Research Paper

POC1A acts as a promising prognostic biomarker associated with high tumor immune cell infiltration in gastric cancer

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ABSTRACT

The effect of POC1 centriolar protein A (POC1A) on gastric cancer (GC) has not been clearly defined. In this study, POC1A expression and clinical information in patients with GC were analyzed. Multiple databases were used to investigate the genes that were co-expressed with POC1A and genes whose changes co-occurred with genetic alternations of POC1A. Moreover, the TISIDB and TIMER databases were used to analyze immune infiltration. The GSE54129 GC dataset and LASSO regression model (tumor vs. normal) were employed, and 6 significant differentially expressed genes (LAMP5, CEBPB, ARMC9, PAOX, VMP1, POC1A) were identified. POC1A was selected for its high expression in adjacent tissues, which was confirmed with IHC. High POC1A expression was related to better overall and recurrence-free survival. GO and KEGG analyses demonstrated that POC1A may regulate the cell cycle, DNA replication and cell growth. Furthermore, POC1A was found to be correlated with immune infiltration levels in GC according to the TISIDB and TIMER databases. These findings indicate that POC1A acts as a tumor suppressor in GC by regulating the cell cycle and cell growth. In addition, POC1A preferentially regulates the immune infiltration of GC via several immune genes. However, the specific mechanism requires further study.

INTRODUCTION

Gastric cancer (GC) is still one of the leading causes of cancer-related death worldwide [1]. Even though there are multiple surgical treatment options for GC, approximately 679,100 new cases and 498,000 new deaths pre-year demonstrated that the outcomes of GC are remain not optimistic [2, 3]. With further investigation was applied to the occurrence and progress of GC, the importance of underlying molecular mechanism attracted the attention of scientists. To improve the diagnosis and therapeutic response of GC,

alternative biomarkers with higher accuracy and specificity are urgently needed.

With the advent of advanced techniques, a great number of proteins have been discovered to have an impact on GC. For instance, Wang et al. [4] found that Hsp90ab1, a member of the heat shock protein family, was found to be overexpressed and correlated with poor prognosis, proliferation and invasion in GC. Furthermore, MICAL-L2 was reported to promote the stability of EGFR to increase GC cell migration [5]. However, the known proteins that regulate GC development are still limited,

which suggests that more innovative proteins should be discovered and further studied.

As a protein located in the centrosome, spindle apparatus and microtubule, POC1A (POC1 centriolar protein homolog A), which is also called WDR51A, is characterized by a conserved structure that consists of a 7-bladed β-propeller formed by an N-terminal WD40 domain and a C-terminal coiled-coil. In biological processes, POC1A plays an important role in the formation and steady state of centrioles, as well as in ciliogenesis. In addition, early steps of centriole duplication and later steps of centriole length control are also functions of POC1A [6, 7]. Many studies have shown that POC1A is associated with short stature, onychodysplasia, facial dysmorphism and hypo-trichosis (SOFT) syndrome, which is related to abnormalities in cell mitosis [7, 8]. All these studies indicate that POC1A may play a critical role in cell proliferation by regulating mitosis. Furthermore, by RT-qPCR and immunohistochemistry staining, we discovered that POC1A, which is highly expressed in adjacent tissues of GC, acted as a tumor suppressor in GC. In recent decades, several studies have revealed that mitosis is an important factor in GC progression. Li et al. [9] discovered that KIF23 participates in the progression of cell mitosis and that its depletion inhibits cell proliferation in GC. Moreover, similar results were revealed by Zhang et al. [10]: PRKDC regulates the DNA damage response to affect proliferation induced by mitosis in GC. As demonstrated by several results above, we suspect that POC1A impacts the development of GC by regulating mitosis, which has never been reported. Therefore, our study aimed to investigate the regulation of POC1A on the progression of GC.

Immune infiltration was one of the several factors inducing GC. The chronic immune inflammation could lead to microbial infections, which may provide suitable environment for helicobacter pylori so that induce the development of GC [11]. Besides, as Chen et al. suggested, high destiny of T-bet+ tumor infiltrating lymphocytes (TILs) induced better survival in gastric cancer including overall survival (OS) and disease-free survival (DFS), which demonstrated that TILs could significantly impact the survival of gastric cancer [12]. Furthermore, Chen et al. indicated that over expression of the chemokine receptor CXCR3 could lead to better prognosis and higher immune infiltration levels including dendritic cells and CD8+ cells [13]. Several researches showed that the immune infiltration induced by immune signatures was significantly correlated with the prognosis of GC. Therefore, we focused on the immune infiltration in GC to investigate the correlated signatures regulating GC progress. In the progression of GC, endogenous genes and the exogenous tumor microenvironment (TME) are both significant factors [14–17], which indicates that investigating the relationship between biomarkers and the TME is meaningful. As components of the TME, tumor-infiltrating lymphocytes (TILs) and tumor-associated macrophages (TAMs) are also reported to have an important impact on the prognosis of GC [18, 19]. Therefore, investigation of the impact of different immune cells and immunotherapies on GC is extremely meaningful, and the immune microenvironment attracted our attention for exploring the function of POC1A.

RESULTS

Acquisition of differentially expressed genes

To determine significantly altered genes, a GEO dataset (GSE54129) was selected (Supplementary Figure 1A, 1B), and the Limma [20] R package was used for differential expression analysis ($|logFC|>2$, $p<0.05$). Thereby, we discovered 164 upregulated genes and 217 downregulated genes (Figure 1A, 1B). After using LASSO regression analysis, we established a statistical model by cross validation. As misclassification error was regarded as the minimized target parameter, we acquired 6 significantly differentially expressed genes, Lysosomal Associated Membrane Protein Family Member 5 (LAMP5), CCAAT Enhancer Binding Protein Beta (CEBPB), Armadillo Repeat Containing 9 (ARMC9), Polyamine Oxidase (PAOX), Vacuole Membrane Protein 1 (VMP1), POC1 centriolar protein A (POC1A) for further research (Figure 1C). In these 6 genes, only POC1A and PAOX were demonstrated to have down-regulated expression and other 4 genes were up-regulated.

The expression of genes in GC tissues

To discover the most significant gene, RT-qPCR was used to evaluate the mRNA expression of 6 genes in 101 pairs of tumor and normal tissues. As scatter plots showed, POC1A had the most significant difference in expression in GC (p=0.0001) (Figure 2A–2F). In addition, as exhibited by the trend in Figure 2G–2L, POC1A was the most obviously downregulated gene. Furthermore, we analyzed POC1A expression in GSE54129 and further revealed significantly high POC1A mRNA expression in adjacent tissues (p<0.05) (Figure 2M). In addition, as shown in Figure 2N, POC1A protein expression was higher in normal tissues than in tumor tissues from the same patient. All the results above demonstrated that POC1A was not only corresponded with trend in bioinformatic analysis but also highly expressed in adjacent tissues compared with GC tissues.

Correlation between POC1A expression and clinico-pathological features

For further investigation, we analyzed the relationship between POC1A expression and several clinic-pathological characteristics from 3 groups. In the RT-qPCR cohort, we identified a clinically significant cut-off point (0.02) for POC1A mRNA expression. Patients were divided into 2 groups based on high POC1A expression (-2.13±4.46, n=23) and low POC1A expression (-7.75±1.23, n=78). POC1A expression was inversely correlated with tumor size (p=0.043) and lymph node metastasis (p=0.001) in GC patients (Supplementary Table 2). To acquire more reliable data, patients from the immunohistochemistry group were analyzed, and multiple factors, including N stage (p=0.04), tumor size (p=0.05) and lymphatic or nervous invasion (p=0.036), were found to have a negative correlation with POC1A protein expression (Supplementary Table 3). Moreover, with the same method applied to define POC1A high/low expression, the results of the correlation analysis in GSE84433 demonstrated that POC1A mRNA expression was negatively correlated with T stage (p=0.002) and lymph node status (p=0.008) (Supplementary Table 4).

POC1A expression is correlated with lymphatic metastasis and GC

Considering the results above, lymph node status was suggested to have a significant correlation with POC1A expression among the 3 groups, which demonstrated that POC1A could be regarded as a meaningful factor impacting lymph node metastasis in GC. Therefore, the
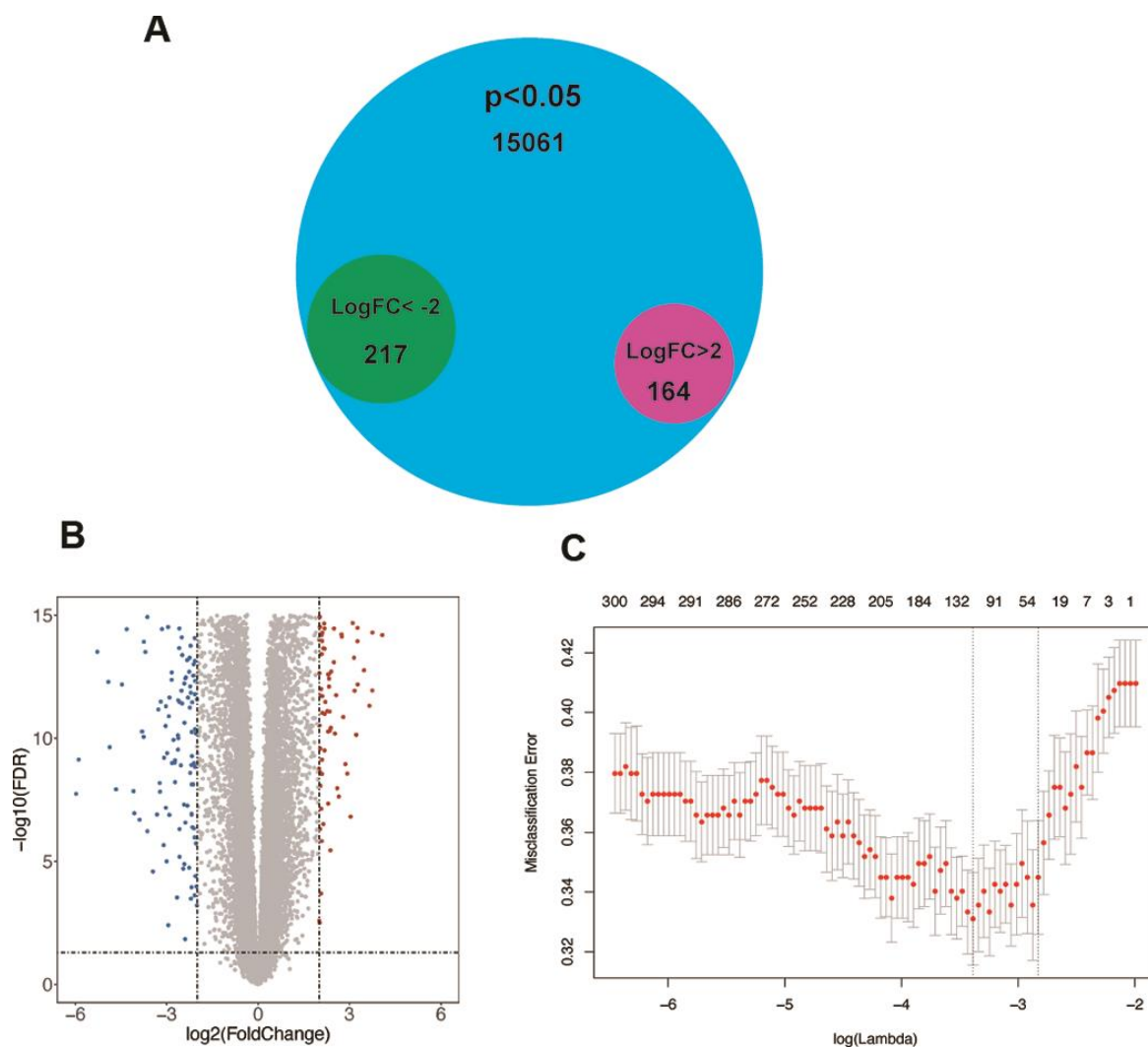

Figure 1. The screening of significant genes. (**A**–**B**) Venn plot and volcano plot demonstrating the expression of 164 upregulated genes and 217 downregulated genes in GSE54129 after differential expression analysis. (**C**) Six genes with the lowest misclassification error remained after LASSO regression analysis.
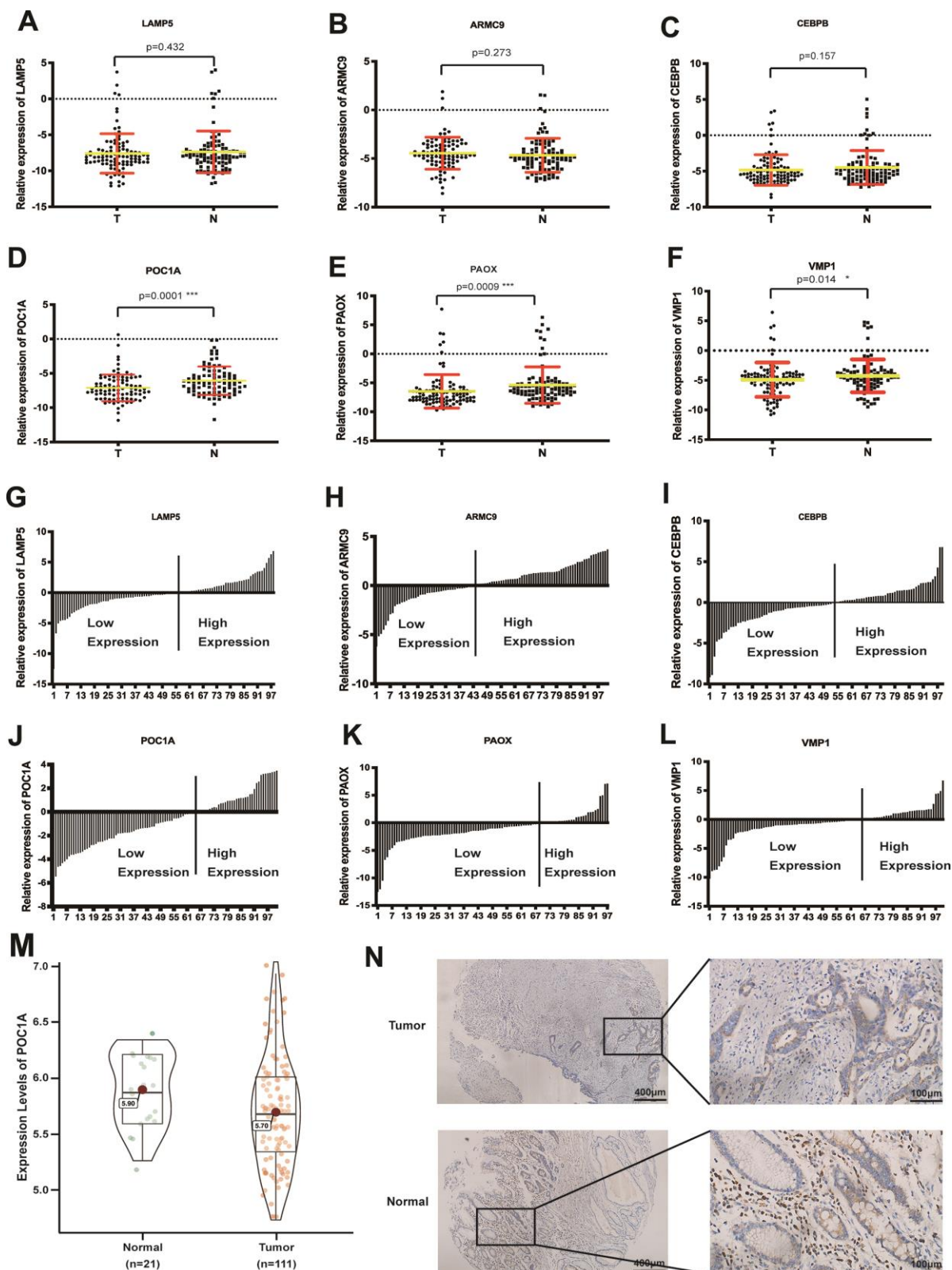
Figure 2. POC1A was a significant anti-tumor gene in gastric cancer. (**A**–**F**) Comparison of mRNA expression of 6 significant genes (LAMP5, CEBPB, ARMC9, POC1A, PAOX, VMP1) from LASSO regression analysis in tumor and adjacent tissues (n=101). (**G**–**L**). Ratio the mRNA expression of 6 genes comparing $2^{(-CT)}$ in tumor tissues with $2^{(-CT)}$ in matched normal tissues (n=101). (**M**) mRNA expression of POC1A in tumor (n=111) tissues was significantly higher than normal (n=21) tissues from GSE54129. (**N**) Intensity of immunohistochemistry staining of POC1A in normal tissue was higher than intensity in tumor tissue from the same patient.

GSE84433 dataset was selected, and patients were divided according to the presence of lymph node metastasis (N0, N1-3). The results suggested that patients in the N0 group, who had no lymph node metastasis, had higher POC1A mRNA expression than patients in the N1-3 group (Figure 3A). Furthermore, similar results were acquired from immunohistochemistry staining of POC1A protein (Figure 3B). High POC1A expression in patients without lymph node metastasis indicated that POC1A may act as a suppressor of lymph node metastasis in GC.

Prognostic potential of POC1A in GC

To understand the role of POC1A in survival outcomes, we analyzed the impact of POC1A on prognosis in GC. In the overall survival analysis of multiple cohorts (GSE15459, GSE84433 and TCGA-STAD) (p=0.028, p=0.004, p=0.007), patients in the high POC1A expression group tended to have better overall survival than those in the low POC1A expression group (Figure 4A–4C), as did patients in our department (FJMUUH, Figure 4D). In addition, Kaplan-Meier analysis of immunohistochemistry results, which included 83 patients with OS data, revealed that high POC1A

protein expression (IHS>=3, n=14) was related to better overall survival than low POC1A expression in GC (IHS<3, n=69) (Figure 4E).

Furthermore, the same method was adopted for analyzing recurrence-free survival (RFS). We applied another GEO dataset (GSE26253) for further investigation and found that degradation of POC1A was significantly related to poor RFS in GC (Figure 4F). Thus, all survival analyses indicated that POC1A acts as a tumor suppressor in GC.

Genes coexpressed with POC1A in STAD

To determine the biological role of POC1A, we selected LinkedOmics to examine genes coexpressed with POC1A in STAD. As the results in Figure 5A suggest, 261 genes (red dots) were demonstrated to have a significant strong positive correlation with POC1A, whereas 355 genes (blue dots) had a significant negative correlation with POC1A (false discovery rate, FDR<0.01, |Spearman's correlation|>0.5). The top 50 significant genes that were positively and negatively correlated with POC1A are shown as heat maps (Figure 5B, 5C). Significant Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses
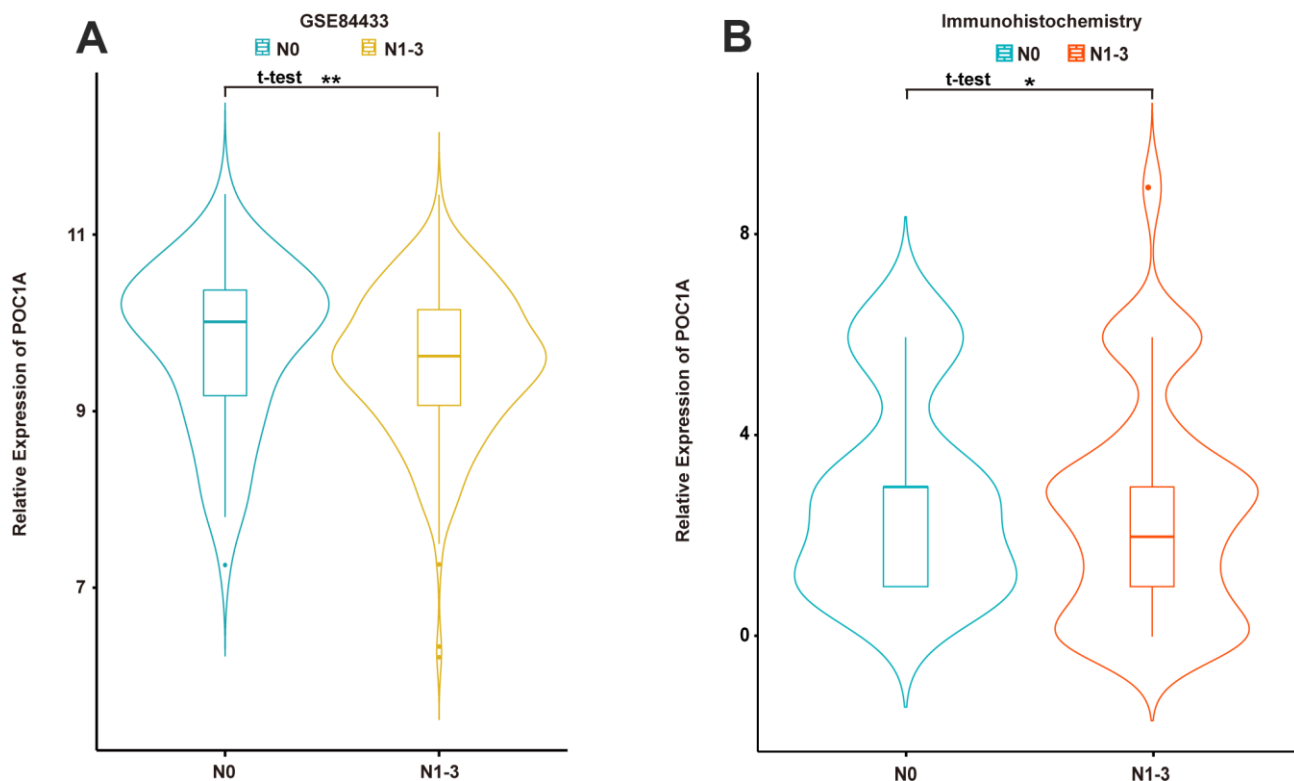

Figure 3. POC1A was significantly correlated with lymph node metastasis. (**A**, **B**) Expression of POC1A in patients with/without lymph node metastasis from GSE84433 analysis (n=357) and immunohistochemistry analysis (n=91).

were performed (Figure 5D, Supplementary Figure 1C–1E). The KEGG results showed that the cell cycle, DNA replication, p53 signaling pathway, etc. (Figure 5D and Supplementary Table 5) were suggested to be enriched pathways. The results of GO analysis indicated that chromosome segregation, DNA replication, cell cycle DNA replication, etc. (Supplementary Figure 1C–1E and Supplementary Table 6) were involved. Furthermore, we
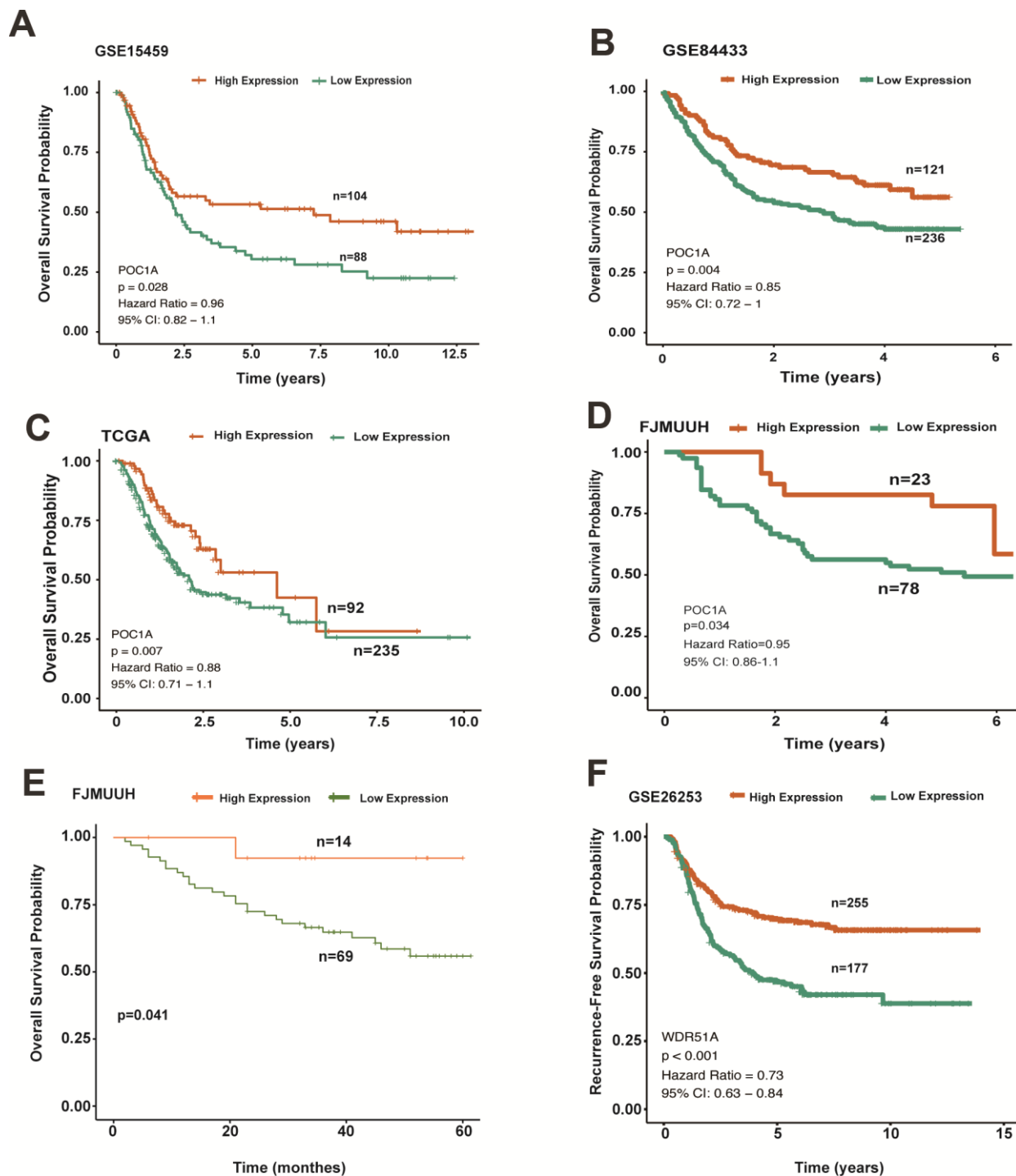

Figure 4. High POC1A expression significantly induced better survival outcomes. (**A–C**) Survival curves of OS in 3 cohorts (GSE15459, GSE84433, STAD of TCGA) (cut-off: 7.97,n=192, p=0.028; cut-off: 9.3, n=357, p=0.004; cut-off: 9.45, n=327, p=0.007) demonstrated that high POC1A mRNA expression led to better overall survival in GC. (**D**) High POC1A expression was significantly correlated with better overall survival (OS) prognosis in the RT-qPCR cohort (n=101, p=0.034). (**E**) Low POC1A protein expression led to poor OS prognosis in the immunohistochemistry cohort (n=83). (**F**) RFS curves of patients from GSE26253 (cut-off: 7.67, n= 432).

used the STRING database to analyze these strongly coexpressed genes of POC1A and acquired the protein-protein interaction (PPI) network (Supplementary Figure 1F). All these results demonstrated that POC1A may affect the cell cycle and DNA replication as a part of the centrosome.

Changes in gene expression cooccurring with POC1A deletion in STAD

Considering that genomic alterations of POC1A are pathogenetic, copy number alteration (CNA) of POC1A attracted our attention. With the cBioPortal database, we discovered that POC1A deletion was significantly correlated with its decreased mRNA expression in STAD (Figure 6A, 6B). Thus, we further examined genes with changes occurring with POC1A deletion in STAD, revealing 212 genes (Supplementary Table 7). Then, we performed KEGG pathway analysis of these genes and found that glycine, serine and threonine metabolism and some other pathways were enriched (Figure 6C, Supplementary Table 8). In addition, analysis of GO enrichment demonstrated that these genes primarily induce cell growth and regulate of cell growth (Figure 6D, Supplementary Table 9), which corresponded to the result that POC1A expression was correlated with tumor size (Supplementary Tables 2, 3).

The impact of POC1A in tumor-infiltrating lymphocytes

Tumor-infiltrating lymphocytes (TILs) are an important factor affecting lymph node status in several cancers [21]. As POC1A can impact lymph node metastasis, immune infiltration attracted our attention. According to the TIMER database, multiple types of copy number alterations (CNAs) of POC1A, especially the POC1A deletion, had significant correlations with the infiltration levels of several immune cells in stomach adenocarcinoma (Figure 7A and Supplementary Table 10). The results above indicated that POC1A deletion in STAD induced low immune infiltration of multiple immune cells. However, the amplification of POC1A also induced the lower immune infiltration level, which need further discussion. Then, we selected and downloaded multiple immune genes from the TIMER database to study immune infiltrating marker sets and performed correlation analysis for further investigation (Supplementary Table 11). As shown in Figure 7B, after adjusting for purity, 7 genes (CCNB1, ESCO2, EXO1, KIF11, NUF2, PRC1, CCL14) were suggested to have a strong and significant relationship with POC1A expression ($|r|>0.5$, $p<0.001$). We further verified the markers above through GEPIA, whose results were similar (Figure 7C). Among the 7 genes, 6 genes (CCNB1, ESCO2, EXO1, KIF11, NUF2, PRC1) were

expressed in CD4+ T cells, and only CCL14 was featured in macrophages, which indicated that CD4+ T cells may play an important role in the impact of POC1A on immune infiltration.

The potential of POC1A in immune therapy

While multiple immune markers were found to be correlated with POC1A expression, enough attention has not been paid to the most significantly related genes. Therefore, we further employed the TISIDB database [22] to further understand POC1A and other biomarkers in immune therapy. By dividing GC into 6 groups (wound healing, IFN-gamma dominant, inflammatory, lymphocyte depleted, immunologically quiet, TGF-b dominant) [23], we found that POC1A expression was the highest in the IFN-gamma dominant subtype and the lowest in the inflammatory subtype (Figure 7D). Furthermore, we analyzed the correlation through TISIDB and stetted filter standard ($|r|>0.1$ and $p<0.05$). In total, 12 MHC molecules, 16 immunoinhibitors and 30 immunostimulators that had a significant correlation with POC1A expression in STAD were discovered (Supplementary Figures 2–4). After defining the thresholds as $|r|>0.5$ and $p<0.01$ to acquire effective immuno-modulators, only ENTPD1, an MHC molecule, was found to have a strong correlation with POC1A expression (Figure 7E). This demonstrated that POC1A has the potential to impact the immune therapy induced by ENTPD1 in STAD. All these results indicated that POC1A has the potential to regulate immune infiltration and the response to immunotherapy, but these hypotheses need to be verified by further studies.

DISCUSSION

As the core component of the centrosome, POC1A is regarded as a factor that regulates the cell cycle; mutation of POC1A can lead to abnormal mitotic mechanics, multipolar spindles and impaired ciliogenesis which can induce short stature, onychodysplasia, facial dysmorphism, and hypo-trichosis (SOFT) [24, 25]. Many articles have reported the function of the centrosome in cancer, and centrosome regulation is a significant factor in the development of cancer processes. Arunabha Bose et al. [26] revealed that centrosome amplification is a significant characteristic in multiple cancer types and contributes to both tumor initiation and tumor progression. As Wang et al. [27] discovered, SCL/TAL1 interrupting locus (STIL) induced tumor progression by promoting centriolar replication and cell cycle progression by regulating the mitotic centrosome. Therefore, we wandered whether POC1A, as a centrosome regulator, could act as a sensitive biomarker in GC. Herein, we investigated POC1A expression and
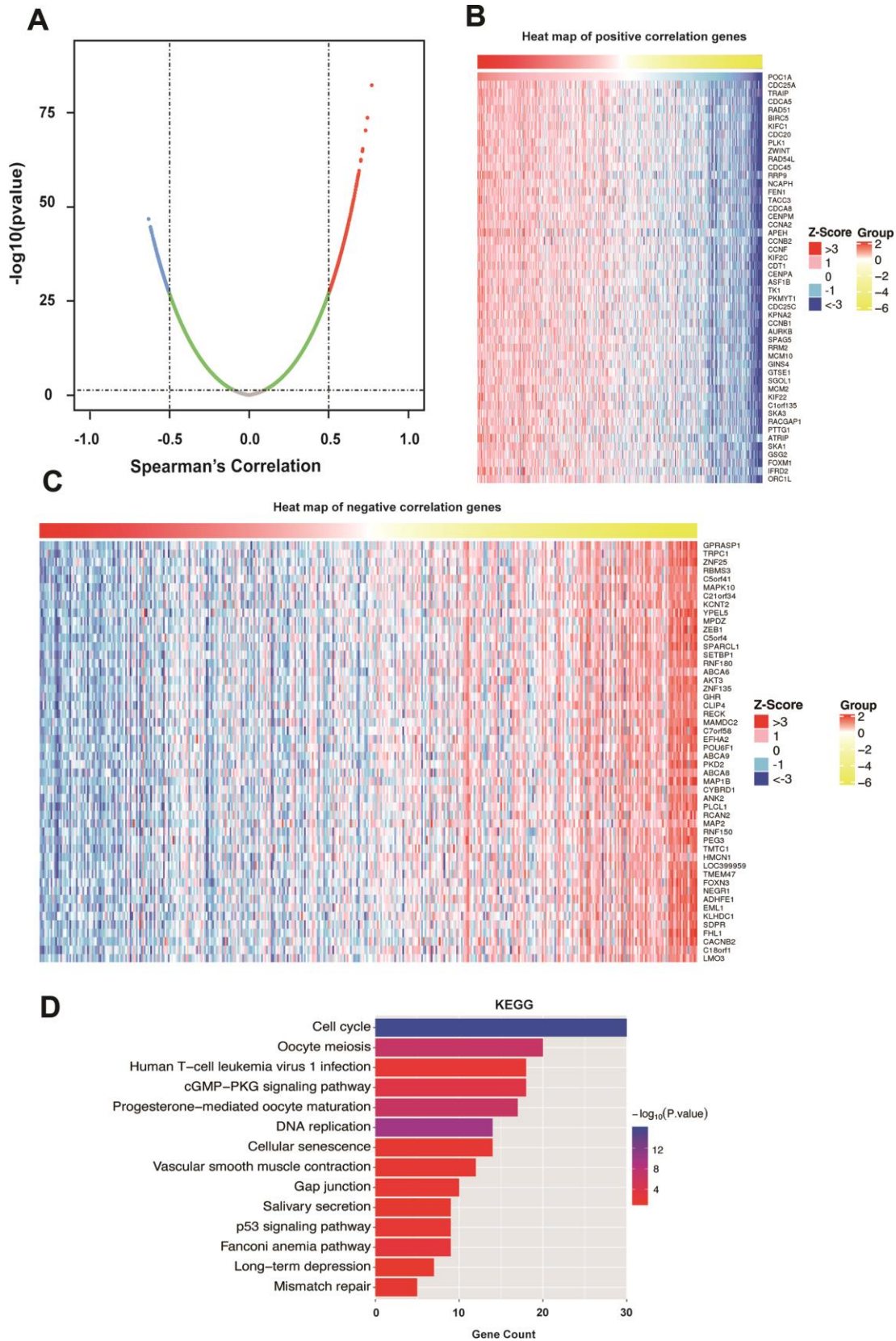
Figure 5. Coexpressed genes of POC1A. (**A**) Strongly coexpressed genes of POC1A identified by Spearman's test in GC. (**B–C**) Heat maps exhibited the top 50 genes that have positive and negative correlations with POC1A in GC. Red represents genes with strong positive correlation, and blue indicates genes with strong negative correlation. (**D**) KEGG pathway enrichment of genes coexpressed with POC1A.
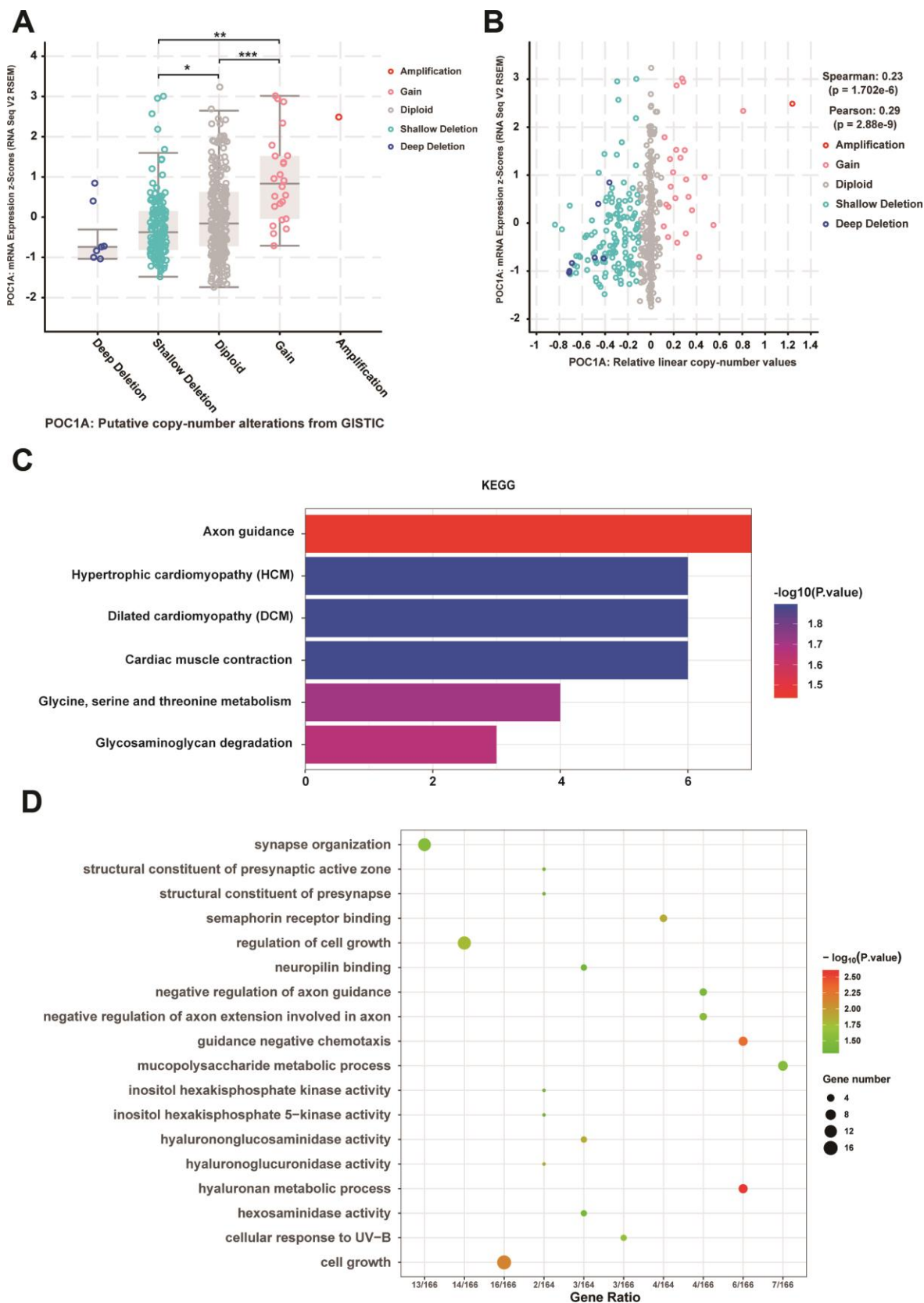
Figure 6. CNV co-occurrence profiles of POC1A in STAD. (A–B) POC1A deletion was significantly correlated with low POC1A expression (Spearman: r=0.23, p=8.129e-6; Pearson: r=0.29, p=8.53e-9), which indicated that POC1A deletion may act as a significant pathogenic factor. (C–D) GO and KEGG analysis of POC1A co-occurrence genes (p<0.05).
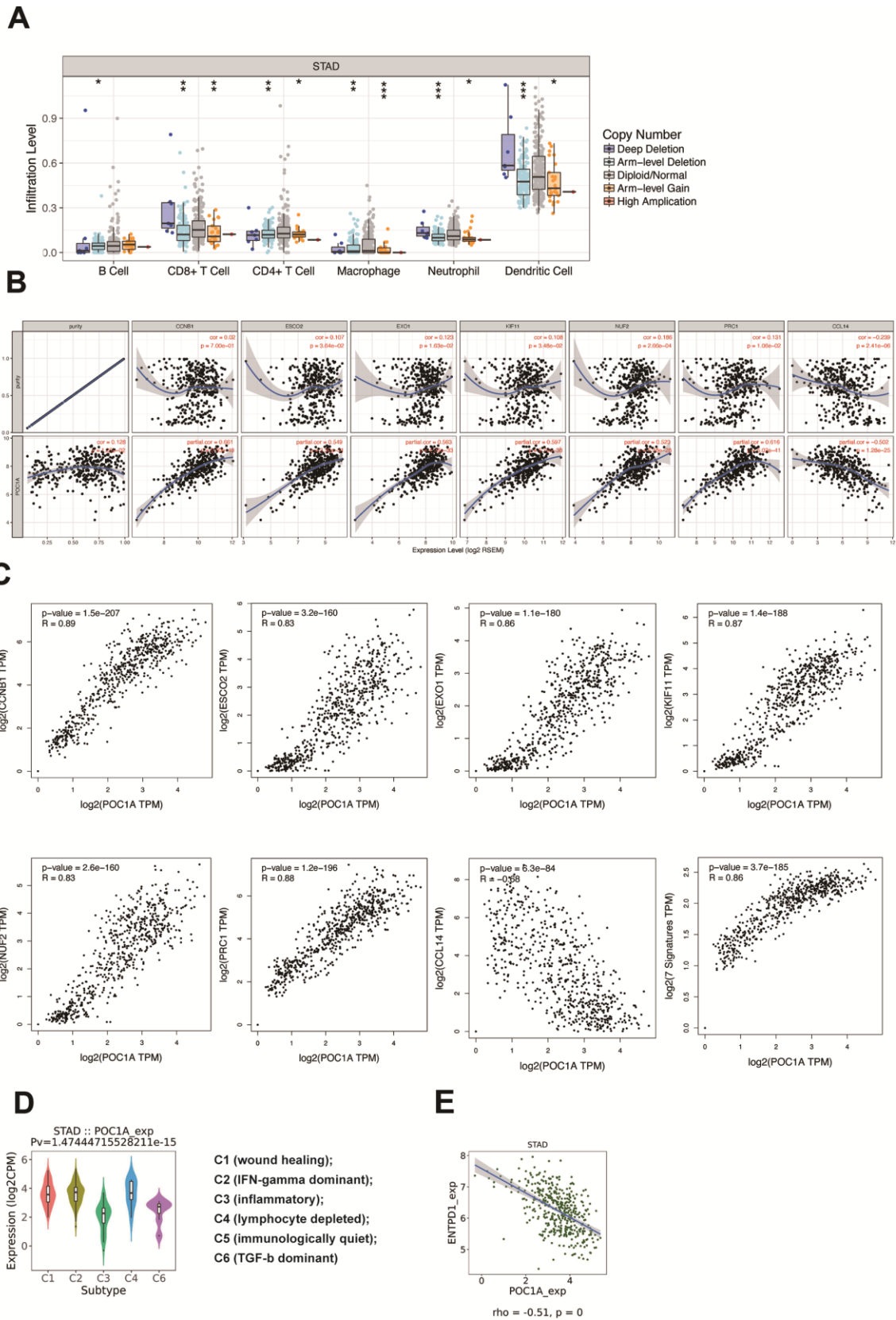
Figure 7. POC1A could impact immune infiltration significantly. (**A**) Copy number alteration (CNA) of POC1A was significantly correlated with immune infiltration levels of several immune cell types in GC. Deletion was found to have a highly reliable significant correlation with the infiltration level in neutrophils and dendritic cells (p<0.001), and arm-level gain was related to the infiltration level of macrophages with high reliability (p<0.001). (**B**) In the TIMER database, scatterplots of strong (|r|>0.5) and significant (p<0.001) correlations

between POC1A expression and 7 immune genes (CCNB1, ESCO2, EXO1, KIF11, NUF2, PRC1, CCL14) after adjusting for purity. (**C**) In the GEPIA database, scatterplots of correlations between POC1A expression and 7 immune genes from TIMER. (**D**) Expression of POC1A in different immune subtypes of GC. (**E**) ENTPD1 had a strong significant correlation with POC1A (r=-0.51, p<0.001).

its effects on prognosis in GC. To our knowledge, this is the first study to highlight POC1A not only as a tumor suppressor but also as an immune-infiltrating protein in GC.

First, we identified several differentially expressed genes from a GEO dataset (GSE54129) by applying the Limma R package, and LASSO regression analysis revealed 6 significant genes. Then, RT-qPCR was applied to analyze these genes, and POC1A was selected for its significantly high expression in adjacent tissues compared to cancer tissues. As the results of RT-qPCR, immunohistochemistry and several GEO datasets show, not only the mRNA expression but also the protein expression of POC1A is higher in cancer-adjacent tissues than in tumor tissues. In addition, we also found that POC1A expression was significantly correlated with tumor size and lymphatic metastasis in 3 cohorts (Supplementary Table 2–4). Moreover, after Kaplan-Meier survival analysis, high overall survival and high recurrence-free survival were shown to correlate with high POC1A expression in GC, which indicates that POC1A produces an antitumor effect in GC. As POC1A could impact prognosis of GC patients, we further analyzed POC1A in different stages. However, there were no significant difference among different stages in STAD. Therefore, we further employed TISIDB to investigate the correlation between POC1A and different stages, which demonstrated that POC1A expression was decreasing with the development of TNM stages in STAD and the correlation was significant (rho=-0.1, p=0.02) (Supplementary Fig5 a). Thus, we hold the view that POC1A has the potential to be a diagnostic and prognostic marker.

In addition, to further investigate the biological function of POC1A in the development of GC, we discovered multiple genes coexpression with POC1A genes, which were further subjected to GO and KEGG enrichment analyses. Our results show that POC1A may produce effects through these factors to regulate the cell cycle and DNA replication. Then, a protein-protein inter-action network of these coexpressed genes was established to provide a basis for investigating the mechanism underlying the antitumor effect of POC1A.

However, analysis of coexpressed genes cannot explain the impact of POC1A on tumor volume and lymphocyte metastasis. Therefore, we focused on genomic alterations. It has been reported that copy number

alteration (CNA) can lead to abnormal gene expression, which induces several genetic diseases. Therefore, as the majority of studies of POC1A reported its gene alteration could lead to genetic disease [7, 8, 24, 25], we verified the positive relationship between POC1A CNA and POC1A expression and investigated changes in gene expression that occurred with POC1A CNA in STAD. A total of 212 genes had expression changes that cooccurred with POC1A deletion. The GO and KEGG analyses of these genes suggested that POC1A deletion may induce cell growth, which is consistent with our results that POC1A expression is significantly correlated with tumor size.

Another aspect of our study is that POC1A is correlated with immune infiltration, which is a significant factor in the microenvironment of GC. As many articles have reported, a great deal of immune cells, including lymphocytes and macrophages, induce an immune microenvironment of GC, which consequently affects the prognosis of GC patients [18, 28–30]. In addition, the association between increased expression of POC1A and the prognosis of patients who have GC with lymph node metastasis indicates that POC1A can be regarded as a significant factor in tumor metastatic progression. It has also been reported that lymphocyte metastasis may also be impacted by tumor-infiltrating lymphocytes [21]. Thus, we analyzed the relationship between POC1A genomic alterations and immune infiltration in GC. However, as POC1A has no significant correlations with tumor purity, we considered that POC1A mutation may lead to genetic diseases [8, 25, 27], so we wanted to determine whether the genomic alteration of POC1A was correlated with immune infiltration levels. With the TIMER database, our results first demonstrated that deletion of POC1A copy number alteration (CNA) had a meaningful correlation with the immune infiltration level of multiple immune cell types, which provides a new foundation and direction for further research of POC1A in GC. We employed the cBioPortal database to measure POC1A mRNA expression in different CNA types and revealed that the CNA of POC1A is positively related to POC1A mRNA expression. As shown above, POC1A deletion significantly reduced immune infiltration, which indicated that the anticancer effect of POC1A in GC is regulated by immune infiltration of the microenvironment via genomic alteration. Besides, we found that amplification also induced the lower immune infiltration level. Through the further investigation, we found that the correlation of POC1A among different subtypes of the same

immune cell was different. For example, POC1A was positively correlated with macrophage M0 and M1 in multiple databases while it was negatively correlated with macrophage M2 (Supplementary Figure 5B–5F), which may induce the negative correlation between POC1A expression and macrophage. Therefore, even though the alteration of copy number could significantly regulate the POC1A expression, the infiltration levels of several cells in Figure 7A may not be corresponding.

Immune signatures are significant biomarkers that predict prognosis in malignant cancer. Further study of immunogenomics will enhance immunotherapy treatment [31]. Long et al. [32] demonstrated that EXO1 is an immune gene that significantly affects prognosis in HCC. Dai et al. [33] found that Epstein–Barr virus (EBV) in GC can lead to PD-L1 positivity and high TIL density, which reduce survival benefits in GC patients. Therefore, to provide more support for our research on immune infiltration, we decided to find the association between POC1A and multiple classic immune biomarkers. Ultimately, 7 significant signatures (CCNB1, ESCO2, EXO1, KIF11, NUF2, PRC1, CCL14) were selected for their strong correlation. These results indicated that POC1A has the potential to act as a significant biomarker to impact the prognosis of GC by regulating immune infiltration levels via these immune signatures. Moreover, as POC1A was reported to have a significant relationship with the lack of response to atezolizumab in urothelial cancer [34], we wondered whether POC1A could function in the response to immune infiltration therapy and focused our attention on the association between POC1A expression and immunomodulator expression. Data from Charoentong et al. [35] and the TISIDB were used to investigate the correlation between immunomodulators and POC1A, and 58 significantly correlated immune immunomodulators were discovered. After filtering these signatures, only ENTPD1 exhibited a strong correlation, which provides a new direction of POC1A in GC immunotherapy. In previous studies, ENTPD1 (also known as CD39) was reported to act as an immune signature which was presented on the surface of several immune cells, including activated cells, T cells, regulatory T cells, endothelial cells and lymphocytes [36–39]. As an immune signature, ENTPD1 was reported to regulate immune infiltration. In the study of Rissiek et al. [40], ENTPD1(+) Tregs could suppress T cells proliferation and inflammatory cytokine production. Furthermore, as Cai et al. [41] reported, high level ENTPD1 may induce poor prognosis in gastric cancer and high CD39+/CD8+ could lead to poor overall survival, which was corresponding to the negative correlation between POC1A and ENTPD1. Therefore, we speculate that POC1A may impact outcome of GC patients though immune regulation which may be induced by ENTPD1.

However, our study has limitations. The reliability of the analyses of molecular mechanism and immune infiltration levels, especially the correlation between immune signatures and POC1A, is not supported by experiments in vivo and in vitro. More experiments for verifying the functions of these small molecules can benefit the in-depth understanding of POC1A in GC and encourage progress in diagnosis and therapy. Therefore, the molecular mechanism of immune infiltration still needs further study.

CONCLUSIONS

In summary, we discovered POC1A as a tumor suppressor and found its potential role in affecting the prognosis of GC. In addition, coexpressed genes and genes with changes concurrent with POC1A CNA were predicted to regulate the cell cycle, DNA replication and cell growth in GC. Moreover, CD4+ T cell infiltration and an immunostimulator were found to have a significant strong correlation with POC1A, which provides a new direction for investigating this new biomarker in GC.

MATERIALS AND METHODS

Patients and specimens

The study employed 2 independent groups of fresh tumor and adjacent tissues from patients with GC who underwent surgery at Fujian Medical University Union Hospital (FJMUUH, Fuzhou, China). The POC1A mRNA expression group included 101 patients who underwent surgery between October 2013 and December 2018. Another group used for POC1A immunohistochemistry included 91 patients with gastrectomies between March 2013 and May 2015. None of the GC patients had received any preoperative chemotherapy or radiotherapy. All clinical pathological information, including sex, age, tumor size, tumor site, differentiation grade, and TNM stage (AJCC 7th edition) [42] were reassessed independently by 2 pathologists. The time from surgery to death or the last follow-up was regarded as overall survival (OS). Recurrence-free survival (RFS) was defined as the time from surgery to recurrence. All specimens were stored in liquid nitrogen at -80 °C. The use of human samples was approved by the Institutional Review Board of FJMUUH. Written informed consent was obtained from our patients to acknowledge the use of their resected tissues for research purposes.

Quantitative reverse transcriptase PCR

We selected 101 pairs of fresh tumor and adjacent tissues to measure POC1A mRNA expression and used TRIzol reagent for extracting total RNA, which was further transcribed to cDNA through reverse transcription. RT-qPCR was performed with SYBR qPCR mix (Takara Bio Inc) in a 7500 real-time PCR System (Thermo Fisher Scientific). GAPDH, whose primers were 5'-CTCGCTTCGGCAGCACA-3' (forward) and 5'-AACGCTTCACGAATTTGCGT-3' (reverse), was regarded as an endogenous reference for normalization. Other primer information is shown in Supplementary Table 1. Finally, we used the 2-ΔCt formula to represent the expression of the genes above.

Tissue microarray and immunochemistry tissue

Tissues from 91 patients were fixed with formalin. After embedding with paraffin, tissues were made into a tissue microarray. Then, we used xylene for deparaffinization, and a grade alcohol series was used for hydration. After citric acid solution was applied for antigen retrieval, we used 3% H2O2 to inhibit endogenous peroxidase. Then, we incubated sections with 10% normal goat serum for 30 minutes. Primary antibody against POC1A (diluted 1:100, Sigma) was applied overnight. Subsequently, goat anti-rabbit IgG (1:1000, Boster Biological Technology) was used as the secondary antibody for incubation. After staining, 2 pathologists evaluated the results of each specimen according to the intensity of expression. The immunohistochemical score (IHS) was qualified by calculating the product of a 4-value intensity score (0, 1, 2, 3) and a 4-value percentage score, which included scores of 0 (0-5%), 1 (6-25%), 2 (51-75%), and 3 (76-100%). Afterwards, IHS<3 was defined as low expression, and high POC1A expression ranged from 3-9 [43].

Immune infiltration database

We used the TIMER database (https://cistrome.shinyapps.io/timer/) to analyze the correlation between POC1A copy number alteration (CNA) and immune infiltration level. Moreover, the relationship between POC1A expression and immune infiltrating genes was also revealed. Furthermore, we employed the TISIDB database to evaluate whether POC1A was related to immune infiltration therapy and subtypes in stomach adenocarcinoma (STAD).

TCGA and GEO database analysis

GSE54129 was selected from the Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo/) database, which was used to find significantly differentially expressed genes. Moreover, GAE8433, GSE15459 and GSE26253 were separately used for overall survival and recurrence-free survival analysis in terms of POC1A, whose expression between tumor and normal tissues was verified by GSE54129. In addition, we also acquired data from The Cancer Genome Atlas (TCGA) to analyze the prognosis of POC1A in STAD.

GEPIA database analysis

As an online database, Gene Expression Profiling Interactive Analysis (GEPIA) (http://gepia.cancer-pku.cn/) is a database that involves tumor and normal samples from TCGA and GTEx [33]. We analyzed the relationship between POC1A and several immune signatures through the database.

cBioPortal database analysis

cBioPortal (https://www.cbioportal.org/) [31, 44] is an open-access database that was sourced from TCGA and 225 cancer studies. We employed it to acquire copy number alteration (CNA) and cooccurrence genes of POC1A in STAD. We then performed GO and KEGG analysis of these cooccurrence genes through the "clusterProfiler" package in R [45], which evaluated cellular components (CCs), biological processes (BPs), molecular functions (MFs), and KEGG pathways.

LinkedOmics database analysis

LinkedOmics (http://www.linkedomics.org/login.php) [46] is a cancer-associated database for analyzing TCGA. We used it to discover genes in TCGA STAD that were correlated with POC1A according to Spearman correlation and selected *LinkFinder* in LinkedOmics to establish a heatmap plot for the coexpressed genes. After acquiring strongly correlated coexpressed genes, we performed GO and KEGG analyses of these genes with the clusterProfiler R package [45].

STRING database analysis

To investigate the interaction among the co-occurrence altered genes, we used the STRING v11 database [47] (https://string-db.org/) to analyze the relationship among 212 genes and construct a PPI network.

Statistical analysis

As all Kaplan-Meier plots had P-values, survival curves were generated by the survival R package. In addition, the correlation between POC1A expression and clinical pathological characteristics of patients from RT-qPCR, immunohistochemistry and

GSE84433 was analyzed with SPSS version 25.0 software. Furthermore, determination of the best cut-off point and LASSO regression analysis were separately performed with the survivalROC [48] and glmnet [49] R packages.

Ethics approval

The study was approved by Fujian Medical University Union Hospital, China.

Abbreviations

POC1A: POC1 centriolar protein homolog A; GC: gastric cancer; TME: tumor microenvironment; TILs: tumor-infiltrating lymphocytes; STAD: stomach adenocarcinoma; EBV: Epstein–Barr virus; GEO: Gene Expression Omnibus; TCGA: The Cancer Genome Atlas; GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; CNA: copy number alteration; CCs: evaluated cellular components; BPs: biological processes; MFs: molecular functions; HIS: immunohistochemical score; RFS: recurrence-free survival; OS: overall survival.

AUTHOR CONTRIBUTIONS

J.L, X.Y.H, C.M.H and C.H.Z contributed to the design and conduct. J.L, X.Y.H, Y.H.W, P.L and J.W.X contributed to the acquisition of the data. J.L, X.Y.H, and Y.H.W contributed to the analysis. J.B.W, J.X.L, Q.Y.C and L.L.C contributed to material support. C.H.Z supervised the study. All authors contributed substantial to the interpretation of data and were involved in drafting and revising the manuscript. All authors approved the final version of the manuscript.

CONFLICTS OF INTEREST

All the authors declare that they have no conflicts of interest.

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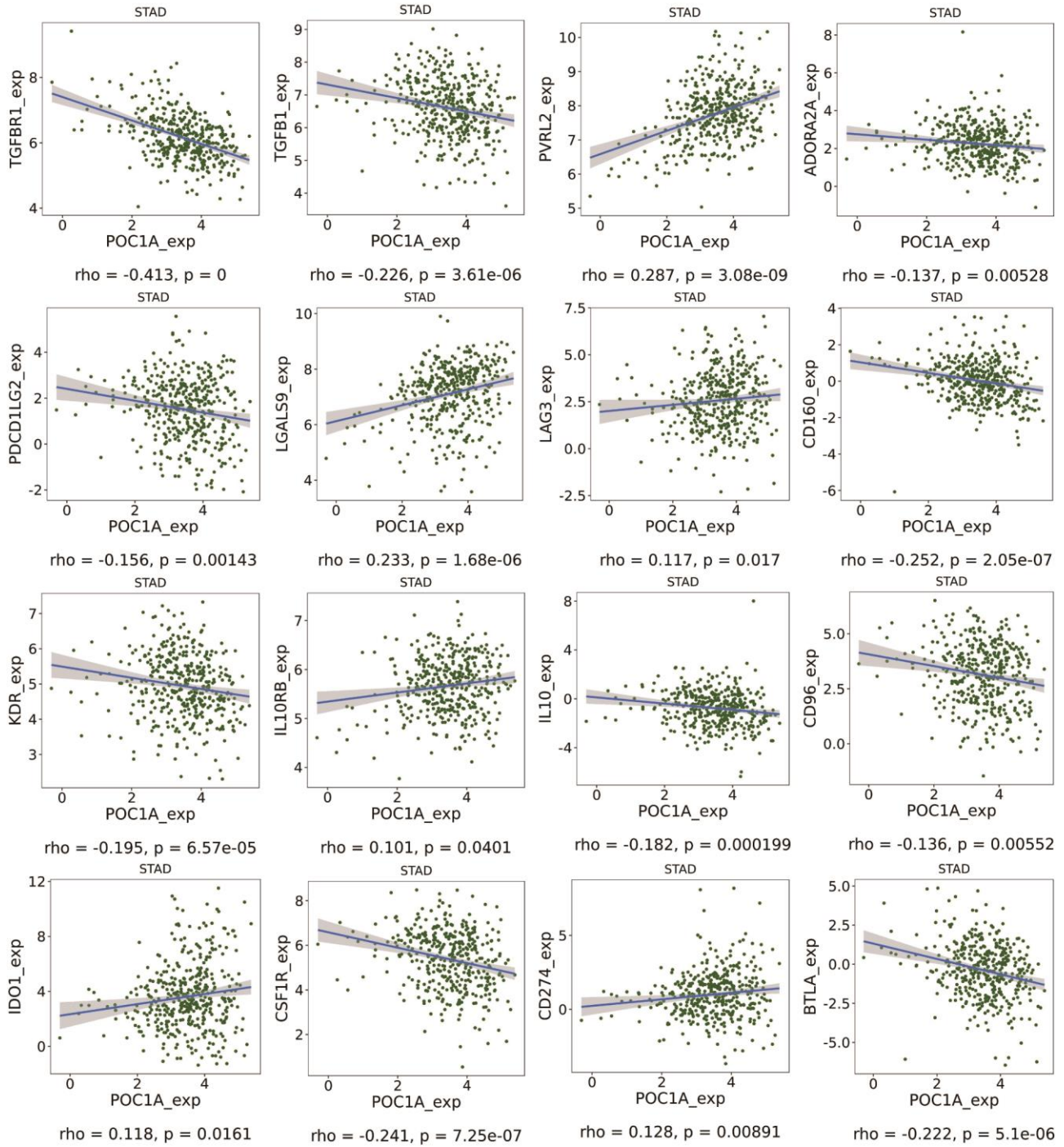PMID:[28035468](https://pubmed.ncbi.nlm.nih.gov/28035468)
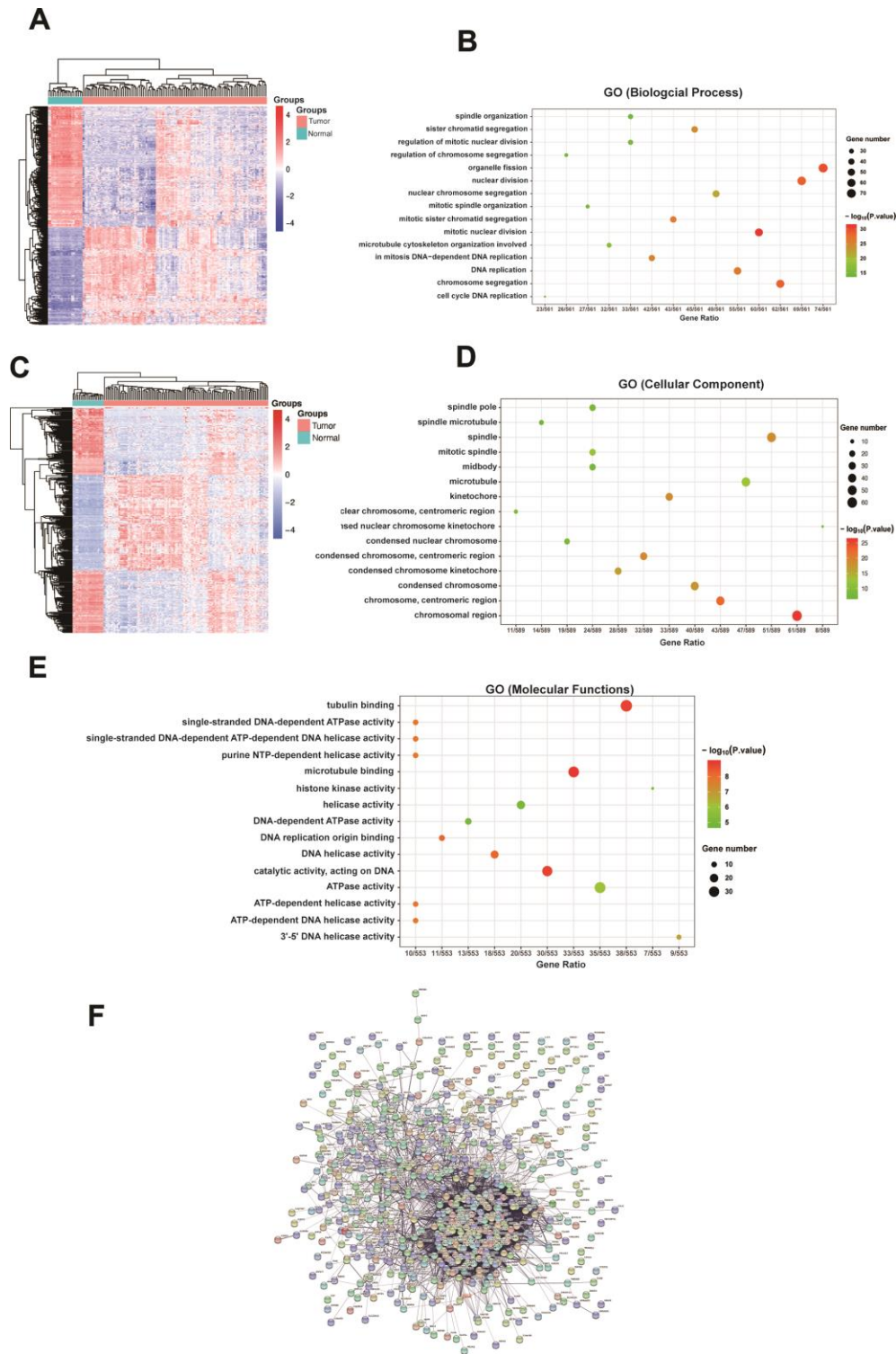
Supplementary Figure 1. The bioinformatic analysis of POC1A. (**A**, **B**) Heat map showing the expression of all genes and differently expressed genes in GSE54129; (**C**–**E**) GO enrichment analysis of POC1A co-expression genes; (**F**) Protein-protein interaction (PPI) network of genes strongly coexpressed with POC1A.
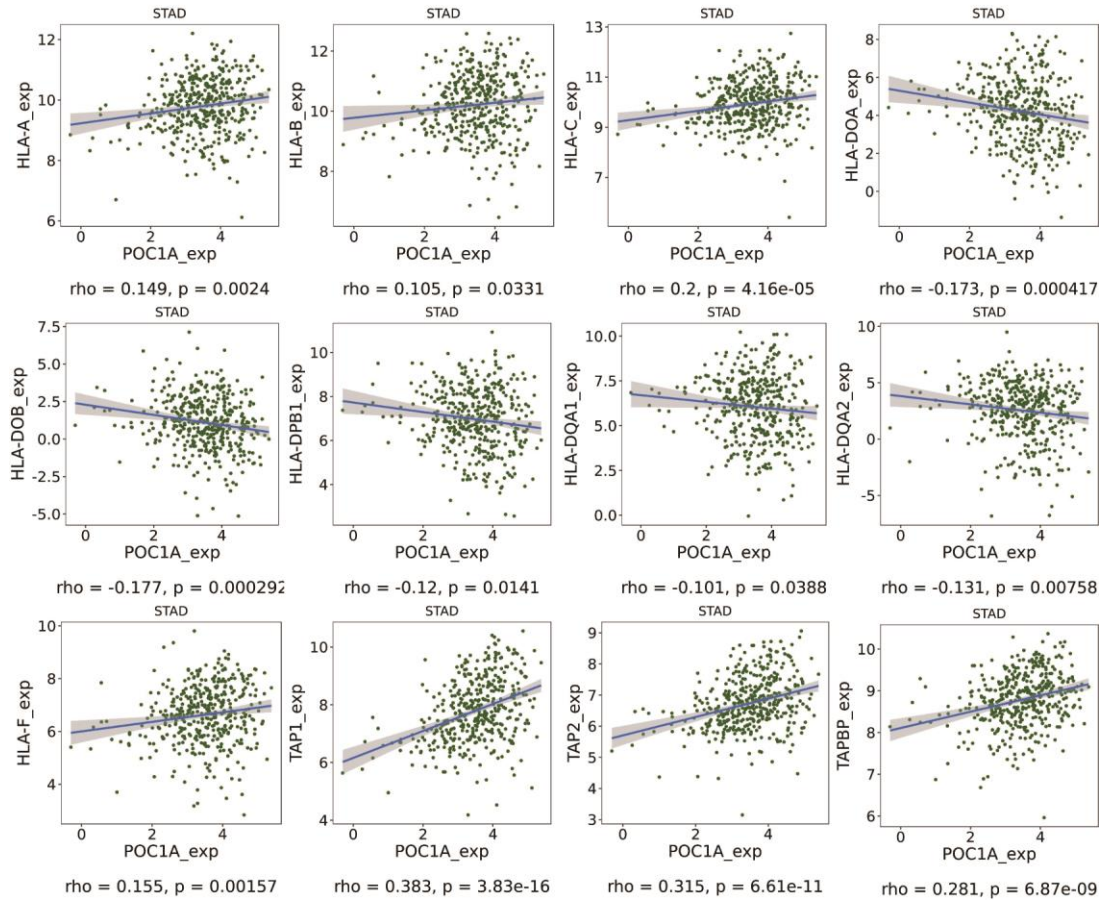
Supplementary Figure 2. Significant correlation between POC1A and several immunoinhibitors.
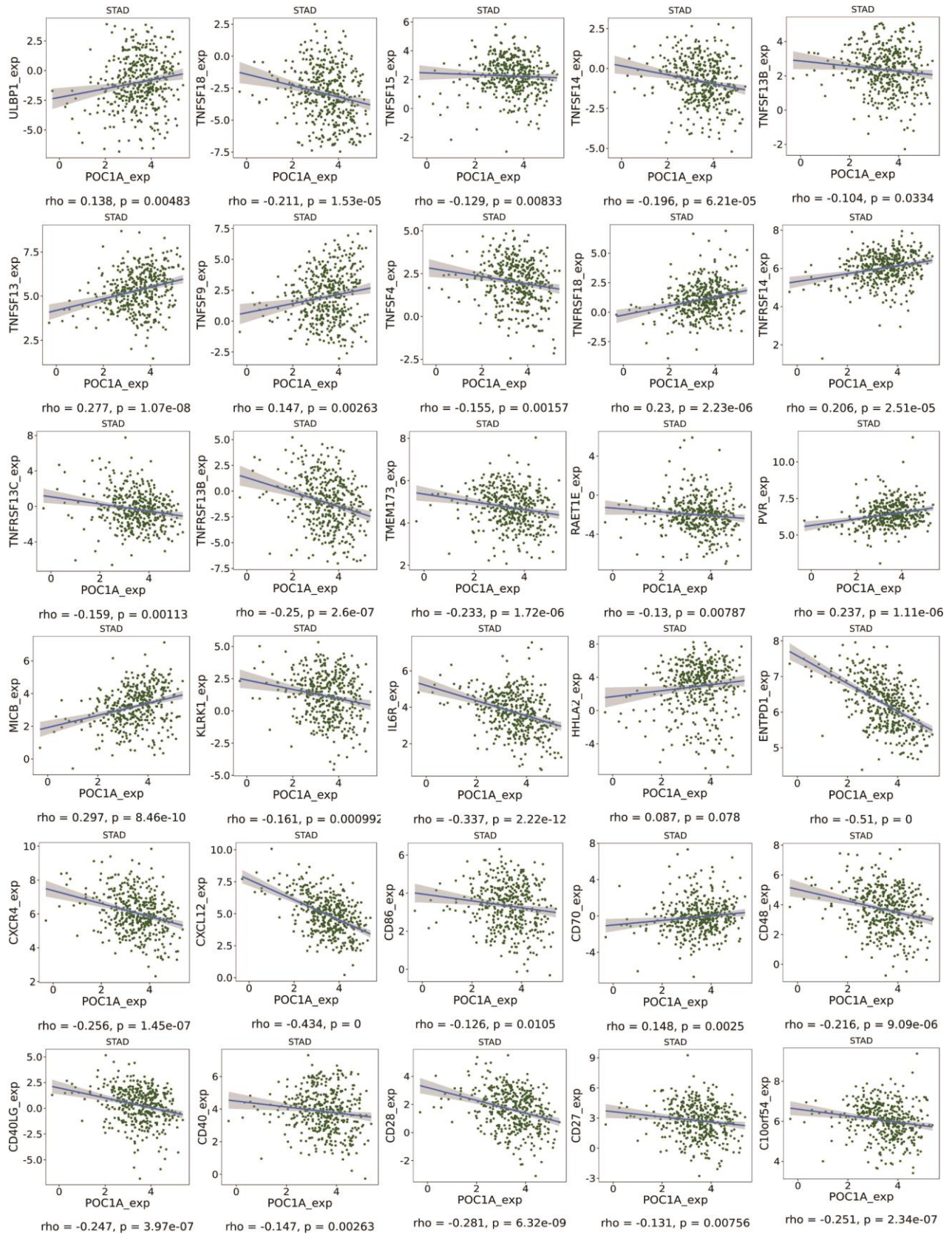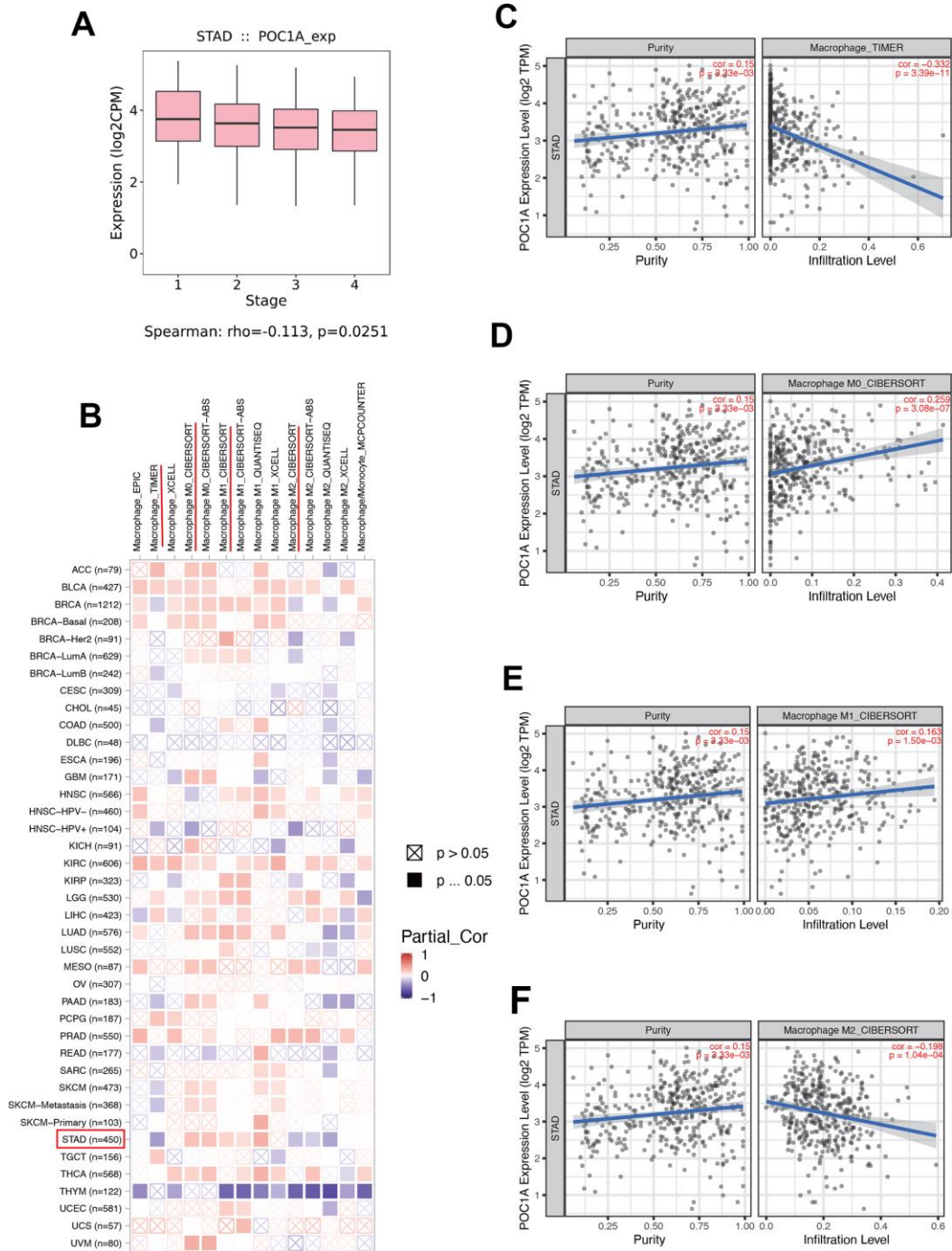
Supplementary Figure 3. Significant correlation between POC1A and several immunostimulators.

MHC molecules

Supplementary Figure 4. Significant correlation between POC1A and several MHC molecular.

Supplementary Figure 5. The sense POC1A in clinical prognosis and immune infiltration. (**A**) Expression of POC1A had significant negative correlation with TNM stages (R=-0.1, p=0.02) according to the TISIDB; (**B**) Heatmap of correlation between POC1A expression and immune infiltration level of macrophages among different datasets and different cancer types; (**C–F**) Scatter plots of correlation between POC1A expression and immune infiltration of different macrophages in STAD.

Supplementary Tables

Please browse Full Text version to see the data of Supplementary Tables 6, 7, 11.

Supplementary Table 1. Primer sequence of 6 genes for RT-qPCR.

Genes	Sequence (5'->3')	
	Forward Primer	**Reverse Primer**
VMP1	GGTAGCTGCAGCCAGTACAA	TGCAACAACAGCAAGGGTTG
LAMP5	CCCCTGATTTTGGGGCTCAT	TACTGGGATCTGTCCCGAGG
ARMC9	CCGGATGCTACCACTTCTCG	AAAATCTTTGCGGGAAGCAGC
CEBPB	TTTGTCCAAACCAACCGCAC	CCCCCAAAAGGCTTTGTAACC
POC1A	GTGACAGCCTCTGACGACAA	AGAAAGTTGGGCATGGTGTTG
PAOX	CTGCTTCGGGGCTATCAAGG	TCGTGGAGTAAAACGTGCGA

Supplementary Table 2. Associations between POC1A mRNA levels and clinicopathological characteristics.

		Number	POC1A		p
			Low	High	
Gender					0.205
	Female	25	17	8	
	Male	76	61	15	
Age (Years)					0.709
	<=65	67	51	16	
	>65	34	27	7	
T stage					0.085
	T1	11	6	5	
	T2	6	4	2	
	T3	34	25	9	
	T4	50	43	7	
N stage					**0.001**
	N0	27	21	6	
	N1	9	2	7	
	N2	24	19	5	
	N3	31	36	5	
TNM stage					0.142
	I	14	8	6	
	II	22	17	5	
	III	65	53	12	
Tumor Location					0.486
	Upper	37	27	10	
	Middle	14	13	1	
	Lower	30	22	8	
	Diffuse	20	16	4	
Tumor Size (mm)					**0.043**
	<50	13	6	7	
	>=50	28	23	5	
Differentiation grade					0.249
	G1	14	13	1	
	G2	58	42	16	
	G3	29	23	6	

Supplementary Table 3. Associations between POC1A expression and clinicopathological characteristics in immunohistochemistry.

	Number	POC1A		p
		Low	High	
Gender				0.646
Female	18	16	2	
Male	73	59	14	
Age (Years)				0.368
<=65	31	24	7	
>65	60	51	9	
BMI (kg/m2)				0.646
<=25	73	59	14	
>25	18	16	2	
T stage				0.087
T1	1	0	1	
T2	10	7	3	
T3	78	33	4	
T4	2	35	8	
N stage				**0.04**
N0	13	10	3	
N1	16	14	2	
N2	28	19	9	
N3	34	32	2	
M stage				0.545
M0	87	72	15	
M1	4	3	1	
TNM stage				0.363
I	4	3	1	
II	23	17	6	
III	60	52	8	
IV	4	3	1	
Position				0.731
Upper	31	25	6	
Middle	17	14	3	
Lower	36	31	5	
Diffuse	7	5	2	
Tumor Size (mm)				**0.05**
<50	48	36	12	
>=50	43	39	4	
Differentiation grade				0.777
Well-moderate	37	31	6	
Poor-undifferentiation	54	44	10	
Lymphatic or nervous invasion				**0.036**
Negative	59	45	14	
Positive	32	30	2	
Borrmann				0.893
Borrmann I-II	16	13	3	
Borrmann III-IV	75	62	13	

Supplementary Table 4. Correlation between POC1A mRNA expression and clinical pathological features in GSE84433.

	Number	POC1A		p
		Low	High	
Gender				0.996
Female	115	76	39	
Male	242	160	82	
Age (Years)				0.836
<=65	241	158	83	
>65	116	78	38	
T stage				**0.002**
T1	11	5	6	
T2	35	16	19	
T3	67	39	28	
T4	244	176	68	
N stage				**0.008**
N0	71	35	36	
N1	155	107	48	
N2	99	69	30	
N3	32	25	7	

Supplementary Table 5. KEGG pathways of co-expression genes of POC1A.

ID	Description	GeneRatio	BgRatio	pvalue	p.adjust	qvalue	Count
hsa04110	Cell cycle	30/253	124/8016	6.54E-19	1.63E-16	1.45E-16	30
hsa03030	DNA replication	14/253	36/8016	1.39E-12	1.73E-10	1.54E-10	14
hsa04114	Oocyte meiosis	20/253	128/8016	2.59E-09	2.15E-07	1.91E-07	20
hsa04914	Progesterone-mediated oocyte maturation	17/253	99/8016	9.75E-09	6.07E-07	5.39E-07	17
hsa04022	cGMP-PKG signaling pathway	18/253	167/8016	4.83E-06	0.00024056	0.00021356	18
hsa03460	Fanconi anemia pathway	9/253	54/8016	4.14E-05	0.00171614	0.00152353	9
hsa05166	Human T-cell leukemia virus 1 infection	18/253	219/8016	0.00018402	0.00654571	0.00581103	18
hsa04115	p53 signaling pathway	9/253	72/8016	0.00040351	0.01175142	0.01043245	9
hsa04540	Gap junction	10/253	88/8016	0.00042475	0.01175142	0.01043245	10
hsa04218	Cellular senescence	14/253	160/8016	0.00051219	0.01275354	0.01132211	14
hsa03430	Mismatch repair	5/253	23/8016	0.00063523	0.01437923	0.01276532	5
hsa04270	Vascular smooth muscle contraction	12/253	132/8016	0.00091174	0.01891871	0.0167953	12
hsa04970	Salivary secretion	9/253	90/8016	0.00204635	0.03919542	0.03479619	9
hsa04730	Long-term depression	7/253	60/8016	0.00265927	0.04729696	0.04198842	7

Supplementary Table 6. GO annotations of co-expression genes of POC1A.

Supplementary Table 7. Genomic alteration genes of POC1A in STAD.

Supplementary Table 8. Significant KEGG pathways of alteration genes of POC1A.

ID	Description	GeneRatio	BgRatio	pvalue	p.adjust	qvalue	Count
hsa04260	Cardiac muscle contraction	6/73	86/8016	0.00012251	0.01296701	0.01246601	6
hsa05410	Hypertrophic cardiomyopathy (HCM)	6/73	90/8016	0.00015765	0.01296701	0.01246601	6
hsa05414	Dilated cardiomyopathy (DCM)	6/73	96/8016	0.00022486	0.01296701	0.01246601	6
hsa00260	Glycine, serine and threonine metabolism	4/73	40/8016	0.00045152	0.01952831	0.01877379	4
hsa00531	Glycosaminoglycan degradation	3/73	19/8016	0.00063237	0.02187986	0.02103448	3
hsa04360	Axon guidance	7/73	181/8016	0.00123623	0.03564476	0.03426755	7

Supplementary Table 9. Significant GO annotations of alteration genes of POC1A (Biological Process and Mollecular Functions).

ID	Description	GeneRatio	BgRatio	pvalue	p.adjust	qvalue	Count
GO:0030212	hyaluronan metabolic process	6/166	37/18670	8.35E-07	0.00263953	0.00228095	6
GO:0050919	negative chemotaxis	6/166	46/18670	3.15E-06	0.00497728	0.00430112	6
GO:0016049	cell growth	16/166	484/18670	6.90E-06	0.00726886	0.00628138	16
GO:0001558	regulation of cell growth	14/166	416/18670	2.18E-05	0.01721154	0.01487336	14
GO:0071493	cellular response to UV-B	3/166	8/18670	3.74E-05	0.02365151	0.02043846	3
GO:1903510	mucopolysaccharide metabolic process	7/166	112/18670	6.41E-05	0.03056102	0.02640931	7
GO:0050808	synapse organization	13/166	408/18670	7.53E-05	0.03056102	0.02640931	13
GO:0048843	negative regulation of axon extension involved in axon guidance	4/166	26/18670	7.73E-05	0.03056102	0.02640931	4
GO:1902668	negative regulation of axon guidance	4/166	28/18670	0.00010447	0.03669279	0.03170808	4
GO:0004415	hyaluronononglucosaminidase activity	3/164	8/17697	4.23E-05	0.01355295	0.01137836	3
GO:0030215	semaphorin receptor binding	4/164	23/17697	5.49E-05	0.01355295	0.01137836	4
GO:0033906	hyaluronoglucuronidase activity	2/164	2/17697	8.54E-05	0.01405602	0.01180071	2
GO:0015929	hexosaminidase activity	3/164	15/17697	0.00032759	0.04045704	0.03396564	3
GO:0098882	structural constituent of presynaptic active zone	2/164	4/17697	0.00050593	0.04600134	0.03862035	2
GO:0038191	neuropilin binding	3/164	18/17697	0.00057562	0.04600134	0.03862035	3
GO:0000828	inositol hexakisphosphate kinase activity	2/164	5/17697	0.00083808	0.04600134	0.03862035	2
GO:0000832	inositol hexakisphosphate 5-kinase activity	2/164	5/17697	0.00083808	0.04600134	0.03862035	2
GO:0099181	structural constituent of presynapse	2/164	5/17697	0.00083808	0.04600134	0.03862035	2

Supplementary Table 10. Correlations between immune infiltration levels of different immune cells and copy number alteration (CNA) of POC1A.

Cancer	Immune Cells	CNA	P value
STAD	B Cell	Deep Deletion	0.51849727
STAD	B Cell	Arm-level Deletion	0.01064684
STAD	B Cell	Diploid/Normal	1
STAD	B Cell	Arm-level Gain	0.22262003
STAD	B Cell	High Amplication	1
STAD	CD8+ T Cell	Deep Deletion	0.21722101
STAD	CD8+ T Cell	Arm-level Deletion	0.00358451
STAD	CD8+ T Cell	Diploid/Normal	1
STAD	CD8+ T Cell	Arm-level Gain	0.00282367
STAD	CD8+ T Cell	High Amplication	1
STAD	CD4+ T Cell	Deep Deletion	0.45450308
STAD	CD4+ T Cell	Arm-level Deletion	0.00141845
STAD	CD4+ T Cell	Diploid/Normal	1
STAD	CD4+ T Cell	Arm-level Gain	0.01199774
STAD	CD4+ T Cell	High Amplication	1
STAD	Macrophage	Deep Deletion	0.23318168
STAD	Macrophage	Arm-level Deletion	0.00456658
STAD	Macrophage	Diploid/Normal	1
STAD	Macrophage	Arm-level Gain	0.0007265
STAD	Macrophage	High Amplication	1
STAD	Neutrophil	Deep Deletion	0.28499674
STAD	Neutrophil	Arm-level Deletion	4.14E-05
STAD	Neutrophil	Diploid/Normal	1
STAD	Neutrophil	Arm-level Gain	0.0389077
STAD	Neutrophil	High Amplication	1
STAD	Dendritic Cell	Deep Deletion	0.14183773
STAD	Dendritic Cell	Arm-level Deletion	1.55E-05
STAD	Dendritic Cell	Diploid/Normal	1
STAD	Dendritic Cell	Arm-level Gain	0.01030807
STAD	Dendritic Cell	High Amplication	1

Supplementary Table 11. Correlation analysis between POC1A and relate genes and markers of immune cells in TIMER.