

# Dynamic immunity gene and lncRNA regulatory cooperative pairs revealed prognostic signatures in the development of colon cancer

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## Research

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# Abstract

**Background:** The pathological development of colon cancer is a complex progression that depends on multiple alterations of coding and non-coding genes. Colon cancer are challenged by the immune system and acquire features to evade its surveillance during the whole process of occurrence and development. Therefore, it is important to capture the immune regulatory events during the progression of colon cancer development and to identify reliable markers for predicting clinical outcomes in patients.

**Methods:** Here, a standardized computational procedures was developed to evaluate immune cell populations-associated gene (immuneCPa gene)-lncRNA relationships in diverse stages (I, II, III and IV) of colon cancer based on genes and lncRNAs expression. Stage-specific immuneCPa genes and lncRNAs were identified in each colon cancer stage.

**Results:** Dynamic stage-specific immuneCPa gene-lncRNA regulatory networks were constructed and characterized in colon cancer development. A immuneCPa gene-lncRNA activity profile across different stages revealed that lncRNAs were highly stage-selective in regulating immuneCPa genes in colon cancer. Specify scores of immune indicated that diverse kinds of immune cells were temporally-specific in colon cancer. NK CD56bright cell showed strongest stage common features in colon cancer. Further survival analysis indicated that some stage specific immuneCPa gene-lncRNA relationships may have the potential for predicting colon cancer prognosis.

**Conclusions:** Collectively, our study leads to a novel starting point for future functional explorations, the identification of immune-related biomarkers, and lncRNA-based targeted therapy for colon cancer.

## Background

Colon cancer represents the third most frequent cancer in the world, with 147,950 novel cases worldwide in 2020 [1]. 30% of patients would occur recurrence when there is nodal involvement (stage III) due to micrometastatic spreading although the patients are diagnosed at a localized stage [2]. Colon cancer is a potentially preventable disease and early-stage colon cancer is usually curable [3]. Although the survival of colon cancer patients improve follow the combination of different therapeutic approaches including surgery, endoscopic ablation, radiation, and chemotherapy, individualization of patients varies greatly. Especially, patients with advanced cancer show worse prognosis. Therefore, it is important to understand the mechanisms regulating the progression of colon cancer and to identify efficacious therapeutic targets and prognostic/predictive biomarkers for predicting clinical outcomes in patients.

The past years have witnessed promising clinical feedback for anti-cancer immunotherapies. Immune cells contribute to invasion by secreting a cornucopia of inflammatory factors that promote epithelial-to-mesenchymal transition and remodeling of the stroma [4]. Previous study reports that immunoscore is a robust and validated clinical assay leveraging immune scoring to predict recurrence risk of colon cancer patient [5]. Immune-related genes play regulatory roles in the immune system and are involved in the initiation and progression of colon cancer [6]. Exploring the roles of immune in diverse stages of colon

cancer could provide assistance for immunotherapy of colon cancer. However, most current researches only focus on immune-related coding genes in cancers.

In recent years, emerging evidence has shown the importance of long non-coding RNAs (lncRNAs) as a new regulator of many physical or pathological processes [7, 8]. lncRNAs also play essential roles in colon cancer. For example, LINC00662 overexpression promoted the occurrence and development of colon cancer [9]. LINC00460 might function as an oncogenic lncRNA in colon cancer development and could be explored as a potential biomarker and therapeutic target for colon cancer. The functions of lncRNA in immune response for cancers are also identified. lncRNA could control the modulation of immune checkpoint molecules in cancer [10]. For example, lncRNA NKILA promotes tumor immune evasion by sensitizing T cells to activation-induced cell death [11]. Interference lncRNA SNHG1 could inhibit the differentiation of Treg cells by promoting miR-448 expression, thereby impeding the immune escape of breast cancer [12]. These studies have significantly enhanced our understanding of the lncRNA mechanisms in immune and underlying disease progression. Unfortunately, there has only been limited work studying the dynamic immune-related gene-lncRNA interactions involved in colon cancer development.

To address this issue, the present study designed an integrated computational approach to identify immuneCPa gene-lncRNA pairs in diverse stages of colon cancer based on genes and lncRNAs expression. Stage-specific immuneCPa genes and lncRNAs were extracted in each colon cancer stage. Dynamic stage-specific immuneCPa gene-lncRNA regulatory networks were constructed and characterized in colon cancer development. A immuneCPa gene-lncRNA activity profile across different stages revealed that lncRNAs were highly stage-selective in regulating immuneCPa genes in colon cancer. Specify scores of immune indicated that diverse kinds of immune cells were temporally-specific in colon cancer. NK CD56bright cell showed strongest stage common features in colon cancer. Further survival analysis indicated that some stage specific immuneCPa gene-lncRNA relationships may have the potential for predicting prognosis in specific stage. In summary, our systematic analysis not only sheds new light on dynamic regulatory mechanisms of immuneCPa gene-lncRNA interactions, but may also help in colon cancer prognosis stratification and discovery of therapeutic targets.

## Materials And Methods

### Obtain of high-throughput expression profiles of lncRNAs and genes for colon cancer

Gene and lncRNA expression profiles (Level 2) were obtained from The Cancer Genome Atlas (TCGA, <https://www.cancer.gov/about-nci/organization/ccg/research/structural-genomics/tcga>). 464 colon cancer tissues and 101 tumor adjacent normal control tissues were got and clinical follow-up information including cancer stages and survival data were retained for further analysis. Lastly, 90, 226, 160 and 81 stage I, II, III and IV colon patients samples were obtained. The genes and lncRNAs which their expression values were 0 in all the samples would be removed. All expression values had been transformed by  $\log_2$  to satisfy normal distribution.

## **Collecting multiple kinds of immune cell populations-associated genes**

17 selected immune cell populations including B cells, Eosinophils, Macrophages, Mast cells, NK CD56bright cells, NK CD56dim cells, Neutrophils, T helper cells, Tcm cells, Tem cells, Tfh cells, aDC, iDC, Activated CD8 T cell, Gamma delta T cell, Regulatory T cell and Cytotoxic cells were selected [13]. Multiple kinds of immune cell populations-associated genes (immuneCPa genes) were also obtained from previous study [14, 15]. All the ImmuneCPa genes were retained for further analysis.

## **Identifying colon cancer stage-specific immuneCPa genes and lncRNAs based on expression profiles**

In order to identify colon cancer stage-specific immuneCPa genes and lncRNAs, t-test was used to perform differential expression analysis between expression profiles of colon cancer and normal control samples in four diverse stages. The significant immuneCPa genes ( $P < 0.05$ ) were considered as colon cancer stage-specific immuneCPa genes. The significant lncRNAs ( $P < 0.01$ ) were considered as colon cancer stage-specific lncRNAs. Only the colon cancer stage-specific immuneCPa genes and lncRNAs were used for follow analyses.

## **Construction of stage-specific immuneCPa gene-lncRNA regulatory networks in colon cancer development**

For each kind of immune cell populations, Pearson correlation coefficients (PCCs) were calculated for each colon cancer stage-specific immuneCPa genes and lncRNAs in colon cancer patients with diverse stages, respectively. The PCCs and P values were obtained for each stage-specific immuneCPa gene-lncRNA pair. Only the stage-specific immuneCPa gene and lncRNA pairs with absolute values which more than 0.5 were used for follow analyses and network construction. The stage-specific immuneCPa gene-lncRNA regulatory networks were constructed by Cytoscape 3.3.0 (<https://cytoscape.org/>). Degree analysis was also performed by Cytoscape 3.3.0.

## **The specificity score of stage-specific immuneCPa gene-lncRNA pairs for colon cancer**

The specificity of each stage-specific immuneCPa gene-lncRNA pair across different stages of colon cancer was determined by the specificity score as: (see Equation 1 in the Supplemental Files)

Where  $N$  is the number of colon cancer stages and  $PCC_i$  is a component normalized to the maximum value of the PCC value. Specificity score was used to evaluate stage specific for stage-specific immuneCPa gene-lncRNA pair in colon cancer.

## **Survival analyses for stage-specific immuneCPa gene-lncRNA pairs in colon cancer**

In order to evaluate the prognosis about stage-specific immuneCPa gene-lncRNA pairs in colon cancer, we performed survival analysis for these four stage groups. The two groups were used as constructing and validating model, respectively. We used the regression coefficient of each immuneCPa gene and lncRNA in the stage-specific immuneCPa gene-lncRNA pair related to patient survival based on gene and lncRNA

expression data to verify if these pairs were associated with survival. First, the colon cancer patients in each stage were randomly divided into two groups and the samples in these two groups are independent. Second, a multivariate Cox regression model was applied to each immuneCPa gene and its interacted lncRNAs in diverse colon cancer stage to obtain a standardized Cox regression coefficient for the first group. Age, cancer stage and sex were also considered as confounders in this process. Third, a risk score formula was constructed based on expression values of each immuneCPa gene and its interacted lncRNAs for the held-out group weighed by their estimated regression coefficients, following the above multivariate Cox regression analysis. Fourth, median of the risk score was used as the threshold value to divide the colon cancer patients into high-risk and low-risk groups. Finally, Kaplan-Meier (K-M) survival analysis was used for the diverse risk groups. Statistical significance assessed using the log-rank test. All analyses were performed within the R 2.6.6 framework.

## Results

### **Some stage-specific immuneCPa genes and lncRNAs were identified in colon cancer**

We first identified colon cancer-specific 17 kinds of immuneCPa genes for colon cancer in all stages. More than 70% of the 15 kinds of immuneCPa genes were differentially expressed (Figure 1A). 96.97% B cells associated genes were differentially expressed in all the colon cancer patients. The result indicated that immuneCPa genes play important roles in colon cancer. Next, stage-specific immuneCPa genes were identified in diverse colon cancer stages. In each kind of immuneCPa gene, percent of differential expressed immuneCPa genes showed similar pattern (Figure 1B). Most stage-specific immuneCPa genes were presented in patients with stage II colon cancer which is a critical stage of cancer progression. The result suggested that stage II was active stage of immune system to fight cancer cells. Diverse stage also shared some common immuneCPa genes (Figure 1C). For example, four stages had 14, 9 and 10 common immuneCPa genes in B cells, Gamma delta T cell and Eosinophils. We also extracted differential expressed lncRNAs (61%) for all colon cancer patients (Figure 1D). Similar to immuneCPa genes, there were most differential expressed lncRNAs in stage II of colon cancer. Four diverse stages shared more common differential expressed lncRNAs compared with immuneCPa genes (Figure 1E). We inferred some key lncRNAs maybe functions essentially in specific colon cancer stage.

### **Identification of stage-specific immuneCPa gene-lncRNA regulatory pairs in colon cancer development**

Two major factors including interactions and similar expression to extract stage-specific immuneCPa gene-lncRNA regulatory pairs. Experimentally verified gene-lncRNA interaction were considered as candidate regulatory pairs. Then considering that interactions do not directly imply their actual regulation of immuneCPa gene-lncRNA pairs in certain conditions, exploring gene regulation of lncRNAs through co-expression analysis can offer useful information to identify active immuneCPa gene-lncRNA relationships in different colon cancer stages. PCCs were calculated for each potential immuneCPa gene-lncRNA pair based on their expression profiles at different stages (Figure 2A). Stage II and III showed most similar distribution of PCCs for gene-lncRNA pairs. Most PCCs of gene-lncRNA pairs were

concentrated between 0.5 and 1 or -0.5 and -1 (Figure 2B). It indicated that most gene-lncRNA pairs showed strong co-expressed level. The numbers of co-expressed gene-lncRNA pairs were diverse in different kinds of immune cells (Figure 2C). For example, there were most co-expressed gene-lncRNA pairs in Tem and Tfh cells. In addition, most co-expressed gene-lncRNA pairs were present in stage III for almost kinds of immune cells (Figure 2D). The different sizes of these stage-specific immuneCPa gene-lncRNA pairs indicate the heterogeneity of immune associated genes and lncRNAs in the development of colon cancer.

### **Construction of dynamic stage-specific immuneCPa gene-lncRNA regulatory networks in colon cancer development**

We used a  $P < 0.05$  as the thresholds to identify a link between immuneCPa genes and lncRNAs in the regulatory networks. There were 91,962, 106,834, 165,589 and 118,431 immuneCPa gene and lncRNA pairs in stage I, II, III, IV (Figure 3A). We extracted absolute values of PCCs  $> 0.9$  as the thresholds to construct more tighter and closer stage-specific immuneCPa gene-lncRNA regulatory networks in colon cancer development (Figure 3B). Specifically, 19,929 edges between 408 immuneCPa genes and 3,768 lncRNAs, 75,259 edges between 408 immuneCPa genes and 4,920 lncRNAs, 52,635 edges between 409 immuneCPa genes and 3,983 lncRNAs and 118,356 edges between 407 TFs and 4,389 lncRNAs were constructed for stages I, II, III and IV colon cancer patients, respectively. In these stage-specific immuneCPa gene-lncRNA regulatory networks, there were some key hub nodes such as immuneCPa gene DCSTAMP, KIR3DL3 and SND1-IT1 could interacted with more than 400 lncRNAs. In these four stage-specific immuneCPa gene-lncRNA regulatory networks, immuneCPa gene had bigger degree than lncRNAs (Figure 3C). The degree analysis indicated that a common immuneCPa gene could be regulated by diverse and a number of lncRNAs in colon cancer development. lncRNAs maybe play specific regulatory roles for immuneCPa genes in diverse stages of colon cancer.

### **The dynamic activity profiles of immuneCPa gene-lncRNA regulatory pairs**

Although stage-specific networks share common topological properties, the immuneCPa gene-lncRNA regulatory interactions may change in different stages of colon cancer. To evaluate the proportion of common and specific immuneCPa gene-lncRNA regulatory interactions during colon cancer progression, we explored the overlaps of immuneCPa gene-lncRNA regulatory relationships among four stage-specific networks (Figure 4A). Most immuneCPa gene-lncRNA regulatory relationships are stage specific. Stage III had maximum number of immuneCPa gene-lncRNA regulatory relationships (Figure 4B). We inferred that stage III is a key stage which lncRNA participated in immune regulation for colon cancer. For all the immuneCPa gene-lncRNA regulatory relationships, 378,658 immuneCPa gene-lncRNA regulatory relationships were only present in one stage. The four stages only shared 249 common immuneCPa gene-lncRNA regulatory relationships, indicating that relationships were temporally-specific in colon cancer (Figure 4C). The immuneCPa gene-lncRNA regulatory relationships were making dynamic changes in colon cancer development (Figure 4D). To provide an overview of all possible immuneCPa gene-lncRNA relationships and their dynamic regulatory status, we built an activity profile for immuneCPa

gene-lncRNA relationships across different stages of colon cancer. Activity score is the standardized value of PPCs. Based on the activity scores, these immuneCPa gene-lncRNA relationships were grouped by the K-means clustering method (Figure 4E). Different groups of immuneCPa gene-lncRNA relationships were apparently activated at one or more stages. The patterns of immuneCPa gene-lncRNA relationships were diverse in different groups. These groups maybe could as stage-specific biomarkers for colon cancer.

### **Specificity score evaluates the specificity of immuneCPa gene-lncRNA relationships in colon cancer development**

Specificity score was designed to evaluate the specificity of immuneCPa gene-lncRNA relationships in colon cancer development. Most immuneCPa gene-lncRNA relationships showed lower specificity scores and indicated stage specific for these relationships in colon cancer (Figure 5A). In order to extract more stage-specific immuneCPa genes, we used mean specificity scores of all immuneCPa gene-lncRNA relationships to represent the specify of each immuneCPa gene. These stage-specific immuneCPa genes could interact with diverse numbers of lncRNAs in colon cancer (Figure 5B). The mean specificity scores for each kind of immune cells were also obtained. Mast and cytotoxic cells showed the strongest stage specificity in colon cancer (Figure 5C). Specificity scores of most immuneCPa genes in these two kinds of immune cells concentrated between 0.06 and 0.08 (Figure 5D). NK CD56bright cell showed strongest stage common features in colon cancer and indicated that it maybe participate in all immune process for colon cancer development. This results also revealed that diverse kinds of immune cells were temporally-specific in colon cancer.

### **Some immuneCPa gene-lncRNA relationships in colon cancer development has specific prognostic potential**

To evaluate the potential value of immuneCPa gene-lncRNA pairs as prognostic biomarkers in colon cancer with diverse stages, we created a risk-score formula according to the expression of each immuneCPa gene and its corresponding lncRNAs to generate OS (overall survival) prediction (see the Material and Methods section). We used median risk score as the cut-off point to test the survival of the diverse stage colon cancer patients into high-risk or low-risk groups. These three immuneCPa gene-lncRNA clusters were associated with Macrophages, iDC and Neutrophils cell populations (Figure 6A). Two stage II and one stage III specific immuneCPa gene-lncRNA clusters were significantly associated with survival, and they could serve as prognostic biomarkers (Figure 6B).

## **Discussion**

In the past few years, a number of studies have successfully characterized the roles of lncRNAs based on the general assumption that lncRNAs are key regulators of biological processes in many kinds of cancers. Although many studies have been carried out to characterize the complex and multiple functions of lncRNAs including cell growth [16], apoptosis [17], autophagy, epithelial mesenchymal transformation [18] and so on, little is known about the roles of lncRNAs in immune regulations for colon cancer.

Preliminary studies have shown that lncRNAs play important roles in the immune system and had become the focus of immunology [19]. Thus, it is urgent to comprehensively characterize the functions and mechanisms of lncRNAs in immune regulation for cancers. In present study, we used immune related genes as a bridge to identifying immune related lncRNAs in colon cancer.

The pathological development of colon cancer is a complex progression that depends on multiple alterations of coding and non-coding genes. Although our understanding of colon cancer has increased, the precise regulatory mechanisms about immune regulations underlying this complex disease are still not fully known. Therefore, it is important to uncover the immune regulating events during progression of colon cancer and identify reliable markers for predicting the clinical outcome of patients. Specially, exploring regulatory mechanisms of lncRNAs in diverse stage of colon cancer could help us understand the dynamic changes of the immune system in different stages of colon cancer. The present study indicated that stage II was a key immune regulatory stage which there were most dysregulatory immuneCPa genes in colon cancer. Most immuneCPa gene-lncRNA relationships were present in stage III which demonstrated this stage was a critical period for lncRNA to participate in regulation. In short, stage II and III were crucial periods of immunotherapy for colon cancer.

To evaluate if immuneCPa gene-lncRNA pairs could become as stage-specific prognostic biomarkers in colon cancer, we constructed risk scores to identify survival-related immuneCPa gene-lncRNA pairs. In this process, we divided each colon cancer stage patients to two independent sets.

One dataset was used for Cox regression analysis and another held-out dataset was applied for validating the model. This method could help avoiding overfitting. These survival-related immuneCPa gene-lncRNA pairs could become as potential stage-specific prognostic biomarkers for colon cancer.

## Conclusion

Collectively, the present study developed a standardized computational procedures to evaluate immuneCPa gene-lncRNA relationships in diverse stages of colon cancer based on genes and lncRNAs expression. Stage-specific immuneCPa genes and lncRNAs were identified in each colon cancer stage. Dynamic stage-specific immuneCPa gene-lncRNA regulatory networks were constructed and analyzed in colon cancer development. A immuneCPa gene-lncRNA activity profile across different stages revealed that lncRNAs were highly stage-selective in regulating immuneCPa genes in colon cancer. Collectively, our study leads to a novel starting point for future functional explorations, the identification of immune-related biomarkers, and lncRNA-based targeted therapy for colon cancer.

## Abbreviations

immuneCPa gene: immune cell populations-associated gene; lncRNA: long non-coding RNA; TCGA: The Cancer Genome Atlas; PCC: Pearson correlation coefficients.

# Declarations

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## Disclosure of interest

The authors declare that they have no competing interest.

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## Availability of data and material

The data that support the findings of this study are available from the corresponding author upon reasonable request.

## Ethics approval and consent to participate

Not applicable.

## Patient consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

## Consent for publication

Not applicable.

## Authors' contributions

WFJ conceived and designed the experiments, WCJ, YLP, and CH analyzed the data, HYZ and LSL validated the work, and WCJ wrote the manuscript.

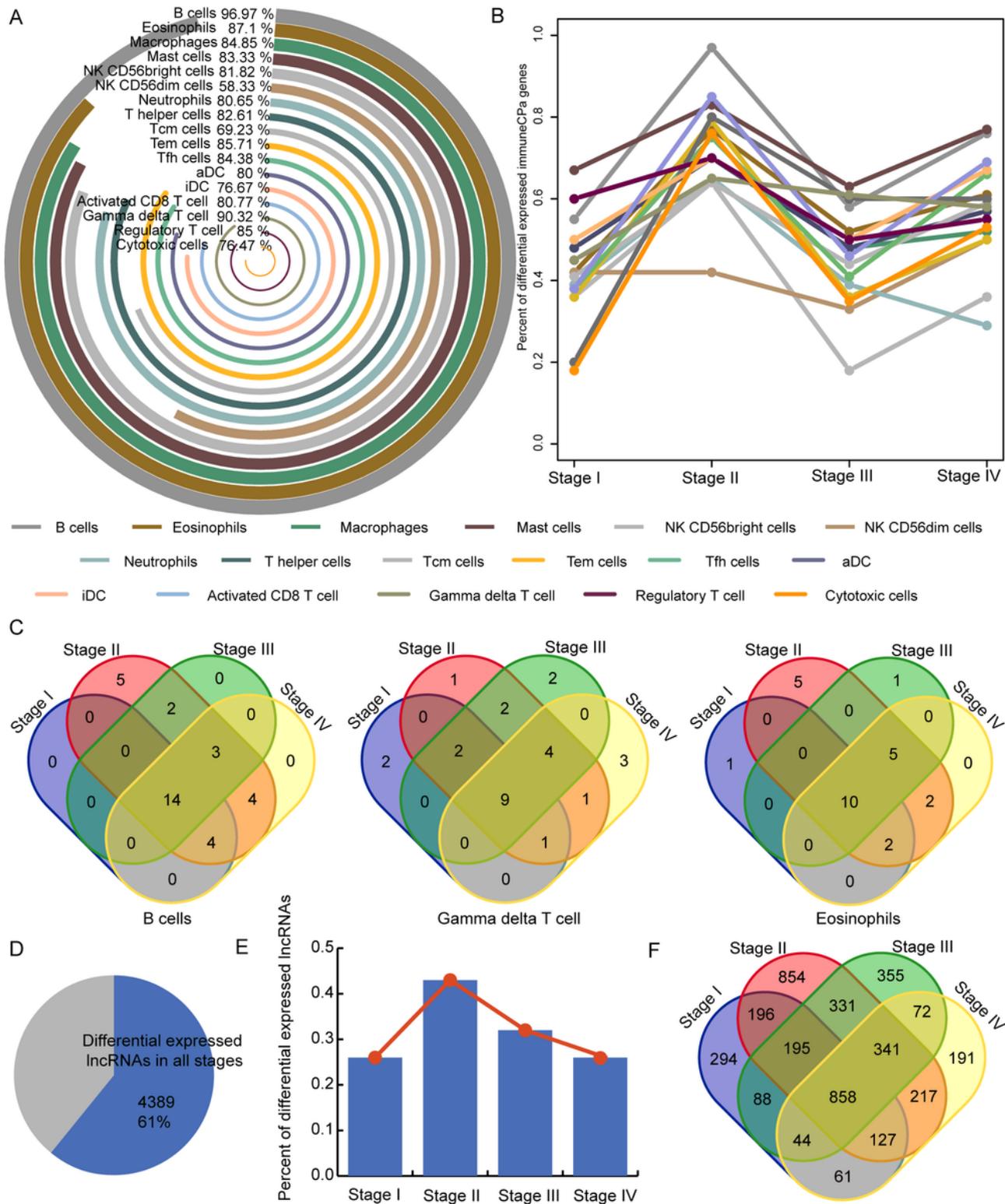
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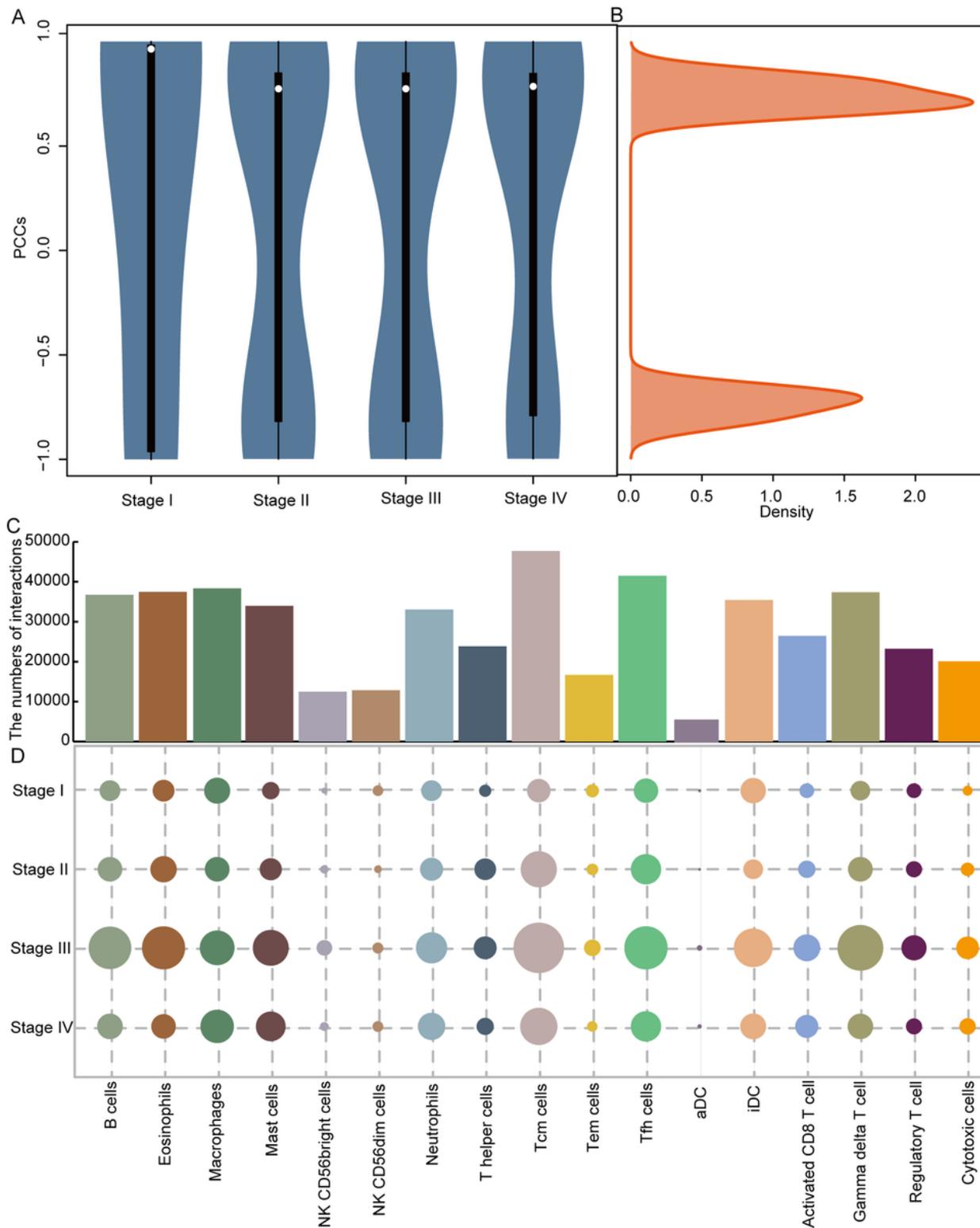
## Figures



**Figure 1**

Stage-specific immuneCPa genes and lncRNAs are identified in colon cancer. (A) The circle diagram shows percent of differential expressed immuneCPa genes for diverse immune cell populations in all colon cancer patients. (B) Line charts show percent of differential expressed immuneCPa genes for diverse immune cell populations in diverse stages. (C) Venn diagrams show the overlap of differential expressed immuneCPa genes about B cells, Gamma delta T cell and Eosinophils in different stages. (D)

Pie chart show percent of differential expressed lncRNAs in all colon cancer patients. (E) Percent of differential expressed immuneCPa lncRNAs for diverse immune cell populations in diverse stages. (F) Venn diagrams show the overlap of differential expressed lncRNAs in different stages.



**Figure 2**

Identification of stage-specific immuneCPa gene-lncRNA regulatory pairs in colon cancer development. (A) Violin plots show PCCs of immuneCPa gene-lncRNA regulatory pairs in diverse stages. (B) The curve

show density distribution for all immuneCPa gene-lncRNA regulatory pairs. (C) The bar plots show numbers of immuneCPa gene-lncRNA regulatory pairs for diverse kinds of immune cell populations. (D) The point plot show numbers of immuneCPa gene-lncRNA regulatory pairs for diverse kinds of immune cell populations in each stage.

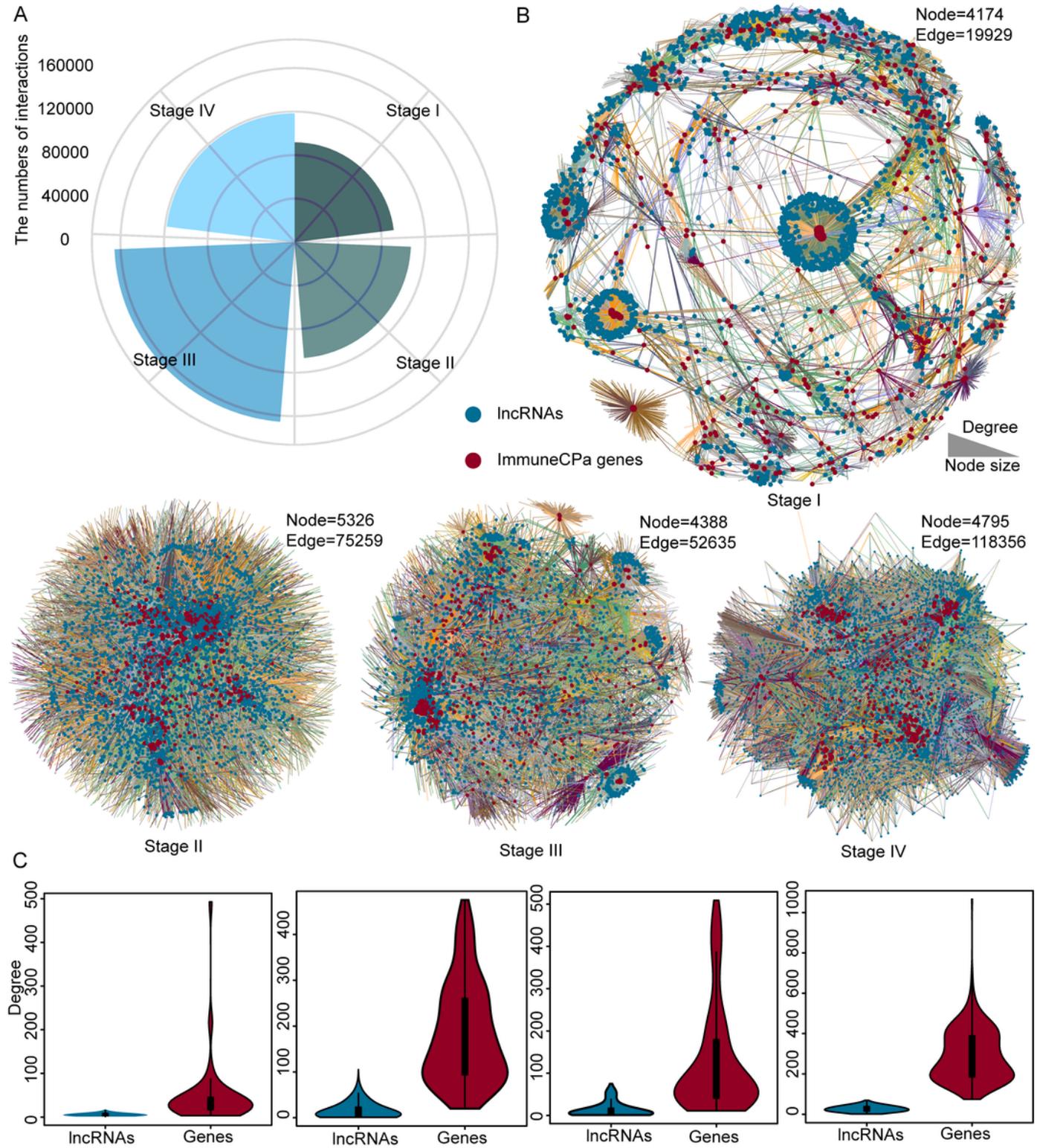


Figure 3

Construction of dynamic stage-specific immuneCPa gene-lncRNA regulatory networks in colon cancer development. (A) The numbers of immuneCPa gene-lncRNA regulatory pairs for networks in diverse stage. (B) Network illustration of the four stage-specific immuneCPa gene-lncRNA regulatory networks. (C) Violin plots show degree of immuneCPa genes and lncRNA in four stages.

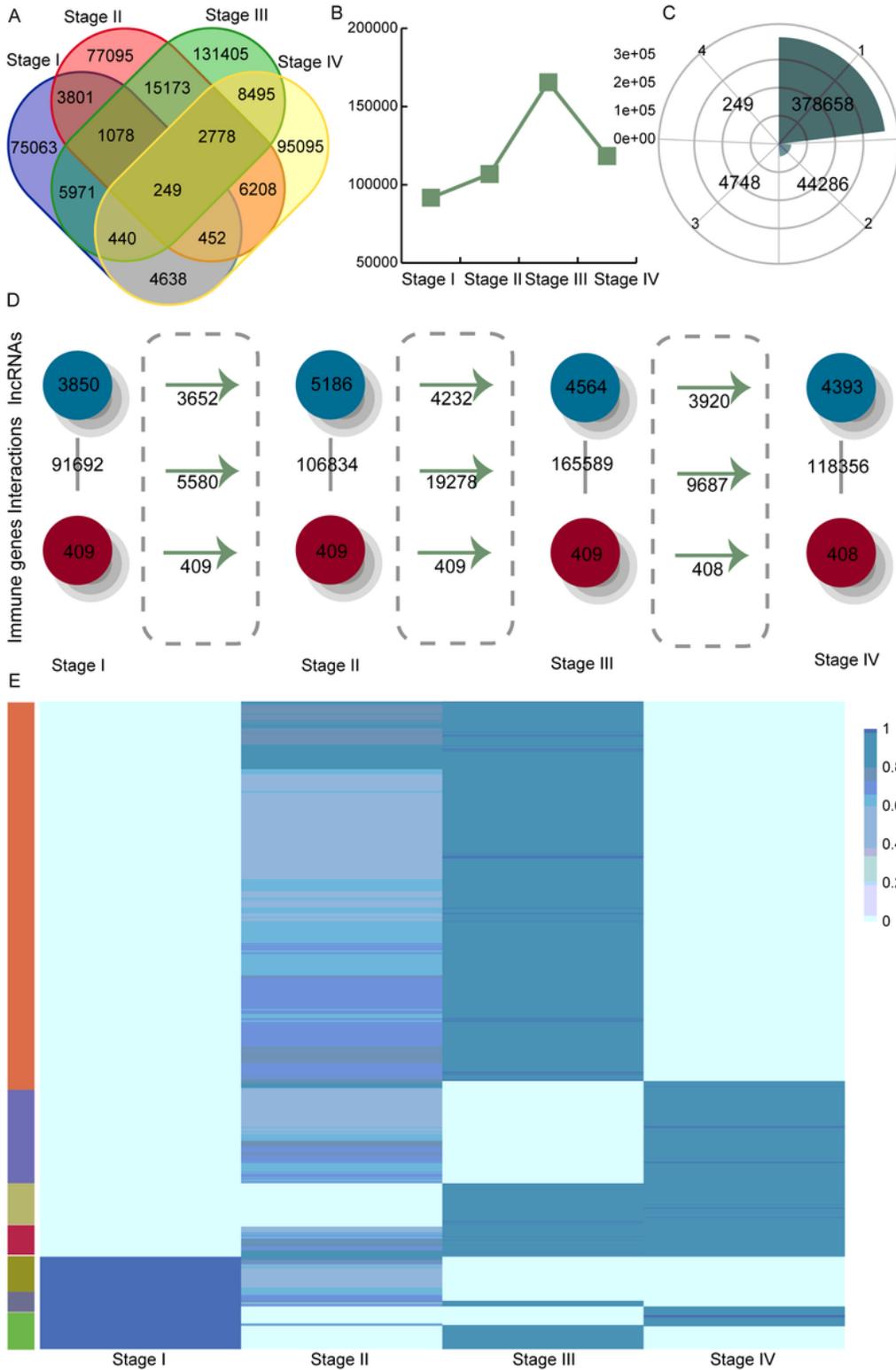
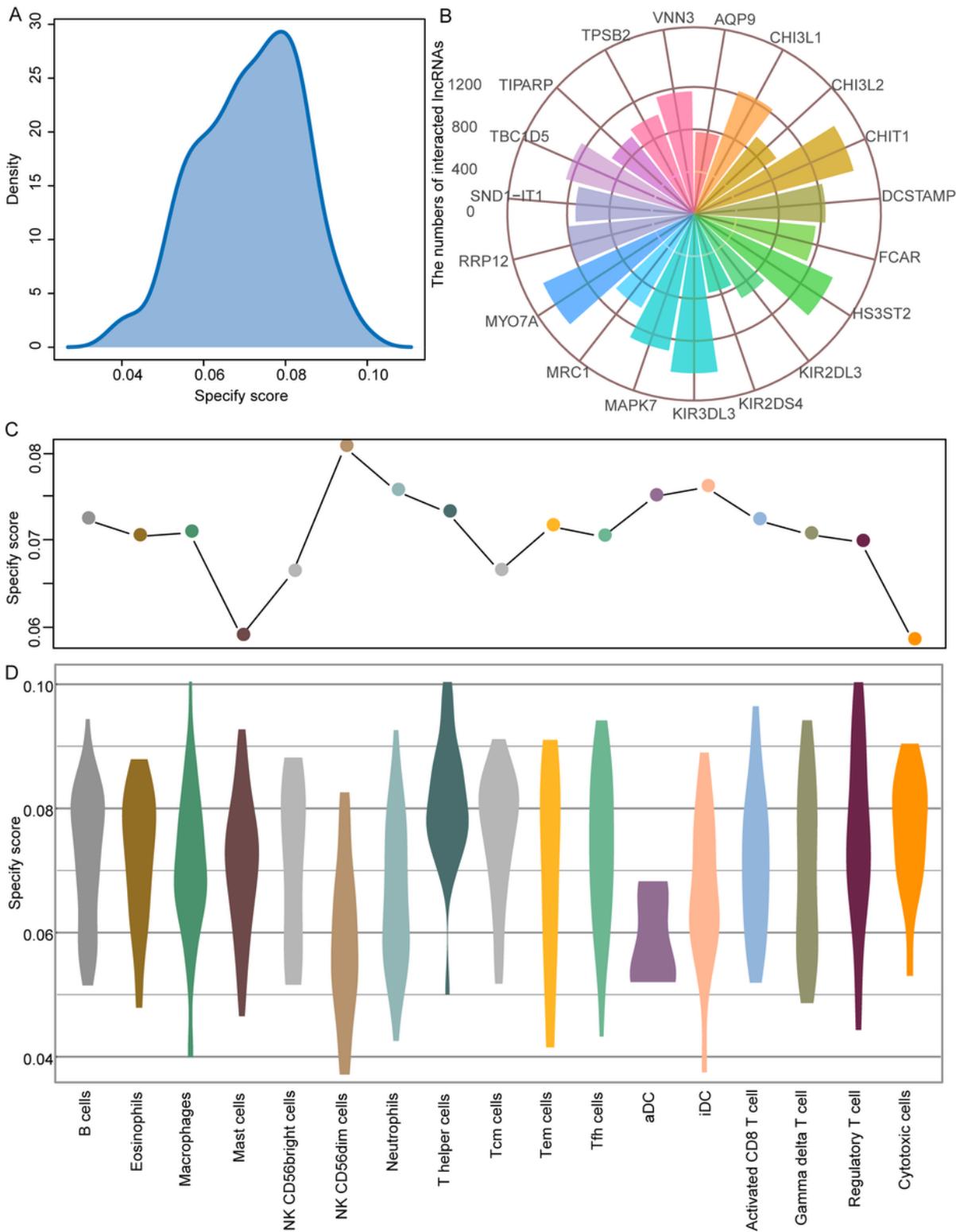


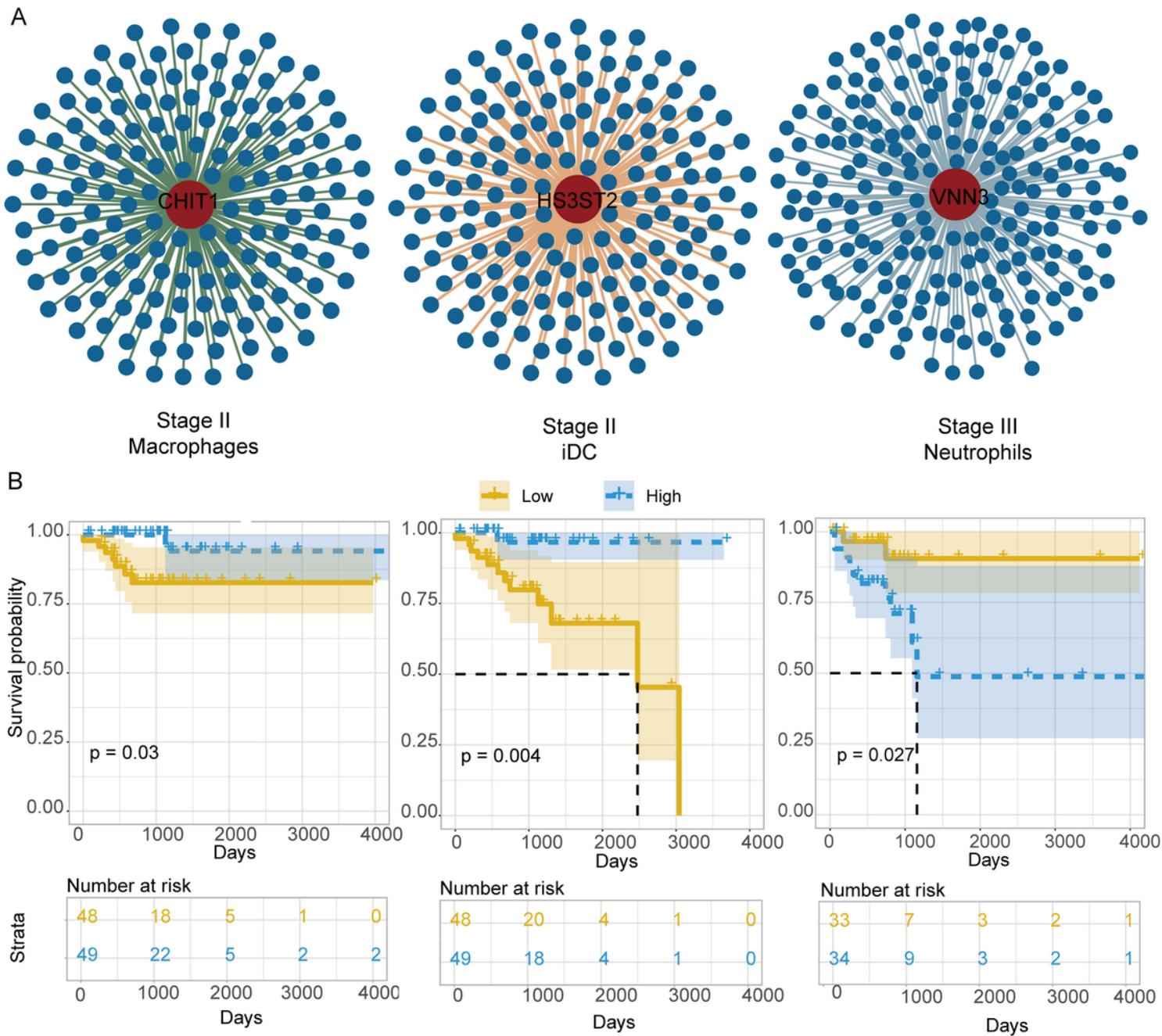
Figure 4

The dynamic activity profiles of immuneCPa gene-lncRNA regulatory pairs. (A) Venn diagrams show the overlap of immuneCPa gene-lncRNA regulatory pairs in different stages. (B) Line chart show percent of immuneCPa gene-lncRNA regulatory pairs in diverse stages. (C) Rose diagram show the numbers of present stages for immuneCPa gene-lncRNA regulatory pairs. (D) The dynamic retaining of immuneCPa gene-lncRNA nodes and regulations across different stages of colon cancer. (E) The activity profile for immuneCPa gene-lncRNA relationships across different stages of colon cancer. The PCCs for immuneCPa gene-lncRNA pairs in each stage of colon cancer were used as activity scores. Clustering analysis revealed that different groups of immuneCPa gene-lncRNA relationships were apparently activated in one or more stages of colon cancer.



**Figure 5**

Specificity score evaluates the specificity of immuneCPa gene-lncRNA relationships in colon cancer development. (A) The curve shows density distribution of specificity scores of immuneCPa gene-lncRNA relationships in colon cancer. (B) The numbers of interacted lncRNAs for immuneCPa genes with lowest specificity scores. (C) Mean specificity scores of multiple kinds of immune cell populations in colon cancers. (D) Specificity scores distribution of multiple kinds of immune cell populations in colon cancers.



**Figure 6**

[No caption was provided with this version.]

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Equation1.pdf](#)