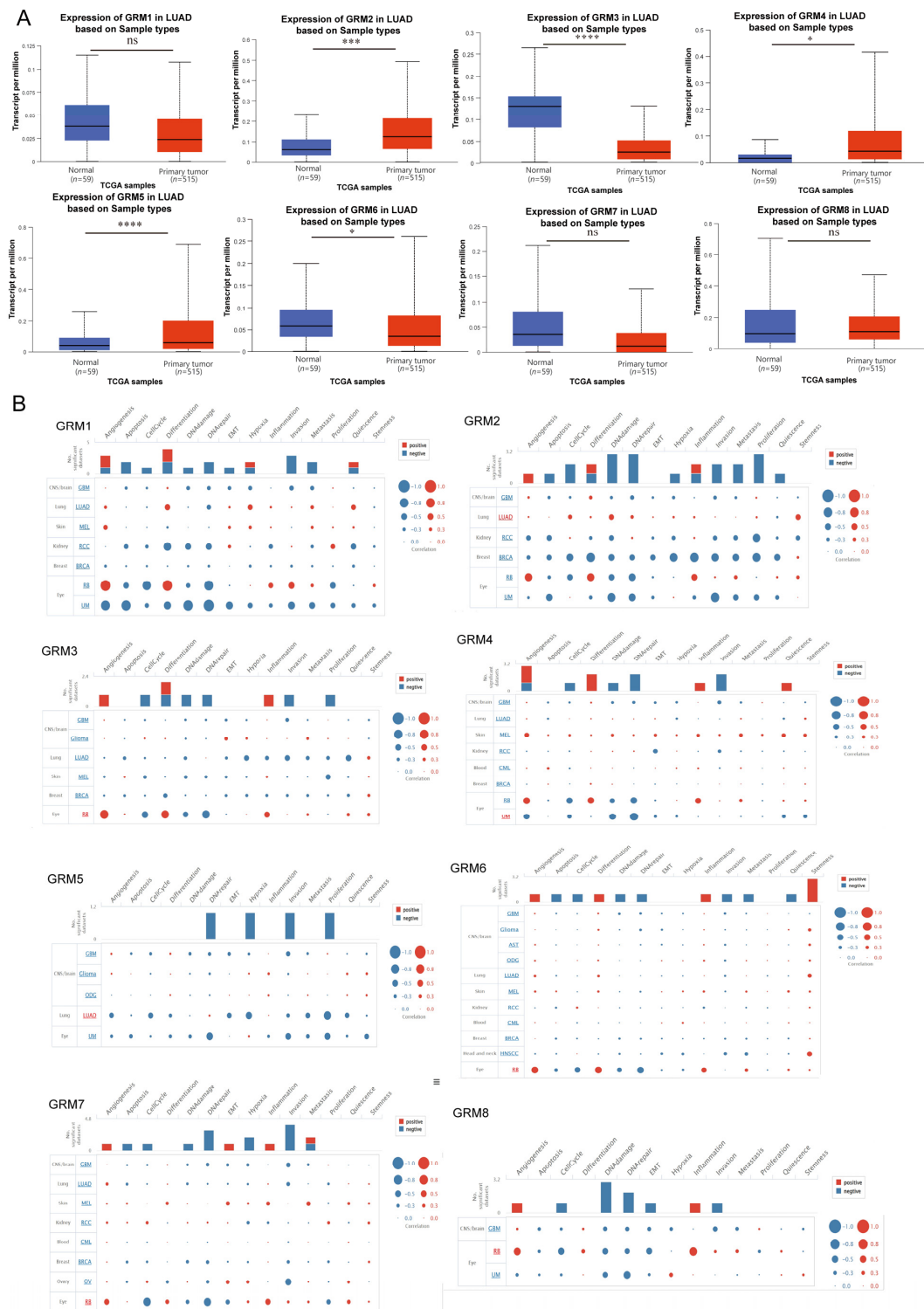


Figure 1. The mGluR family expression and function in LUAD. (A) The expression of the mGluR family in LUAD tissues and normal tissues is derived from UALCAN (ns $p \geq 0.05$, * $p < 0.05$, *** $p < 0.001$, **** $p < 0.0001$). (B) Functional enrichment analysis of the mGluR family via CancerSEA.

2.2. Associations Between Clinical Features and the mGluR Family

To clarify the relationships between the mGluR family and the clinical characteristics of LUAD patients, we evaluated the associations between differentially expressed mGluR

family and the tumor pathological stages of LUAD patients via the UALCAN database and visualized the results (Figure 2A). The results revealed that in various pathological stages (I–IV) of LUAD, the expression levels of GRM2, GRM4, and GRM5 were higher than those in normal lung tissues, while GRM3, GRM6, and GRM7 consistently remained lower than in normal tissues, suggesting their potential roles in promoting or suppressing cancer during the occurrence and development of LUAD. Furthermore, we analyzed survival data from 1161 LUAD patients via the Kaplan–Meier Plotter database (Figure 2B) and found that the OS of patients in the GRM2 overexpression group was significantly longer than that of patients in the low-expression group ($p < 0.05$), whereas the OS of patients in the overexpression groups of other members was markedly shorter than that of patients in their respective low-expression groups ($p < 0.05$). In summary, the expression levels of mGluR family members are correlated with the pathological stage of LUAD and significantly influence patient prognosis.

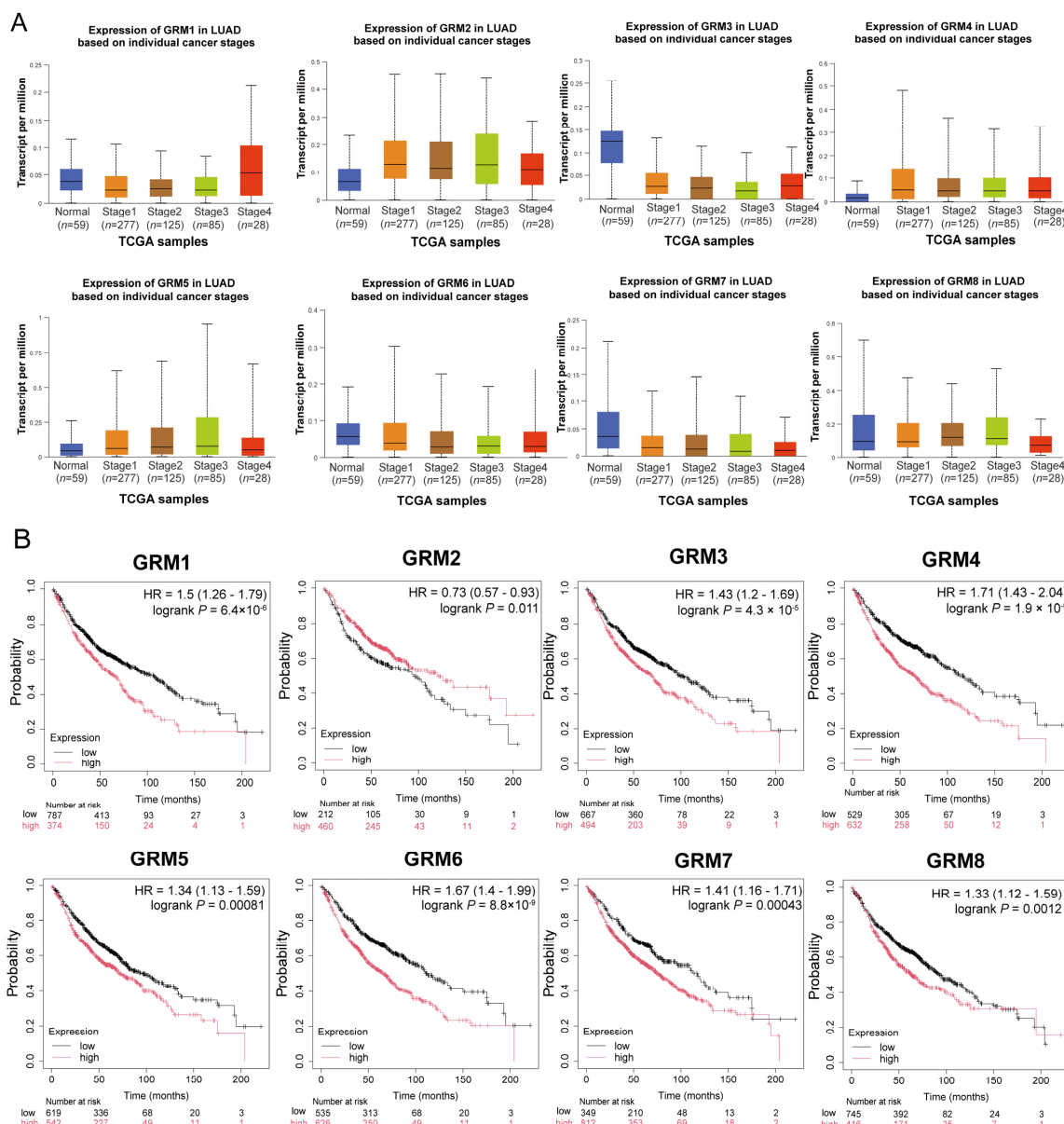


Figure 2. Associations between clinical characteristics and the mGluR family. **(A)** Association between the mGluR family and tumor stage in LUAD. **(B)** The Kaplan–Meier curve demonstrates the impact of high expression (red) and low expression (black) of mGluR family genes on OS in patients with LUAD.

2.3. Panoramic Analysis of Molecular Variations in the mGluR Family in LUAD

In the development and progression of LUAD, genetic molecular variation is a core mechanism driving uncontrolled tumor cell proliferation, invasion, metastasis, and drug resistance [26]. By integrating genomic data from databases with visualization techniques, our analysis of the overall mutation profile revealed that mGluR family gene variations were detected in 258 out of 503 LUAD samples, indicating a high proportion of LUAD patients with at least one genetic variation in this family (Figure 3A). Further analysis focusing on gene-specific variation frequency revealed significant differences in the incidence of somatic mutations among mGluR family members, with GRM5 being the member with the highest variation frequency in LUAD. To elucidate the functional consequences of GRM5 alteration, we examined the correlation between mutation class and transcript abundance, revealing that missense variants significantly increase GRM5 expression (Figure 3B). Overall, these findings suggest that GRM5 is a key molecule that regulates the development and progression of LUAD.

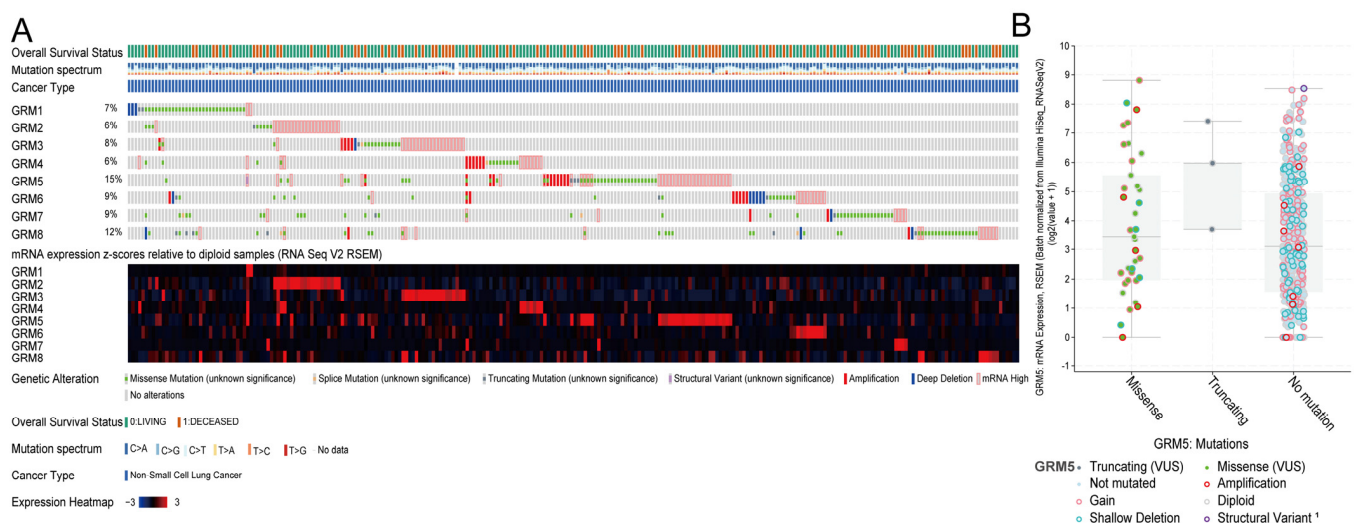


Figure 3. Genetic alterations of the mGluR family genes based on TCGA-LUAD data. **(A)** A bar plot and heatmap showing the mutation profiles of 8 mGluR family genes, where the bar plot presents the mutation frequency of each gene and the heatmap displays the mutation patterns across different samples. **(B)** A scatter plot showing the mRNA expression levels of the GRM5 gene in different mutation types. ¹ Structural variants are shown instead of copy number alterations when a sample has both.

2.4. Correlation Analysis of mGluR Family Expression with Immune Infiltration

Tumor formation is closely associated with the immune system, and tumor-infiltrating immune cells have a significant effect on the therapeutic efficacy and prognosis of lung cancer patients [27]. To explore the relationship between the mGluR family and immune infiltration in LUAD, we conducted a study based on the TIMER database (Figure 4). The results indicate that the mGluR family participates in regulating the LUAD immune microenvironment, with GRM5 being the most broadly associated member of the family across multiple immune cell lineages.

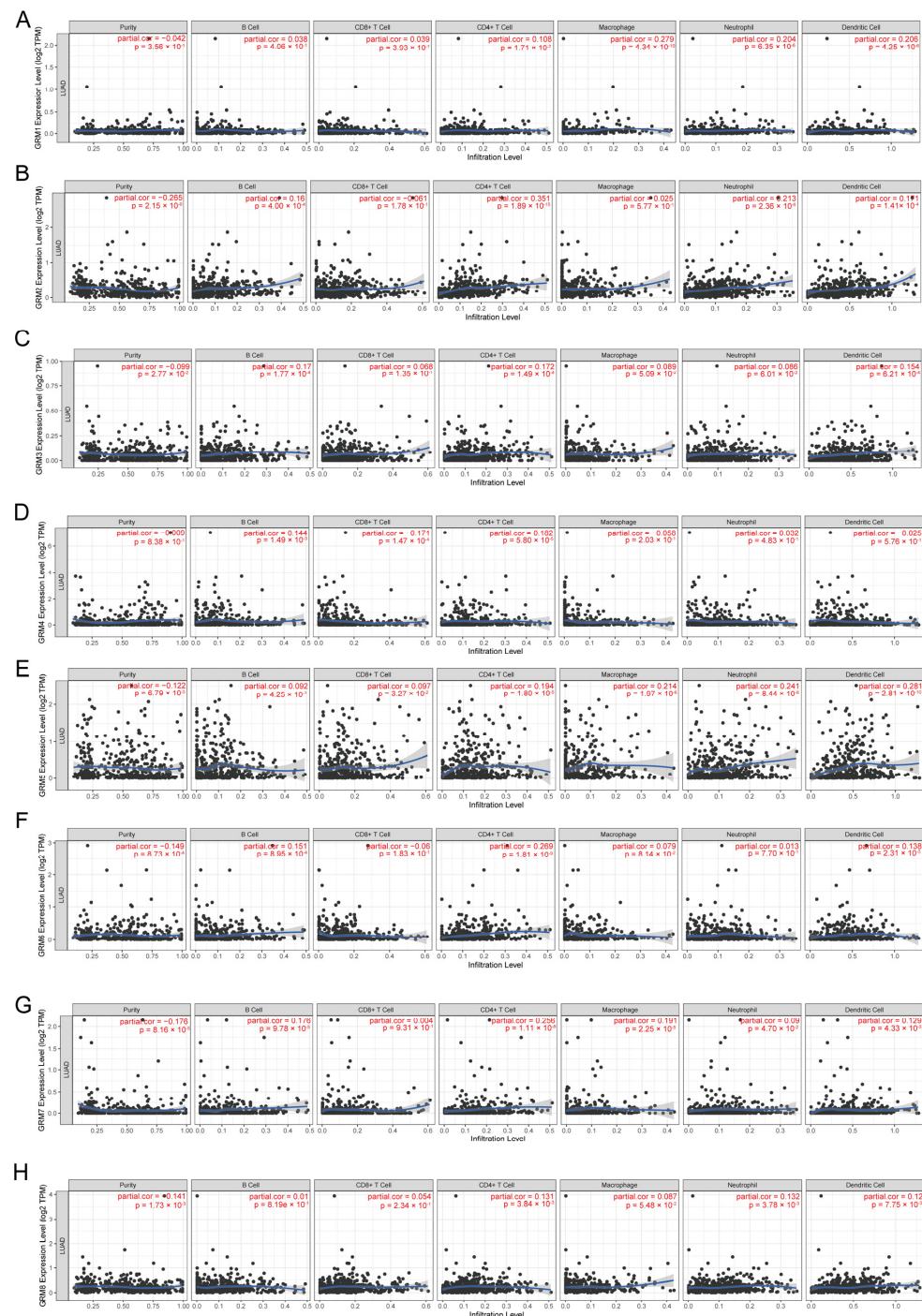


Figure 4. Correlations of mGluR family expression with immune infiltration levels in LUAD. (A) GRM1 expression was positively correlated with the infiltration levels of CD4⁺ T cells, neutrophils and dendritic cells. (B) GRM2 expression was positively correlated with the infiltration levels of B cells, CD4⁺ T cells, neutrophils, and dendritic cells. (C) GRM3 expression was positively correlated with the infiltration levels of B cells, CD4⁺ T cells, and dendritic cells. (D) GRM4 expression was positively correlated with the infiltration levels of B cells and CD4⁺ T cells but negatively correlated with the infiltration levels of CD8⁺ T cells. (E) GRM5 expression was positively correlated with the infiltration levels of B cells, CD8⁺ T cells, CD4⁺ T cells, macrophages, neutrophils, and dendritic cells. (F) GRM6 expression was positively correlated with the infiltration levels of B cells, CD4⁺ T cells, and dendritic cells. (G) GRM7 expression was positively correlated with the infiltration levels of B cells, CD4⁺ T cells, macrophages, neutrophils, and dendritic cells. (H) GRM8 expression was positively correlated with the infiltration levels of CD4⁺ T cells and dendritic cells. Each data point denotes an individual sample; the blue smoothed curve stands for the trend line fitted via the local regression approach, and the gray shaded area represents the 95% confidence interval.

2.5. CN Inhibits the Proliferation and Migration of A549 Cells by Targeting GRM5

Previous bioinformatics analysis revealed a significant correlation between GRM5 and the clinicopathological features, as well as the prognosis of LUAD patients. Combined with our team's previous finding that the small-molecule compound CN binds to the GRM5 protein with high affinity, CN is hypothesized to target GRM5. To investigate the biological function of GRM5 in LUAD and its potential as a drug target, this study employed the A549 cell line as a model. To elucidate GRM5 function, we established a stable GRM5-overexpressing cell line using lentiviral infection technology and validated its overexpression efficiency via WB analysis (Figure 5A). CN was prepared with absolute ethanol as the solvent. After treating A549 cells with different CN concentrations for 48 h, cell viability was determined via CCK-8 assay, and the data demonstrated a concentration-dependent inhibitory effect of CN on A549 cells (Figure 5B). Further analysis showed that cell viability was closer to 50% at 300 μ M (a relatively low concentration), so 300 μ M was selected as the optimal concentration for subsequent functional experiments.

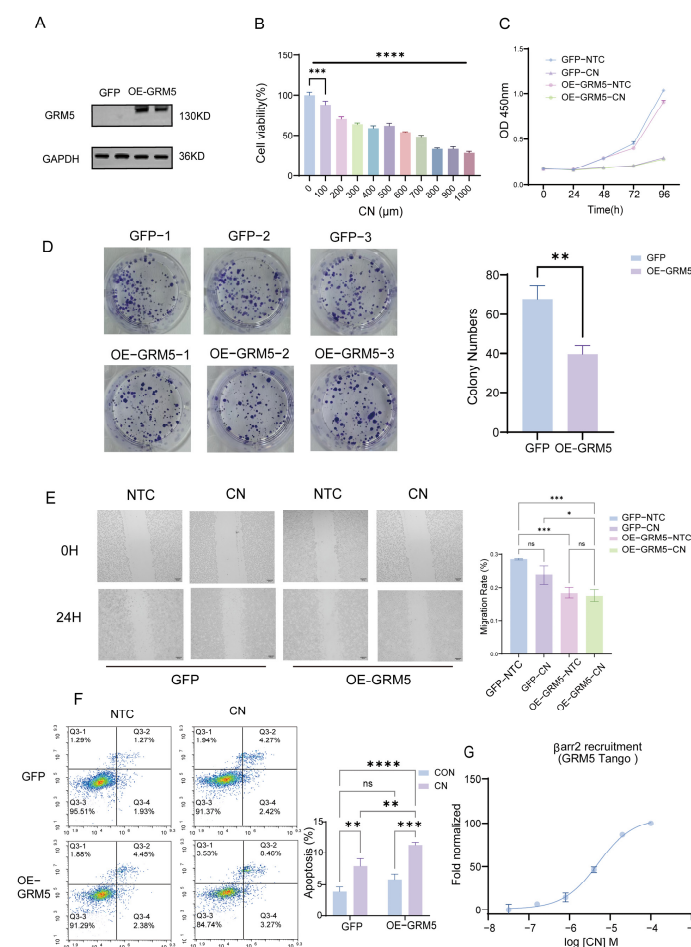


Figure 5. CN inhibits the proliferation and migration of A549 cells by targeting GRM5. (A) Results of the detection of GRM5 protein overexpression level. (B) After treating A549 cells with different concentrations of CN for 48 h, cell viability was determined via the CCK-8 assay. (C) After the cells in the GFP-NTC group and OE-GRM5 group were treated with 300 μ M CN for 24 h, 48 h, 72 h, and 96 h, respectively, cell viability was determined via the CCK-8 assay. (D) After culturing cells in the GFP-NTC group and OE-GRM5 group for 14 days, the effect of GRM5 overexpression on the proliferative capacity of A549 cells was evaluated via the colony formation assay. (E) The effects of CN and GRM5 on the migratory capacity of A549 cells were assessed via the wound healing assay. (F) The regulatory effects of CN and GRM5 on the apoptosis of A549 cells were analyzed by flow cytometry. (G) The mediating effect of CN on GRM5 was detected via the Tango assay (ns $p \geq 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$).

To clarify whether CN exerts its effects by mediating GRM5, we detected the signal activation of the GPCR to which GRM5 belongs via the Tango assay and found that CN activated the functional activity of GRM5 in a concentration-dependent manner (Figure 5G). The GFP-NTC and OE-GRM5 groups were treated with CN and cell viability was measured at different time points. The results showed that the inhibitory effect of CN on cells gradually increased and stabilized as treatment time increased, whereas GRM5 overexpression itself inhibited cell proliferation, and CN exhibited a synergistic effect with OE-GRM5 to enhance this inhibition (Figure 5C). Colony formation assay also indicated that GRM5 overexpression could suppress the proliferation of LUAD cells (Figure 5F). Apoptosis and wound healing assays demonstrated that GRM5 overexpression significantly induced apoptosis and inhibited cell migration after CN treatment (Figure 5D,E). In conclusion, GRM5 overexpression and CN each exert anti-tumor effects in LUAD, with GRM5 being the target of action of CN.

2.6. Transcriptional Characteristics of LUAD Cells After GRM5 Overexpression and CN Treatment

On the basis of previous pharmacodynamic evidence, to elucidate the molecular mechanism by which CN target GRM5, we conducted transcriptome sequencing on drug-treated groups and A549 cells with gene overexpression. By analyzing the gene expression profiles in the raw dataset, we revealed the distribution characteristics of the samples through principal component analysis (PCA). The results showed that the four groups of samples were clearly separated, indicating significant differences in gene expression patterns among the four groups (Figure 6A). Differential gene analysis demonstrated that both GRM5 overexpression and CN treatment significantly reshaped the transcriptome of LUAD cells (Figure 6B–F). Using the GFP-NTC group as a control, 1140 differentially expressed genes (DEGs) (331 upregulated and 809 downregulated) were identified in the OE-GRM5-NTC group, suggesting that GRM5 overexpression alone could significantly alter gene expression in LUAD cells (Figure 6B). Compared with the GFP-CN group, the OE-GRM5-CN group had 1458 DEGs (769 upregulated and 689 downregulated), indicating that in the context of CN treatment, GRM5 overexpression had a stronger regulatory effect on gene expression (Figure 6C). Compared with the GFP-NTC group, the GFP-CN group included 1840 DEGs (510 upregulated and 1294 downregulated) (Figure 6D); compared with the OE-GRM5-NTC group, the OE-GRM5-CN group included 1187 DEGs (582 upregulated and 607 downregulated) (Figure 6E). These findings indicated that CN treatment alone could significantly change the gene expression profile of LUAD cells and that, on the basis of GRM5 overexpression, CN treatment could further regulate gene expression. Furthermore, compared with the GFP-NTC group, the OE-GRM5-CN group presented 1458 DEGs (769 upregulated and 689 downregulated), suggesting that when GRM5 overexpression and CN were combined, they had a more significant remodeling effect on the gene expression profile of LUAD cells, and there was a synergistic effect between them in regulating cell transcription levels (Figure 6F).

Through Venn diagram analysis, we identified 17 commonly found differentially expressed genes, termed core genes (Figure 6G). We then carried out a heatmap analysis to examine the expression patterns of these 17 genes across different tissues (Figure 6H). The genes *IL21R*, *INSIG1*, *ANKRD1*, and *FOLR1* presented the highest expression in the OE-GRM5-CN group and the lowest in the GFP-NTC group. The expression of *A2M* and *ETV1* was greatest in the OE-GRM5-NTC group. The remaining 11 genes presented the highest expression in the GFP-NTC group and the lowest in the OE-GRM5-CN group. Overall, transcriptome analysis has shown that GRM5 overexpression and CN treatment can reshape the transcriptome of LUAD cells both separately and in a cooperative way, and 17 core genes that were potential key targets for their actions were selected.

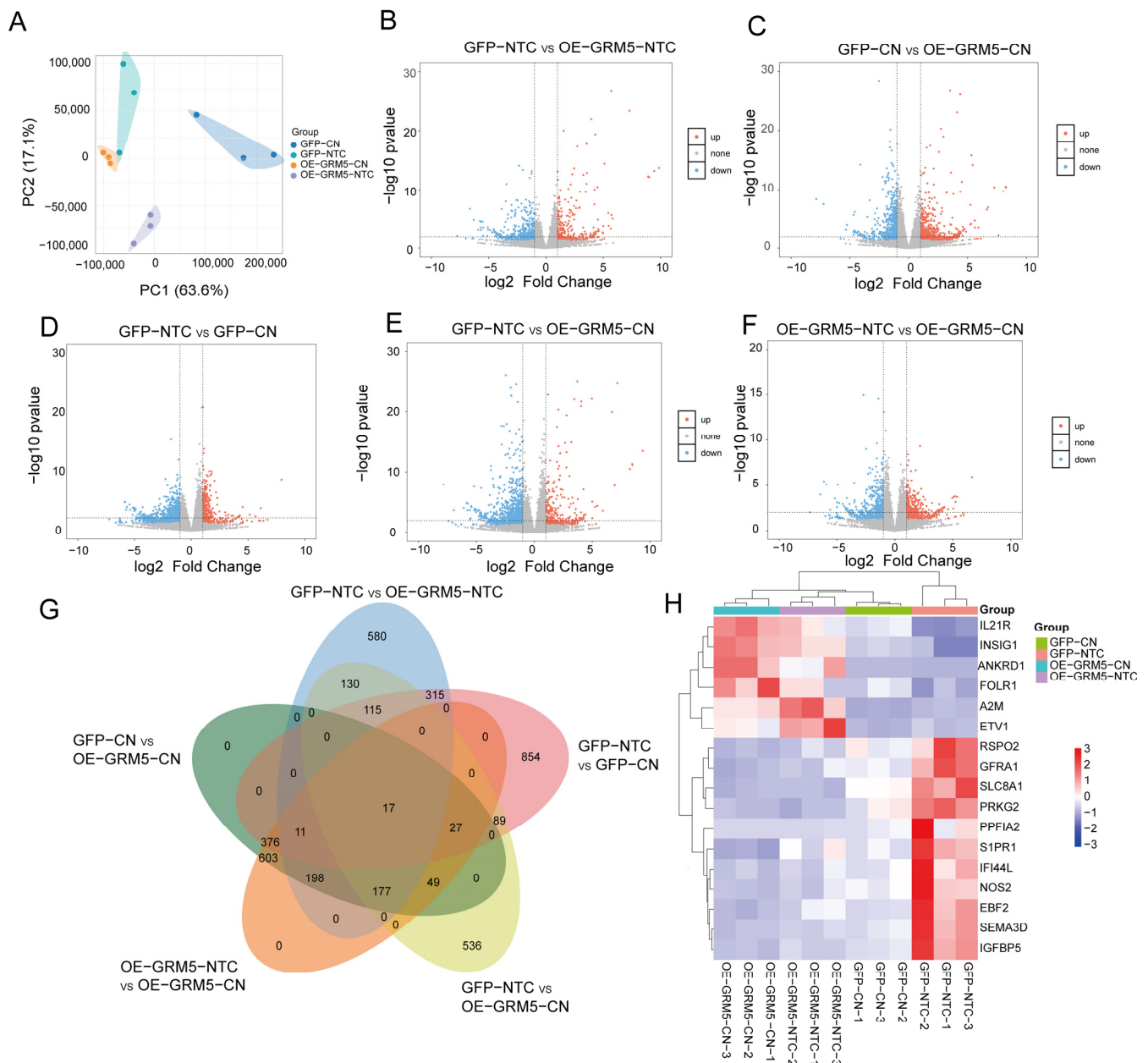


Figure 6. Transcriptomic alterations in LUAD cells following OE-GRM5 and CN treatment. (A) PCA plot of the four experimental groups ($n = 3$). (B) Volcano plot of DEGs between GFP-NTC and OE-GRM5-NTC. (C) Volcano plot of DEGs between GFP-CN and OE-GRM5-CN. (D) Volcano plot of DEGs between GFP-NTC and GFP-CN. (E) Volcano plot of DEGs between GFP-NTC and OE-GRM5-CN. (F) Volcano plot of DEGs between OE-GRM5-NTC and OE-GRM5-CN. (G) Venn diagram of DEGs from the five pairwise comparisons (B–F). (H) Expression heatmap of the 17 core DEGs shared across all five comparisons.

2.7. Transcriptome-Based Validation and Public Database Mining Identify Potential Clinical Targets in LUAD

To pinpoint key targets among the 17 core genes, screening was conducted via GEPIA2 and Kaplan–Meier Plotter. These findings revealed that *A2M*, *GFRA1*, *RSPO2*, and *S1PR1* were differentially expressed between LUAD and normal tissues, with their expression levels significantly correlated with patient overall survival (Figure 7A,B). Furthermore, RT-qPCR experiments confirmed the expression trends observed in the transcriptome analysis (Figure 7C). Overall, through database screening and experimental validation,

we preliminarily identified *A2M*, *GFRA1*, *RSPO2*, and *S1PR1* as critical candidate targets associated with the CN-targeted GRM5 regulatory axis in LUAD.

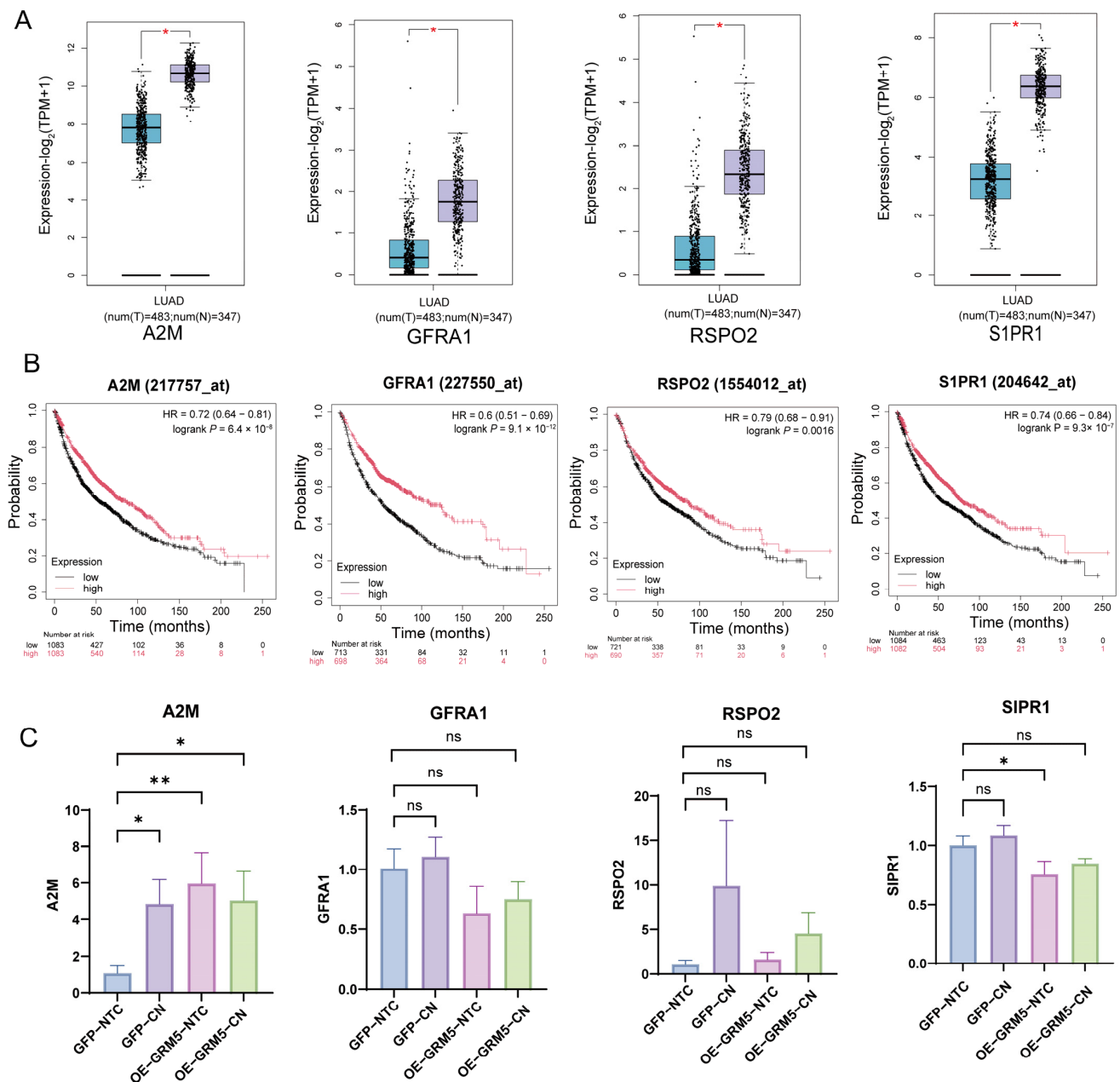


Figure 7. Transcriptome-based validation and public database mining identify potential clinical targets in LUAD. (A) GEPIA2 identification of four core differentially expressed genes (* $p < 0.05$). (B) Kaplan–Meier plotter survival analysis validating the four core genes. (C) RT–qPCR verification of the changes in the expression of the four core genes (ns $p \geq 0.05$, * $p < 0.05$, ** $p < 0.01$).

2.8. Functional Dissection of the GRM5–CN Axis via GO and KEGG Analyses

To gain an in-depth understanding of the molecular mechanisms by which CN targets GRM5, we conducted functional annotations on the DEGs from five pairs of comparative samples. Our aim was to precisely identify the dominant biological processes and key signaling pathways involved. GO enrichment analysis revealed that the DEGs in both the GFP-NTC vs. OE-GRM5 groups and the GFP-CN vs. OE-GRM5-CN groups were significantly enriched in items related to cell morphogenesis and extracellular matrix. This indicates that GRM5 overexpression can regulate cellular structure and its interaction with the microenvironment (Figure 8A,B). The DEGs in the GFP-NTC vs. GFP-CN groups were

mainly enriched in transporter complexes and respiratory chain complexes (Figure 8C). The DEGs in the OE-GRM5-NTC vs. OE-GRM5-CN groups were enriched in items related to the regulation of MAPK cascades and behavior (Figure 8E). Meanwhile, the DEGs in the GFP-NTC vs. OE-GRM5-CN groups were concentrated in cell morphogenesis, tube morphogenesis, and the regulation of MAPK cascades (Figure 8D). Overall, GO functional annotations have shown that GRM5 overexpression and CN treatment can exert their effects by regulating biological processes such as cell morphology, microenvironment interactions, and MAPK cascades.

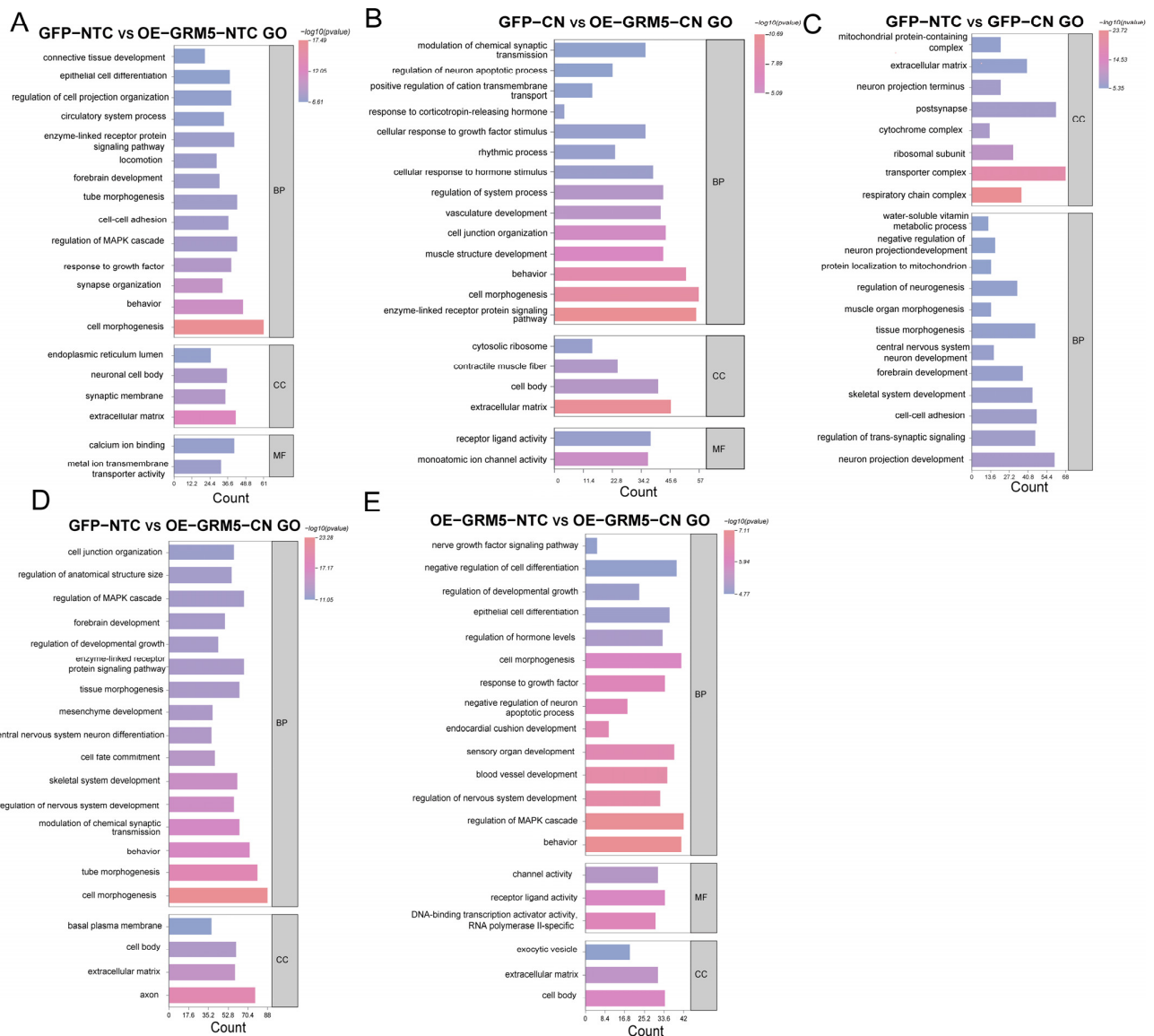


Figure 8. GO enrichment of DEGs across the five comparison groups. (A) GO enrichment plot for GFP-NTC vs. OE-GRM5-NTC. (B) GO enrichment plot for GFP-CN vs. OE-GRM5-CN. (C) GO enrichment plot for GFP-NTC vs. GFP-CN. (D) GO enrichment plot for GFP-NTC vs. OE-GRM5-CN. (E) GO enrichment plot for OE-GRM5-NTC vs. OE-GRM5-CN.

The results of the KEGG enrichment analysis revealed that the DEGs in the GFP-NTC vs. OE-GRM5 groups were enriched mainly concentrated in the cGMP-PKG signaling pathway (Figure 9A). The DEGs in the GFP-CN vs. OE-GRM5-CN groups were significantly enriched in the neuroactive ligand–receptor interactions (Figure 9B). The DEGs in the GFP-NTC vs. GFP-CN groups were enriched primarily in the Huntington’s disease pathway (Figure 9C). The DEGs in the OE-GRM5-NTC vs. OE-GRM5-CN groups were

enriched mainly in the MAPK signaling pathway (Figure 9E). Meanwhile, the DEGs in the GFP-NTC vs. OE-GRM5-CN groups were enriched mainly in the neuroactive ligand–receptor interaction and calcium signaling pathways (Figure 9D). A comprehensive analysis indicated that KEGG enrichment analysis demonstrated that GRM5 overexpression and CN treatment can exert their functions by regulating pathways such as the cGMP-PKG pathway, the MAPK pathway, the calcium signaling pathway, and neuroactive ligand–receptor interactions (Figure 9A–E). Combining the results of the GO functional annotations and KEGG enrichment analysis, the MAPK pathway appears to be of particular importance in the molecular mechanism by which CN targets GRM5.

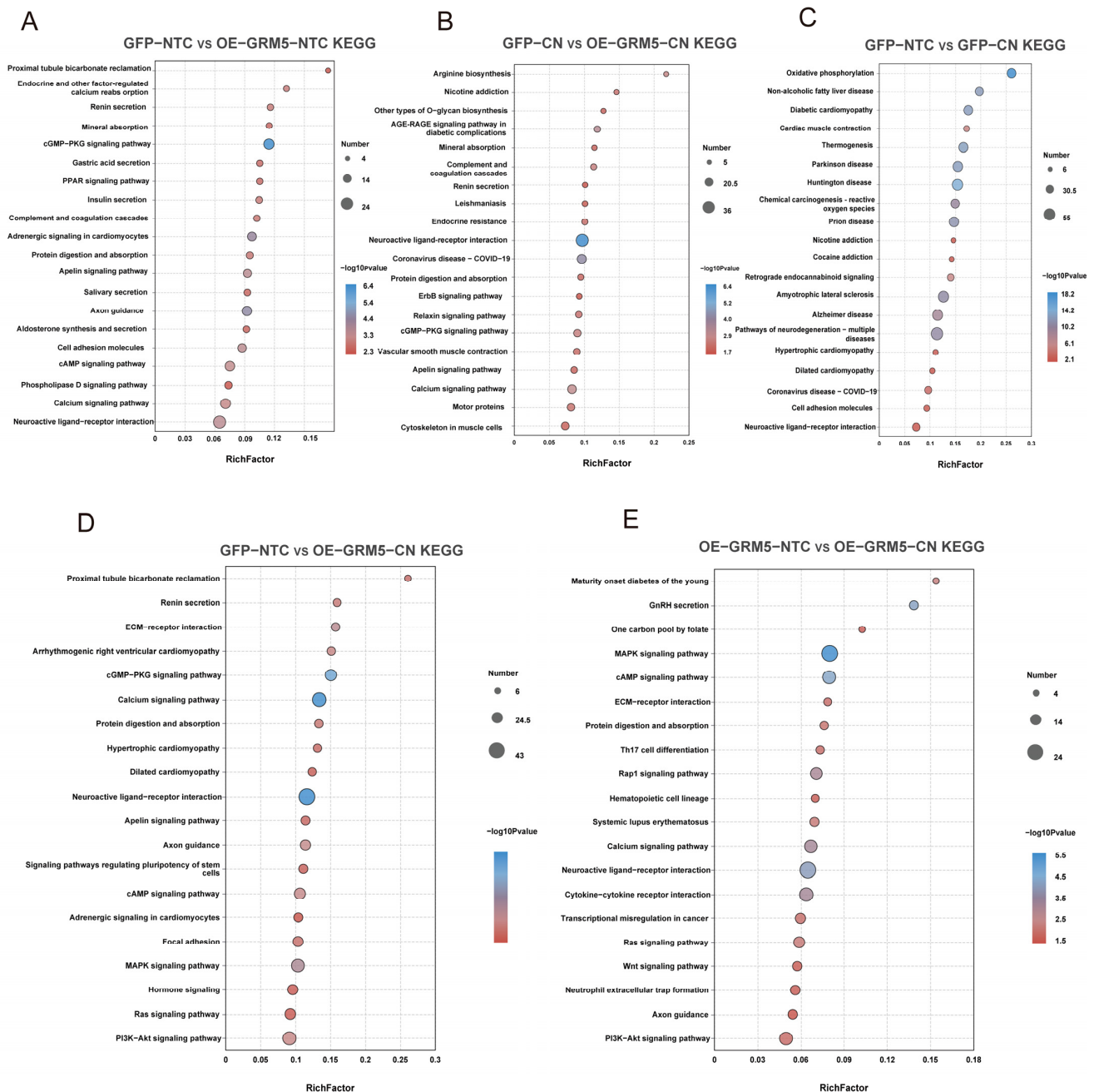


Figure 9. KEGG pathway enrichment of differentially expressed genes across the five comparison groups. (A) KEGG enrichment plot for GFP-NTC vs. OE-GRM5-NTC. (B) KEGG enrichment plot for GFP-CN vs. OE-GRM5-CN. (C) KEGG enrichment plot for GFP-NTC vs. GFP-CN. (D) KEGG enrichment plot for GFP-NTC vs. OE-GRM5-CN. (E) KEGG enrichment plot for OE-GRM5-NTC vs. OE-GRM5-CN.

3. Discussion

Via bioinformatics analysis, this study systematically investigated the expression characteristics, clinical correlations, molecular mutation patterns, and tumor microenvironment immune infiltration associations of the mGluR family in LUAD. Analysis via the UALCAN database revealed that five members of the mGluR family exhibited significantly abnormal differences in expression levels between LUAD tissues and normal lung tissues; analysis via the CancerSEA database revealed that seven members of this family were closely associated with the core biological functions of LUAD (such as proliferation and invasion). Analysis of the clinical characteristics and prognostic correlations revealed that the expression levels of the six mGluR family members were significantly correlated with the clinical stage of LUAD, and Kaplan–Meier survival analysis results demonstrated that the expression of all the mGluR family members was significantly associated with poor prognosis in patients with LUAD. Further evaluation of molecular mutation characteristics showed that all members of the mGluR family had a certain mutation rate in LUAD, among which GRM5 had the highest mutation rate, and there was an association between its mutation rate and expression level.

Subsequent bioinformatics analysis further revealed the potential role of the mGluR family in regulating immune infiltration within the tumor microenvironment. Integrating findings across multiple levels—from tumorigenesis and progression to prognostic prediction and immune regulation—this study demonstrates that GRM5 exhibits significant biological characteristics in these critical pathways of LUAD. In fact, the role of GRM5 in other cancers has been partially confirmed by previous studies. PARK et al. reported that GRM5 is not only frequently overexpressed in oral squamous cell carcinoma but also may be related to tumor progression, and that its expression may be associated with prognosis [16]. Frati C et al. reported that the GRM5 receptor may be involved in the regulation of melanocyte proliferation [28].

Interestingly, this study observed a proliferation-inhibitory effect following GRM5 overexpression in A549 cells, which contradicts the clinical data showing that high GRM5 expression predicts poor prognosis in LUAD patients. We hypothesize that this discrepancy arises from differing complexities between *in vivo* and *in vitro* models. In clinical samples, high GRM5 expression often co-occurs with co-activation of oncogenes like EGFR, where the poor prognosis results from multi-gene synergistic effects. In contrast, the *in vitro* model of single GRM5 overexpression fails to replicate the intricate gene interaction networks found *in vivo*, thus presenting an opposite phenotype. Such *in vivo*-*in vitro* phenotypic discrepancies are common. For instance, low HOXD1 expression in LUAD clinical samples indicates poor prognosis, yet *in vitro* overexpression suppresses the malignant phenotype of A549 cells by upregulating the BMP2/BMP6 pathway. This discrepancy fundamentally stems from the co-silencing effect of HOXD1 with tumor suppressor genes in clinical samples [29]. Pulice, J. et al. [30] confirmed that NKX2-1 co-activates with EGFR to drive tumor progression in clinical settings, whereas its isolated overexpression *in vitro* triggers negative feedback due to dose imbalance, ultimately suppressing cell proliferation. Additionally, our WB detection results indicate that the basal expression level of GRM5 in the A549 cell line is inherently very low, consistent with reports in the literature [31].

Functional experiments confirm that the CN significantly activates the GRM5, thereby inhibiting LUAD cell proliferation and inducing apoptosis, suggesting its potential therapeutic value as a GRM5-targeting agent. However, as a member of the cinchona alkaloid family, CN's chemical structural characteristics indicate it may exert antitumor effects through a multi-target synergistic mechanism. Previous studies indicate that CN can embed into the RING domain of TRAF6, disrupting its interaction with Ubc13. This inhibits the AKT/TAK1 signaling cascade, reducing cell proliferation and promoting apoptosis [24].

While this non-specific multi-target effect may enhance therapeutic efficacy, it may also increase off-target risks and compromise the precision of targeted therapy. We plan to use proteomics, molecular docking, and other technical means to identify the potential off-target proteins of CN in subsequent research, so as to comprehensively distinguish its specific and off-target effects and provide a more complete theoretical basis for the development and application of CN as a potential anti-LUAD drug.

Our study is the first to associate the anti-LUAD effects of CN with GRM5 expression and its downstream transcriptional regulatory network. Results indicate that GRM5 overexpression synergizes with CN treatment to regulate core genes, including *A2M*, *GFR11*, *RSPO2*, and *S1PR1*. These genes not only occupy central positions in the network but also exhibit significant correlations with poor LUAD patient prognosis, suggesting they are key molecules mediating the antitumor function of the CN-GRM5 axis. Enrichment analysis further indicates that the MAPK signaling pathway may participate in this regulatory network. In previous research, Wei Kou et al. verified that GRM5 overexpression promotes the proliferation of MM cells and inhibits apoptosis by activating the Ras-MAPK pathway [32]. Within the NSCLC stromal enrichment protein interaction module, GRM5 was similarly identified as a pivotal node connecting multiple oncogenic pathways, including MAPK and PI3K-Akt [33]. Integrating these findings, we hypothesize that CN disrupts tumor cell proliferation advantages and undermines microenvironment-driven oncogenic signaling by “hijacking” the GRM5-MAPK positive feedback loop, thereby blocking downstream transcriptional reprogramming.

This study preliminarily reveals the mechanism by which CN inhibits LUAD progression through regulating GRM5 expression to affect key genes, providing new insights for therapeutic strategies targeting GRM5. However, three major limitations exist: First, the conclusions are primarily based on the single A549 cell line and have not been validated in other LUAD cell lines (e.g., H1299, PC9), leaving their generalizability to be further confirmed. Second, direct experimental evidence supporting the specific involvement of the MAPK pathway is currently lacking. Future studies should employ WB analysis, pathway inhibitors, or phosphorylation assays for in-depth validation. Third, no in vivo experiments were conducted, and CN’s dosage and administration regimen were not optimized for clinical translation, creating a gap from real-world therapeutic scenarios. Subsequent studies will replicate the functional validation of the CN-GRM5 axis across multiple cell lines, elucidate specific signaling mechanisms, and optimize dosing strategies through animal experiments to advance its preclinical translation.

4. Materials and Methods

4.1. mGluR Family Expression, Clinical Staging, and Prognosis in LUAD

Our study focused on LUAD and integrated multidatabase analysis to characterize mGluR family genes. Pan-cancer differential expression analysis of the mGluR family was conducted via GSCA (Gene Set Cancer Analysis, <https://guolab.wchscu.cn/GSCA/#/>) (accessed on 9 March 2025) [34]. CancerSEA (<http://biocc.hrbmu.edu.cn/CancerSEA/home.jsp>) (accessed on 20 March 2025)) was employed to decipher the functional associations of this family in LUAD [35]. UALCAN (<https://ualcan.path.uab.edu/index.html>) (accessed on 12 April 2025)) enables visualization of mGluR family expression levels in LUAD and correlation analysis with tumor stage [36]. By combining the Kaplan–Meier Plotter database (<https://kmplot.com/analysis/>) (accessed on 27 March 2025)) with Cox regression analysis, we investigated the associations between mGluR family expression levels and clinical–pathological characteristics in LUAD patients.

4.2. Panoramic Analysis of Molecular Alterations in the mGluR Family in LUAD

Through the cBioPortal platform (<https://www.cbioportal.org/> (accessed on 1 April 2025)) [37], multiomics data, including genomic copy number variations, gene point mutations, and transcription abnormalities, from 503 samples in the TCGA-LUAD cohort were integrated. Using OncoPrint visualization technology, a comprehensive analysis of molecular variation patterns across all members of the mGluR family was performed.

4.3. Immune Infiltration Analysis

TIMER (<https://cistrome.shinyapps.io/timer/> (accessed on 17 April 2025)) is a comprehensive web platform for analyzing tumor-infiltrating immune cells, used to investigate the associations between mGluR family expression and tumor-infiltrating immune cell types, including purity cells, B cells, CD8⁺ T cells, CD4⁺ T cells, macrophages, neutrophils, and dendritic cells [38].

4.4. Cell Source and Culture Conditions

Human embryonic kidney 293T cells (HEK-293T) and the human LUAD cell line A549 were both purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA) and authenticated by STR identification. Specifically, A549 cells were cultured in RPMI-1640 medium (Gibco, Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 10% fetal bovine serum (VivaCell, Denzlingen, Germany) and 1% penicillin–streptomycin (Gibco, Thermo Fisher Scientific, Waltham, MA, USA). In contrast, HEK-293T cells were maintained in DMEM (Gibco, Thermo Fisher Scientific, Waltham, MA, USA) containing the same concentrations of FBS and penicillin–streptomycin as described above. Both cell lines were cultured in a humidified incubator at 37 °C with 5% CO₂ under standard adherent culture conditions. For all experiments, cells in the logarithmic growth phase were used to ensure consistency and reliability.

4.5. Lentiviral Construction and Cell Transfection

The transfer vectors, pCDH-3×Flag-GFP-Puro or pCDH-GRM5-3×Flag-GFP-Puro (Youbio, Changsha, China), the psPAX2 (Youbio, Changsha, China) packaging vector, and the pMD2.G (Youbio, Changsha, China) envelope vector, were cotransfected into HEK-293T cells to produce the lentiviruses with Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. Fresh A549 cells in the logarithmic growth phase were infected with the lentiviral mixture. After 48 h, the cells were cultured continuously for 3 days with 2 µg/µL puromycin to select positively infected cells.

4.6. Western Blotting (WB)

Proteins were extracted via RIPA lysis buffer (P0013B, Beyotime, Shanghai, China), and their concentrations were determined via a BCA protein assay kit (catalog number 23227, Thermo Scientific, Waltham, MA, USA). After concentration determination, the proteins were separated via sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and then transferred to a polyvinylidene fluoride (PVDF) membrane. The membrane was blocked with 5% nonfat dry milk, followed by incubation with the primary anti-GRM5 antibody (1:1000, YT2745, Immunoway, Plano, TX, USA) at 4 °C overnight. The next day, incubation with the corresponding secondary antibody was performed, followed by chemiluminescent detection using the eBlot Touch Imager™ (Shanghai, China).

4.7. Cell Counting Kit-8 (CCK8) Assay

A549 cells were seeded at a density of 2×10^3 cells/well in a 96-well plate. After incubation for 24 h, 48 h, or 72 h, detection solution containing 10% CCK-8 (TargetMol, Boston, MA, USA) was added to each well. The plates were incubated at 37 °C in the

dark for 2 h. Absorbance at 450 nm was then measured via a microplate reader (Molecular Devices, San Jose, CA, USA). Cell viability (%) = (Experimental OD – Blank OD)/(Control OD – Blank OD) × 100.

4.8. Wound-Healing Assay

A standardized wound model was established using the Cell Scratch Film (Beyotime FAM112-120, Boston, MA, USA). The cells were then seeded at a density of 6×10^5 cells per well and cultured in complete medium until they reached 90% confluence. The insert was carefully removed with sterile forceps, creating a uniform scratch in each well. After gently washing twice with PBS to remove detached cells, the medium was replaced with 1% FBS-containing medium supplemented with the test drugs or vehicle control. Phase-contrast images of fixed fields were captured at 0 h and 24 h via an Olympus IX73 inverted microscope at fixed fields. Wound closure was analyzed via ImageJ software (version 1.53a), and cell migration rate was calculated via the following formula: Migration rate (%) = [(scratch area at 0 h – scratch area at 24 h)/scratch area at 0 h] × 100. All experiments were independently repeated three times, with three replicate wells in each run to ensure reliability.

4.9. Colony Formation Assay

The cells were seeded at 300 cells/well in 12-well plates supplemented with complete medium and cultured at 37 °C in a 5% CO₂ incubator, and the medium was changed every 3 days. After 2 weeks, the culture was terminated. The medium was discarded, and the cells were washed twice with PBS and fixed with 4% paraformaldehyde (Biosharp, Beijing, China) at room temperature for 30 min. The fixative was discarded, and the cells were washed twice with PBS and stained with 0.1% crystal violet (Solarbio, Beijing, China) at room temperature in the dark for 15 min. The background stains were rinsed away with deionized water. After air-drying, colonies with >50 cells were counted under an optical microscope. Three runs were performed per group.

4.10. Quantitative Real-Time PCR (RT-qPCR)

Total RNA was extracted via TRIzol reagent (Takara, Tokyo, Japan) and reverse-transcribed into cDNA via PrimeScript™ RT Master Mix (Takara, Tokyo, Japan). RT-qPCR was then performed via TB Green Premix Ex Taq™ II (Takara, Tokyo, Japan) on a QuantStudio™ 5 system (Thermo Fisher, USA). Relative gene expression was calculated via the $2^{-\Delta\Delta CT}$ method, with GAPDH used as the internal reference. All primers were synthesized by Sangon Biotech (Shanghai, China), and their sequences are listed in Table 1.

Table 1. RT-qPCR primer information.

Genes	Forward Primer Sequence (5'-3')	Reverse Primer Sequence (5'-3')
GAPDH	GGAGCGAGATCCCTCCAAAAT	GGCTGTTGTCATACTTCTCATGG
A2M	CCTTGCTTTAGGAGTGCAG	CCGTCTCGTAGTAATCATAGAC
GFRA1	GGTCTGAGAATGAAATTCCCAC	AGATAATAGGGTGGACAGAGC
RSPO2	ATGCAGTTTCGCCTTTTCTCC	TCTTGCATCTCCTGGATTTCAG
S1PR1	CCATCGTCATTCTGTACTGC	AGAGTGTAATGATGGGGTTGG

4.11. Apoptosis Assay

The cells were seeded at a density of 7×10^4 cells/well in 12-well plates and cultured for 24 h until they reached 80–90% confluence, after which they were treated with CN for 24 h. The cells were stained via the Annexin V-APC/DAPI (Elabscience, Wuhan, China) kit. Flow cytometry (Agilent NovoCyte Advanteon, San Diego, CA, USA) was used to detect and analyze the data.

4.12. Tango Ligand Activation Assay

When the HEK-293T cells reached 80% confluence, they were cotransfected with GRM5-Tango, β -arrestin2-TEV, and TRE-Tight-Luc at a 2:1:1 ratio in a 6 cm dish. After 24 h, the cells were seeded at 3×10^4 cells/well into a 96-well plate and allowed to adhere overnight. The next day, 100 μ L of basal medium was added, $10\times$ CN working solution prepared with basal medium was added, the plate was gently tapped to mix, and the mixture was incubated for 18 h. 50 μ L of diluted Bright-Glo (Promega, Madison, WI, USA, #E2610) was added to each well, the mixture was gently tap to mix, and the luminescence value was immediately read on a microplate reader (Molecular Devices, USA).

4.13. mRNA Sequencing and Transcriptome Analysis

After 48 h of CN treatment, cell samples were collected from the GFP control group and the OE-GRM5 overexpression group and sent to Wuhan Benagen for mRNA sequencing. Transcriptome analysis was performed in the R platform (version 4.4.2) with the aid of dedicated bioinformatics tools (R packages) and online analytical resources. PCA was executed using the prcomp function, and the ggplot2 package was employed to visualize the distributions of four sample groups ($n = 3$ per group) to assess differences in overall expression patterns across datasets. DEGs were identified via the DESeq2 package, with selection criteria set as $|\log_2\text{FoldChange}| > 1$ and an adjusted p -value < 0.05 . Volcano plots were generated via the EnhancedVolcano package to visualize the distribution of DEGs. KEGG pathway enrichment analysis and GO functional annotation of the DEGs were performed via the clusterProfiler package. Venn diagrams were created via the online tool jvenn [39] (<https://www.bioinformatics.com.cn/static/others/jvenn/example.html> (accessed on 16 July 2025)). We identified 17 core genes and generated an expression heatmap via the bioinformatics online platform (<https://www.bioinformatics.com.cn/> (accessed on 8 September 2025)).

4.14. Statistical Analysis

Statistical analysis and data visualization were conducted with GraphPad Prism (version 10.1.2) and the R platform (version 4.4.2). The t -test was used for comparisons between two groups, and one-way analysis of variance (ANOVA) was used for comparisons among multiple groups. Each experiment was independently repeated at least three times to confirm the consistency of the results. The significance level was set at $p < 0.05$, indicating statistical significance.

5. Conclusions

In summary, bioinformatics analysis revealed that GRM5 is significantly overexpressed in LUAD and is closely associated with poor patient prognosis and an immunosuppressive microenvironment. In vitro functional experiments further confirmed that CN effectively inhibits A549 cell proliferation and promotes apoptosis by activating GRM5, suggesting CN as a potential targeted agonist for GRM5. These findings provide novel mechanistic insights and a research foundation for LUAD treatment, while also offering experimental evidence for developing intervention strategies targeting GRM5.

Author Contributions: Conceptualization, Y.X. and W.L.; methodology, Y.X.; software, Y.X.; validation, Y.X., W.L. and Y.W.; formal analysis, Y.W.; investigation, Y.X.; resources, C.L.; data curation, Y.Y. and P.T.; writing—original draft preparation, Y.X.; visualization, Y.X. and W.L.; supervision, S.C. and C.S.; project administration, W.L., Y.W. and Y.X.; writing—review and editing, S.C. and C.S.; funding acquisition, C.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Chief Scientist Research Project of Hubei Shizhen Laboratory (Grant No. HSL2024SX0006), the Key Scientific and Technological Project of Hubei Shizhen Laboratory (Grant No. SZL-2025-KT-06), and the Scientific Research Start-up Fund for Talents of Chengdu University of Traditional Chinese Medicine (Grant No. 030040015).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are openly available from the National Genomics Data Center at <https://ngdc.cncb.ac.cn/search/all?q=PRJNA1357989> (accessed on 6 November 2025), reference number PRJNA1357989.

Acknowledgments: We are grateful to all the laboratory members for their technical advice and helpful discussions.

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

Abbreviations

The following abbreviations are used in this manuscript:

LUAD	Lung adenocarcinoma
mGluR	Metabotropic glutamate receptor
CN	Cinchonine
SCLC	Small-cell lung cancer
NSCLC	Non-small-cell lung cancer
GPCR	G protein-coupled receptor
GSCA	Gene Set Cancer Analysis
GO	Gene Ontology
KEGG	Kyoto Encyclopedia of Genes and Genomes
BP	Biological process
CC	Cellular component
MF	Molecular function
Wb	Western blotting
DEGs	Differentially expressed genes

References

1. Bray, F.; Laversanne, M.; Sung, H.; Ferlay, J.; Siegel, R.L.; Soerjomataram, I.; Jemal, A. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J. Clin.* **2024**, *74*, 229–263. [\[CrossRef\]](#)
2. Gao, S.; Li, N.; Wang, S.; Zhang, F.; Wei, W.; Li, N.; Bi, N.; Wang, Z.; He, J. Lung Cancer in People's Republic of China. *J. Thorac. Oncol.* **2020**, *15*, 1567–1576. [\[CrossRef\]](#)
3. Thai, A.A.; Solomon, B.J.; Sequist, L.V.; Gainor, J.F.; Heist, R.S. Lung cancer. *Lancet* **2021**, *398*, 535–554. [\[CrossRef\]](#) [\[PubMed\]](#)
4. Ling, B.; Liao, X.; Huang, Y.; Liang, L.; Jiang, Y.; Pang, Y.; Qi, G. Identification of prognostic markers of lung cancer through bioinformatics analysis and in vitro experiments. *Int. J. Oncol.* **2020**, *56*, 193–205. [\[CrossRef\]](#)
5. Shu, J.; Jiang, J.; Zhao, G. Identification of novel gene signature for lung adenocarcinoma by machine learning to predict immunotherapy and prognosis. *Front. Immunol.* **2023**, *14*, 1177847. [\[CrossRef\]](#) [\[PubMed\]](#)
6. Bade, B.C.; Dela Cruz, C.S. Lung Cancer 2020: Epidemiology, Etiology, and Prevention. *Clin. Chest Med.* **2020**, *41*, 1–24. [\[CrossRef\]](#) [\[PubMed\]](#)
7. Cleva, R.M.; Olive, M.F. Metabotropic receptors and drug addiction. *Wiley Interdiscip. Rev. Membr. Transp. Signal.* **2012**, *1*, 281–295. [\[CrossRef\]](#) [\[PubMed\]](#)
8. Teh, J.L.; Shah, R.; La Cava, S.; Dolfi, S.C.; Mehta, M.S.; Kongara, S.; Price, S.; Ganesan, S.; Reuhl, K.R.; Hirshfield, K.M.; et al. Metabotropic glutamate receptor 1 disrupts mammary acinar architecture and initiates malignant transformation of mammary epithelial cells. *Breast Cancer Res. Treat.* **2015**, *151*, 57–73. [\[CrossRef\]](#)
9. Xiao, B.; Chen, D.; Zhou, Q.; Hang, J.; Zhang, W.; Kuang, Z.; Sun, Z.; Li, L. Glutamate metabotropic receptor 4 (GRM4) inhibits cell proliferation, migration and invasion in breast cancer and is regulated by miR-328-3p and miR-370-3p. *BMC Cancer* **2019**, *19*, 891. [\[CrossRef\]](#)

10. Chang, H.J.; Yoo, B.C.; Lim, S.B.; Jeong, S.Y.; Kim, W.H.; Park, J.G. Metabotropic glutamate receptor 4 expression in colorectal carcinoma and its prognostic significance. *Clin. Cancer Res.* **2005**, *11*, 3288–3295. [\[CrossRef\]](#)
11. Yoo, B.C.; Jeon, E.; Hong, S.H.; Shin, Y.K.; Chang, H.J.; Park, J.G. Metabotropic glutamate receptor 4-mediated 5-Fluorouracil resistance in a human colon cancer cell line. *Clin. Cancer Res.* **2004**, *10*, 4176–4184. [\[CrossRef\]](#)
12. Zhang, C.; Yuan, X.R.; Li, H.Y.; Zhao, Z.J.; Liao, Y.W.; Wang, X.Y.; Su, J.; Sang, S.S.; Liu, Q. Anti-cancer effect of metabotropic glutamate receptor 1 inhibition in human glioma U87 cells: Involvement of PI3K/Akt/mTOR pathway. *Cell Physiol. Biochem.* **2015**, *35*, 419–432. [\[CrossRef\]](#) [\[PubMed\]](#)
13. D’Onofrio, M.; Arcella, A.; Bruno, V.; Ngomba, R.T.; Battaglia, G.; Lombardi, V.; Ragona, G.; Calogero, A.; Nicoletti, F. Pharmacological blockade of mGlu2/3 metabotropic glutamate receptors reduces cell proliferation in cultured human glioma cells. *J. Neurochem.* **2003**, *84*, 1288–1295. [\[CrossRef\]](#)
14. Martino, J.J.; Wall, B.A.; Mastrantonio, E.; Wilimczyk, B.J.; La Cava, S.N.; Degenhardt, K.; White, E.; Chen, S. Metabotropic glutamate receptor 1 (Grm1) is an oncogene in epithelial cells. *Oncogene* **2013**, *32*, 4366–4376. [\[CrossRef\]](#)
15. Prickett, T.D.; Wei, X.; Cardenas-Navia, I.; Teer, J.K.; Lin, J.C.; Walia, V.; Gartner, J.; Jiang, J.; Cherukuri, P.F.; Molinolo, A.; et al. Exon capture analysis of G protein-coupled receptors identifies activating mutations in GRM3 in melanoma. *Nat. Genet.* **2011**, *43*, 1119–1126. [\[CrossRef\]](#)
16. Park, S.Y.; Lee, S.A.; Han, I.H.; Yoo, B.C.; Lee, S.H.; Park, J.Y.; Cha, I.H.; Kim, J.; Choi, S.W. Clinical significance of metabotropic glutamate receptor 5 expression in oral squamous cell carcinoma. *Oncol. Rep.* **2007**, *17*, 81–87. [\[CrossRef\]](#)
17. Zhang, Z.; Li, N.; Wei, X.; Chen, B.; Zhang, Y.; Zhao, Y.; Hu, X.; Hou, S. GRM4 inhibits the proliferation, migration, and invasion of human osteosarcoma cells through interaction with CBX4. *Biosci. Biotechnol. Biochem.* **2020**, *84*, 279–289. [\[CrossRef\]](#)
18. Ye, Y.; Li, Y.; Wei, Y.; Xu, Y.; Wang, R.; Fu, Z.; Zheng, S.; Zhou, Q.; Zhou, Y.; Chen, R.; et al. Anticancer effect of HOTTIP regulates human pancreatic cancer via the metabotropic glutamate receptor 1 pathway. *Oncol. Lett.* **2018**, *16*, 1937–1942. [\[CrossRef\]](#) [\[PubMed\]](#)
19. Koochekpour, S. Glutamate, a metabolic biomarker of aggressiveness and a potential therapeutic target for prostate cancer. *Asian J. Androl.* **2013**, *15*, 212–213. [\[CrossRef\]](#)
20. Ganor, Y.; Levite, M. The neurotransmitter glutamate and human T cells: Glutamate receptors and glutamate-induced direct and potent effects on normal human T cells, cancerous human leukemia and lymphoma T cells, and autoimmune human T cells. *J. Neural Transm.* **2014**, *121*, 983–1006. [\[CrossRef\]](#) [\[PubMed\]](#)
21. Warhurst, D.C. Cinchona alkaloids and malaria. *Lancet* **1981**, *2*, 1346. [\[CrossRef\]](#) [\[PubMed\]](#)
22. Liang, F.; Lv, Y.; Qiao, X.; Zhang, S.; Shen, S.; Wang, C.; Xu, G.; Zou, X.; Wang, L.; Zhang, B. Cinchonine-induced cell death in pancreatic cancer cells by downregulating RRP15. *Cell Biol. Int.* **2023**, *47*, 907–919. [\[CrossRef\]](#)
23. Wang, C.; Li, Q.; Hou, Y.; Sun, M.; Sun, J.; Lou, Z.; Li, Y. The interaction of cinchonine and immunoglobulin G and the development of a nanocomplex with improved anti-breast cancer activity. *Int. J. Biol. Macromol.* **2025**, *287*, 138152. [\[CrossRef\]](#) [\[PubMed\]](#)
24. Qi, Y.; Pradipta, A.R.; Li, M.; Zhao, X.; Lu, L.; Fu, X.; Wei, J.; Hsung, R.P.; Tanaka, K.; Zhou, L. Cinchonine induces apoptosis of HeLa and A549 cells through targeting TRAF6. *J. Exp. Clin. Cancer Res.* **2017**, *36*, 35. [\[CrossRef\]](#)
25. Jin, Z.L.; Yan, W.; Qu, M.; Ge, C.Z.; Chen, X.; Zhang, S.F. Cinchonine activates endoplasmic reticulum stress-induced apoptosis in human liver cancer cells. *Exp. Ther. Med.* **2018**, *15*, 5046–5050. [\[CrossRef\]](#) [\[PubMed\]](#)
26. Pös, O.; Radvanszky, J.; Buglyó, G.; Pös, Z.; Rusnakova, D.; Nagy, B.; Szemes, T. DNA copy number variation: Main characteristics, evolutionary significance, and pathological aspects. *Biomed. J.* **2021**, *44*, 548–559. [\[CrossRef\]](#)
27. Li, B.; Severson, E.; Pignon, J.C.; Zhao, H.; Li, T.; Novak, J.; Jiang, P.; Shen, H.; Aster, J.C.; Rodig, S.; et al. Comprehensive analyses of tumor immunity: Implications for cancer immunotherapy. *Genome Biol.* **2016**, *17*, 174. [\[CrossRef\]](#)
28. Frati, C.; Marchese, C.; Fisichella, G.; Copani, A.; Nascia, M.R.; Storto, M.; Nicoletti, F. Expression of functional mGlu5 metabotropic glutamate receptors in human melanocytes. *J. Cell Physiol.* **2000**, *183*, 364–372. [\[CrossRef\]](#)
29. Hu, X.; Zhang, S.; Zhang, X.; Liu, H.; Diao, Y.; Li, L. HOXD1 inhibits lung adenocarcinoma progression and is regulated by DNA methylation. *Oncol. Rep.* **2024**, *52*, 173. [\[CrossRef\]](#)
30. Pulice, J.L.; Meyerson, M. Amplified dosage of the NKX2-1 lineage transcription factor controls its oncogenic role in lung adenocarcinoma. *Mol. Cell* **2025**, *85*, 1311–1329.e16. [\[CrossRef\]](#)
31. Li, T.J.; Huang, Y.H.; Chen, X.; Zhou, Z.; Luo, S.W.; Feng, D.D.; Han, J.Z.; Luo, Z.Q. Metabotropic glutamate receptor 8 activation promotes the apoptosis of lung carcinoma A549 cells in vitro. *Sheng Li Xue Bao* **2015**, *67*, 513–520.
32. Kou, W.; Huang, H.; Dai, S.; Tan, X.; Chen, Q.; Huang, R.; Zou, H. mGluR5 promotes the progression of multiple myeloma in vitro via Ras-MAPK signaling pathway. *Adv. Clin. Exp. Med.* **2022**, *31*, 881–888. [\[CrossRef\]](#) [\[PubMed\]](#)
33. Pazhouhandeh, M.; Samiee, F.; Boniadi, T.; Khedmat, A.F.; Vahedi, E.; Mirdamadi, M.; Sigari, N.; Siadat, S.D.; Vaziri, F.; Fateh, A.; et al. Comparative Network Analysis of Patients with Non-Small Cell Lung Cancer and Smokers for Representing Potential Therapeutic Targets. *Sci. Rep.* **2017**, *7*, 13812. [\[CrossRef\]](#)
34. Liu, C.J.; Hu, F.F.; Xia, M.X.; Han, L.; Zhang, Q.; Guo, A.Y. GSCALite: A web server for gene set cancer analysis. *Bioinformatics* **2018**, *34*, 3771–3772. [\[CrossRef\]](#) [\[PubMed\]](#)

35. Yuan, H.; Yan, M.; Zhang, G.; Liu, W.; Deng, C.; Liao, G.; Xu, L.; Luo, T.; Yan, H.; Long, Z.; et al. CancerSEA: A cancer single-cell state atlas. *Nucleic Acids Res.* **2019**, *47*, D900–D908. [[CrossRef](#)]
36. Chandrashekar, D.S.; Bashel, B.; Balasubramanya, S.A.H.; Creighton, C.J.; Ponce-Rodriguez, I.; Chakravarthi, B.; Varambally, S. UALCAN: A Portal for Facilitating Tumor Subgroup Gene Expression and Survival Analyses. *Neoplasia* **2017**, *19*, 649–658. [[CrossRef](#)] [[PubMed](#)]
37. Gao, J.; Aksoy, B.A.; Dogrusoz, U.; Dresdner, G.; Gross, B.; Sumer, S.O.; Sun, Y.; Jacobsen, A.; Sinha, R.; Larsson, E.; et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. *Sci. Signal* **2013**, *6*, pl1. [[CrossRef](#)]
38. Li, T.; Fu, J.; Zeng, Z.; Cohen, D.; Li, J.; Chen, Q.; Li, B.; Liu, X.S. TIMER2.0 for analysis of tumor-infiltrating immune cells. *Nucleic Acids Res.* **2020**, *48*, W509–W514. [[CrossRef](#)]
39. Bardou, P.; Mariette, J.; Escudié, F.; Djemiel, C.; Klopp, C. jvenn: An interactive Venn diagram viewer. *BMC Bioinform.* **2014**, *15*, 293. [[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.