Original Article

A pan-cancer analysis of the expression of gasdermin genes in tumors and their relationship with the immune microenvironment

Yuan-Yuan Wang[1], Lin-Yang Shi[2], Ming-Hao Xu[1], Yu Jing[1], Cui-Cui Sun[1], Jia-Hui Yang[2], Ruo-Nan Wang[2], Ning-Ning Sheng[2], Ca-Fa Zhang[2], Li Zhang[1], Zhi-Tu Zhu[1]^, Qing-Jun Wang[1]

[1]Department of Clinical Trial, The First Affiliated Hospital of Jinzhou Medical University, Jinzhou, China; [2]Department of Oncology, The First Affiliated Hospital of Jinzhou Medical University, Jinzhou, China

Contributions: (I) Conception and design: YY Wang, ZT Zhu, QJ Wang; (II) Administrative support: None; (III) Collection and assembly of data: CF Zhang, NN Sheng, L Zhang, JH Yang; (IV) Provision of study materials or patients: None; (V) Data analysis and interpretation: MH Xu, RN Wang, Y Jing, LY Shi, CC Sun; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

Correspondence to: Qing-Jun Wang; Zhi-Tu Zhu. Department of Clinical Trial, The First Affiliated Hospital of Jinzhou Medical University, Jinzhou 121000, China. Email: qingjunwang999@163.com; zhizhitu@163.com.

Background: Gasdermins (GSDMs) are a class of proteins related to pyrolysis and in humans, consist of GSDMA, GSDMB, GSDMC, GSDMD, DFNA5, and DFNB59. The inflammatory factors and cell contents released during pyrolysis can recruit immune cells and change the microenvironment. However, to date, there is a paucity of studies examining the relationship between GSDMs and the immune microenvironment in tumors. Therefore, this current report analyzed the expression of GSDM genes in tumors and their relationship with the immune microenvironment.

Methods: Apply GSCALite and GEPIA2 online analysis tools to analyze the gene expression levels and the Single nucleotide variant (SNV), copy number variation (CNV), and methylation characteristics of GSDM genes respectively. Use R software or TISIDB online analysis tool to carry out the correlation analysis required in the article. Furthermore, Gene Ontology (GO) enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were conducted to examine the role of these GSDM genes in various cancers.

Results: The results demonstrated that CNV can cause an increase in GSDM gene expression, and methylation can inhibit GSDM gene expression. The elevated expression of GSDMA, GSDMB, GSDMC, GSDMD, and DFNA5 in some or most tumors was often accompanied by elevated immune scores, increased immune cell infiltration, and high expression of major histocompatibility complex (MHC) molecules, chemokines and their receptors, and immune checkpoint-related genes. However, DFNB59 was often negatively correlated with these indicators in tumors. GSDMD was the most highly expressed GSDM protein in various normal tissues and tumors, and showed the strongest correlation with immune microenvironment-related genes. Moreover, the methylation of GSDMD was accompanied by low immune cell infiltration, low expression of MHC molecule-related genes, low expression of chemokines and receptor-related genes, and low expression of immune checkpoint-related genes.

Conclusions: Therefore, the expression of GSDM-related genes is associated with the tumor immune microenvironment. The GSDM genes, especially GSDMD, may be used as therapeutic targets to predict or change the tumor microenvironment and as biomarkers to predict the therapeutic efficacy of immune checkpoint inhibitors.

Keywords: Gasdermins (GSDMs); immune microenvironment; pan-cancer; immune cell infiltration; immune checkpoint

^ ORCID: 0000-0001-5488-6968.

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Introduction

Pyrolysis is a process that is similar to apoptosis and autophagy, with all three processes involved in programmed cell death. However, unlike apoptosis, during pyrolysis, the plasma membrane of the cell is damaged and the cell contents are released outside the cell, thereby causing an inflammatory response (1). While apoptosis is generally accepted as a non-inflammatory process, and has intact plasma membrane (1). During pyrolysis, when the cell is stimulated, the intracellular inflammasome activates the cysteine aspartate specific protease caspase, to cleave the gasdermin (GSDM) proteins, releasing the N-terminal domain which recognizes and punches holes in the cell membrane. This pore-forming activity disrupts the cell's osmotic pressure, and the resultant electrolyte imbalance causes the cell to swell and rupture, releasing large amounts of inflammatory factors and cell contents. This results in the recruitment of immune cells, which further induces the inflammatory response and causes the inflammatory death of the cell (2,3).

In humans, GSDMs consists of GSDMA, GSDMB, GSDMC, GSDMD, DFNA5, and DFNB59. Gasdermin D (GSDMD) was first discovered in 2015 and was identified as the key executive protein of downstream molecular signals after the activation of caspase (4). DFNA5 has also been shown to be involved in the pyrolysis of cancer and normal cells (5). In addition to DFNB59 , almost all N-terminal domains of GSDMs can exert pore-forming activity in the plasma membrane (6). Indeed, GSDMs are related to infectious diseases, autoimmune diseases, neurological diseases, and tumors (7-9).

The high expression GSDMs may affect the tumor microenvironment. Recent research showed that DFNA5 suppresses tumor growth by activating anti-tumor immunity (10). Using The Cancer Genome Atlas (TCGA) and the Genotype-Tissue Expression (GTEx) databases , the present study analyzed the expression of the GSDM genes in tumors and their impact on the immune microenvironment. ESTIMATE (Estimation of Stromal and Immune Cells in Malignant Tumor Tissues using Expression Data) was used to calculate immune and stromal scores (11) and the CIBERSORT (Cell Type Identification by Estimating Relative Subset of known RNA Transcripts) algorithm (12,13) was used to calculate the content of immune-infiltrating cells in each tumor.

This study demonstrated that GSDMD was generally highly expressed in various normal and tumor tissues, followed by GSDMB, DFNA5, DFNB59, GSDMA, and GSDMC. Copy number variation (CNV) caused an increase in the expression of GSDM genes, while methylation inhibited the expression of GSDM genes. Recent research showed that cytokine release syndrome (CRS) caused by chimeric antigen receptor T cells (CAR-T) is related to pyroptosis induced by DFNA5 and GSDMD (14). Furthermore, DFNA5 can suppress tumor growth by activating anti-tumor immunity (10). Therefore, GSDM genes are related to the immune microenvironment in tumors. This comprehensive analysis revealed that with the exception of DFNB59 , which has no punching activity, high expression of other GSDM genes in most tumors is often accompanied with elevated immune scores, increased immune cell infiltration, and high expression of major histocompatibility complex (MHC) molecules, chemokines and their receptors, and immune checkpoint-related genes. In particular, the expression of GSDMD was the highest among all GSDMs in all tumor types examined , and showed the strongest positive correlation with these indicators. High expression of chemokines and their receptors tends to induce an increase in immune cell infiltration (15,16), and high expression of MHC molecules is conducive for immune cells to recognize and kill cancer cells (17). High expression of immune checkpoint-related genes can inhibit the body's anti-tumor immune effect and thus, the application of immune checkpoint inhibitors in such a microenvironment may be favorable.

Therefore, the expression of GSDMs is related to changes in the tumor immune microenvironment. With the exception of DFNB59, high expression of the other GSDMs, especially GSDMD, may indicate that the tumor immune microenvironment represents a tumor that is conducive to immunotherapy. The GSDM genes, especially GSDMD, may be used as clinical biomarkers to predict the therapeutic efficacy of immune checkpoint inhibitors or as therapeutic targets to alter the tumor microenvironment.

We present the following article in accordance with the MDAR reporting checklist (available at https://dx.doi.org/10.21037/tcr-21-1635).

Methods

Data and data processing methods

The gene expression data of each tumor was obtained from TCGA website (https://portal.gdc.cancer.gov/repository). The ESTIMATE (11) and CIBERSORT algorithm (12,13) was used to calculate the immune and stromal scores, and immune cell infiltration content of each tumor. The immune subtypes of each sample were based on the research of Thorsson *et al.* (18). The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

The tumor names and abbreviations in the TCGA database were as follows: adrenocortical carcinoma (ACC), bladder urothelial carcinoma (BLCA), breast invasive carcinoma (BRCA), cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC), cholangiocarcinoma (CHOL), colon adenocarcinoma (COAD), lymphoid neoplasm diffuse large B-cell lymphoma (DLBC), esophageal carcinoma (ESCA), glioblastoma multiforme (GBM), head and neck squamous cell carcinoma (HNSC), kidney chromophobe (KICH), kidney renal clear cell carcinoma (KIRC), kidney renal papillary cell carcinoma (KIRP), acute myeloid leukemia (LAML), brain low-grade glioma (LGG), liver hepatocellular carcinoma (LIHC), lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), mesothelioma (MESO), ovarian serous cystadenocarcinoma (OV), pancreatic adenocarcinoma (PAAD), pheochromocytoma and paraganglioma (PCPG), prostate adenocarcinoma (PRAD), rectum adenocarcinoma (READ), sarcoma (SARC), skin cutaneous melanoma (SKCM), stomach adenocarcinoma (STAD), testicular germ cell tumor (TGCT), thyroid carcinoma (THCA), thymoma (THYM), uterine corpus endometrial carcinoma (UCEC), uterine carcinosarcoma (UCS), and uveal melanoma (UVM).

Online website analysis

The GSCALite web server (19) (http://bioinfo.life.hust.edu.cn/web/GSCALite/) was used to analyze the expression of GSDM genes in each tissue in the GTEx database (https://www.ncbi.nlm.nih.gov/geo/). The SNV, CNV, and methylation characteristics of the GSDM genes was analyzed in 33 tumors in the TCGA database along with their relationships with gene expression. GEPIA2 (20) (http://gepia.cancer-pku.cn/) was used to analyze the gene expression levels in normal and tumor tissues in the TCGA and GTEX databases and draw gene expression heat maps for different tumor tissues and corresponding normal tissues. The TISIDB online analysis tool (http://cis.hku.hk/TISIDB/) (21) was used to analyze the correlation between GSDM gene methylation and immune cell infiltration, immune checkpoint-related gene expression, MHC molecules, chemokines and their receptor-related gene expression.

Correlation analysis

The R packages of corrplot, pheatmap, limma, ggplot2, reshape2, RColorBrewer, and others were used for correlation analysis. These packages were also used to construct the heat maps for the correlation between gene expression levels, the relationship between gene expression and immune score, the relationship between gene expression and immune subtypes, the relationship between gene expression and immune cell infiltration, the relationship between gene expression and immune checkpoint-related gene expression, and the relationship between immune cell infiltration and immune checkpoint-related gene expression. $P<0.05$ indicated statistical significance.

GO and KEGG pathway enrichment

GO biological processes (BP) terms of Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways were downloaded from the Gene Set Enrichment Analysis (GSEA) platform (https://www.gsea-msigdb.org/gsea/). GO enrichment and KEGG pathway enrichment of the GSDM genes in various cancers were investigated by GSEA. $P<0.05$ indicated statistical significance. Heat maps were constructed for the meaningful results from the GO and KEGG pathway enrichment analysis. Normalized enrichment scores (NES) >1 was indicated by red, whereas NES <1 was indicated by blue. GO enrichment results were clustered using the hclust method.

Statistical analysis

For GEPIA2 online analysis websites, $P<0.05$ was selected, which was statistically significant. Spearman correlation coefficient was used for the correlation between methylation and gene expression, and Pearson correlation coefficient was used for other correlation analysis. $P<0.05$ was statistically significant.

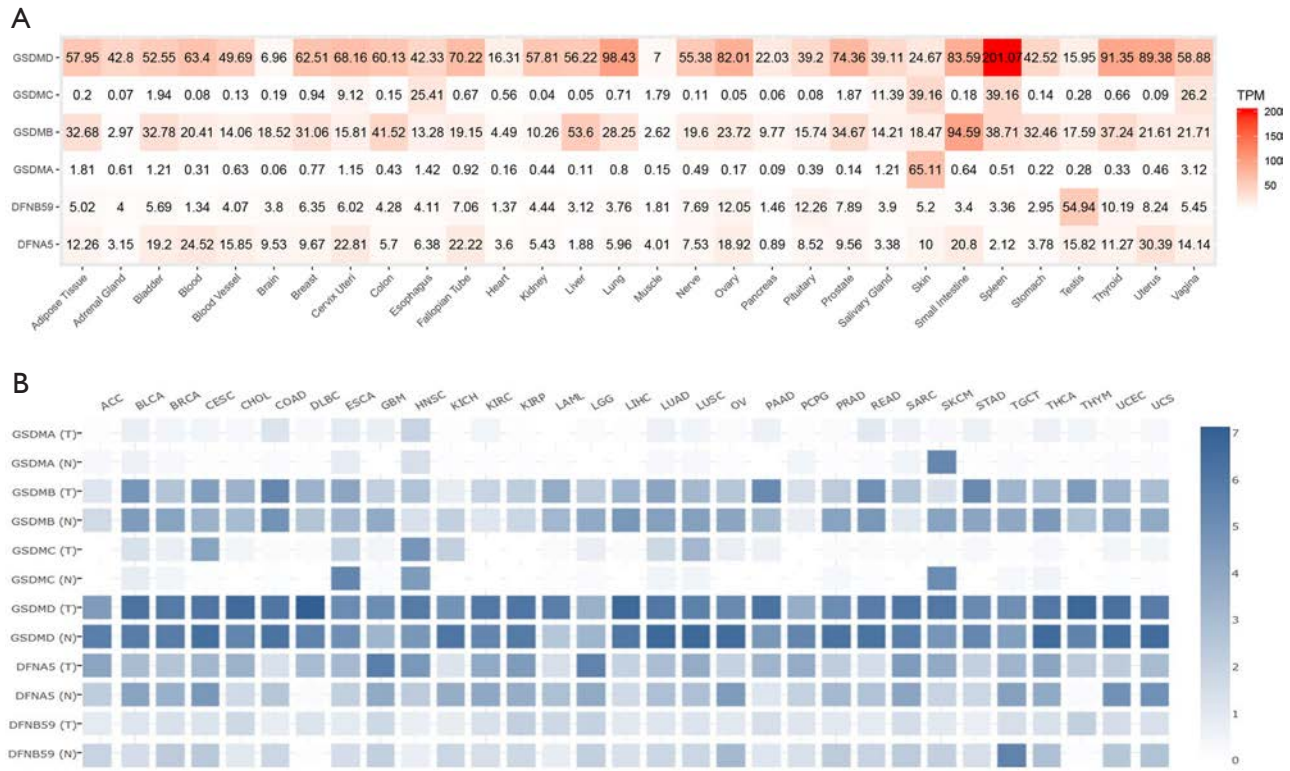


Figure 1 The expression level of the genes of GSDMs in normal tissues and tumors. (A) The expression levels of the genes of GSDMs in various tissues of the GTEx database. (B) The gene expression of tumor tissues and normal tissues based on the TCGA and GTEx database. GSDMs, gasdermins; GTEx, Genotype-Tissue Expression; TCGA, The Cancer Genome Atlas.

Results

The expression of GSDM genes in normal tissues and tumors

Analysis of the GTEx database showed that GSDMD was the most highly expressed GSDM in most normal tissues, followed by GSDMB, DFNA5, DFNB59, GSDMA, and GSDMC. The highest expression of GSDMD was detected in the spleen. GSDMA was highly expressed in skin tissues, but its expression in other tissues was very low. GSDMB had relatively high expression in most tissues, and had the highest expression of all GSDM genes in the small intestines. GSDMC had the lowest expression level in most tissues, being mainly expressed in the skin, spleen, vagina, esophagus, salivary gland, and cervix uteri. The expression of DFNA5 in various tissues was second only to that of GSDMB, with the highest expression detected in the uterus. DFNB59 was expressed in most tissues, with its highest expression detected in the testis, followed by the pituitary and ovary. The specific expression level of each GSDM is shown in *Figure 1A*.

GSDMD was also expressed in all tumor tissues and was the highest expressing GSDM gene in tumors, followed by GSDMB, DFNA5, DFNB59, GSDMA, and GSDMC (*Figure 1B* and Figure S1A). Comparative analysis of the expression level of the genes of GSDMs in tumor tissues and normal tissues base on the TCGA (Figure S1B) or TCGA and GTEx database (*Figure 1B* and Figure S2) showed similar results. GSDMA was highly expressed in COAD and poorly expressed in SKCM, and the difference was statistically significant. GSDMB was lowly expressed in BRCA, GBM, KICH, LGG, LUSC, OV, PRAD, SKCM, and THCA, but highly expressed in HNSC, PAAD, SARC, STAD, and THYM. GSDMC was poorly expressed in ESCA and SKCM, and highly expressed in CESC, KICH, LUAD, and LUSC. GSDMD was highly expressed in CHOL, DLBC, GBM, HNSC, LAML, PAAD, SKCM, and THYM, but poorly expressed in ACC, KICH, LUSC, OV, PCPG, and PRAD. DFNA5 was poorly expressed in BLCA, CESC, COAD, KICH, LAML, OV, READ, UCEC,

and UCS, but highly expressed in ACC, CHOL, DLBC, GBM, HNSC, LGG, PAAD, PCPG, SKCM, and THYM. DFNB59 was highly expressed in DLBC and THYM, but poorly expressed in ACC, CESC, COAD, KICH, OV, PRAD, SKCM, TGCT, THCA, UCEC, and UCS.

Single nucleotide variant (SNV) characteristics of the GSDM genes in tumors

SNV analysis of the GSDM genes in tumor tissues showed that the highest mutations were in UCEC and SKCM (*Figure 2A*). However, the overall incidence of SNV was very low. The GSDM genes with the most to the least number of mutation were GSDMC, DNF5A, DNFB59, GSDMA, GSDMD, and GSDMB (*Figure 2B*). Single nucleotide polymorphisms (SNPs) were the main variant type, followed by deletions (DEL) and insertions (INS). Missense mutation in variant classification was the main type, followed by frameshift insertion, in-frame deletion, splice site, frame shift deletion, nonsense mutation, and nonstop mutation. Most of the SNV classes were C>T, followed by C>A, T>C, C>G, T>G, and T>G (*Figure 2C*).

CNV characteristics of the GSDM genes in tumors

CNV analysis revealed few mutations in LAML and THCA (*Figure 3A*). In all samples, heterozygous amplification mutations were the most common among the six genes, followed by heterozygous deletion and homozygous amplification (*Figure 3A-3C*). The incidence of homozygous deletion was very low (*Figure 3A*,*3C*). Heterozygous amplification mutations were the main mutations observed in GSDMC, GSDMD, and DFNA5 in all tumors (*Figure 3A*). GSDMA and GSDMB showed heterozygous deletion mutations in KICH, PCPG, SARC, OV, UCECC, ACC, BRCA, SKCM, and UCS, but heterozygous amplification in other tumors. DFNB59 was a heterozygous deletion mutation in KICH, SARC, ACC, CESC, BRCA, and BLCA, but a heterozygous amplification in other tumors. Heterozygous amplification occurred more often in GSDMD, GSDMC, and DNF5A than in other genes. Heterozygous deletion occurred in GSDMA, GSDMB, and DNFB59. Homozygous amplification occurred in GSDMD and GSDMC (*Figure 3A*,*3B*). The occurrence of CNV also increased the expression levels of the genes. This correlation was observed in the greatest number of tumors for GSDMD, followed by GSDMB, DFNB59, DFNA5, GSDMC, and GSDMA (*Figure 3D*).

Methylation characteristics of the GSDM genes in tumors

The methylation analysis of the GSDM genes in the TCGA database showed that gene methylation was associated with low expression of genes in most tumors. This correlation was most obvious in DFNA5, GSDMD, and GSDMB (*Figure 4A*). The genes' methylation change between tumor and normal samples in each cancers are shown in *Figure 4B*. Overall, gene methylation was decreased in tumor tissues, however, gene methylation of DFNA5 was elevated in some tumor tissues.

Pan-cancer analysis of the relationship between GSDM genes and the immune microenvironment

GSDMA, GSDMB, GSDMC, and GSDMD showed either a positive correlation with each other or no correlation at all. GSDMA was correlated with both GSDMB and GSDMC (R=0.17 and 0.38, respectively), and GSDMB was correlated with GSDMD (R=0.28). DFNA5 was negatively correlated with GSDMB and GSDMD, but positively correlated with GSDMA and GSDMC. DFNB59 was negatively correlated with GSDMA and GSDMC, but positively correlated with GSDMB. The expression of DFNA5 and DFNB59 was positively correlated with a correlation coefficient of 0.15 (*Figure 5A*). The specific correlation analysis results in each tumor are shown in Figure S3.

GSDMA, GSDMB, GSDMC, and GSDMD were positively correlated with immune scores, whereas DFNA5 and DFNB59 were poorly correlated with immune scores. GSDMA, GSDMD, and DFNA5 were positively correlated with stromal scores, while GSDMB and DFNB59 were negatively correlated with stromal scores. GSDMC had no correlation with stromal scores. GSDMA, GSDMD, and DFNA5 were negatively correlated with tumor purity, whereas DFNB59 was positively correlated with tumor purity. GSDMD showed the strongest positive correlation with immune and stromal scores, and the strongest negative correlation with tumor purity (*Figure 5B*).

GSDMA, GSDMB, GSDMC, and GSDMD were similarly correlated with different types of immune infiltrating cells, and DFNA5 and DFNB59 had similar correlations with different types of immune infiltrating cells. In particular, activated memory CD4 T cells demonstrated a strong positive correlation with GSDMA, GSDMB, GSDMC, and GSDMD and a strong negative correlation with DFNA5 and DFNB59. Monocytes and M2 macrophages had a strong negative correlation with

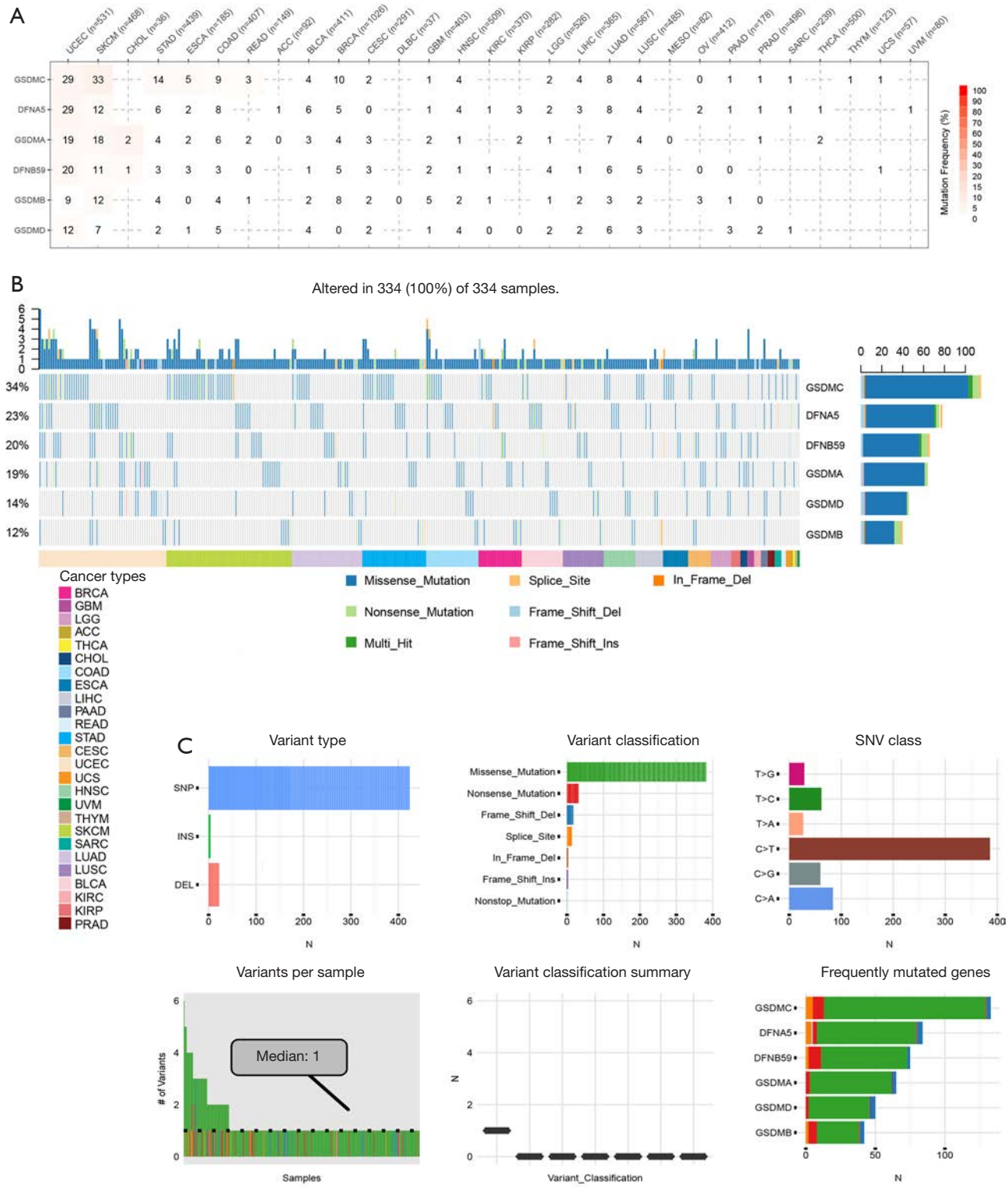


Figure 2 The SNV characteristics of the genes of GSDMs in tumors. (A) The SNV frequency of genes in each cancers. (B) The waterfall plots of SNV. (C) The summary plot displays number of variants in each sample. SNV, single nucleotide variant; GSDMs, gasdermins.

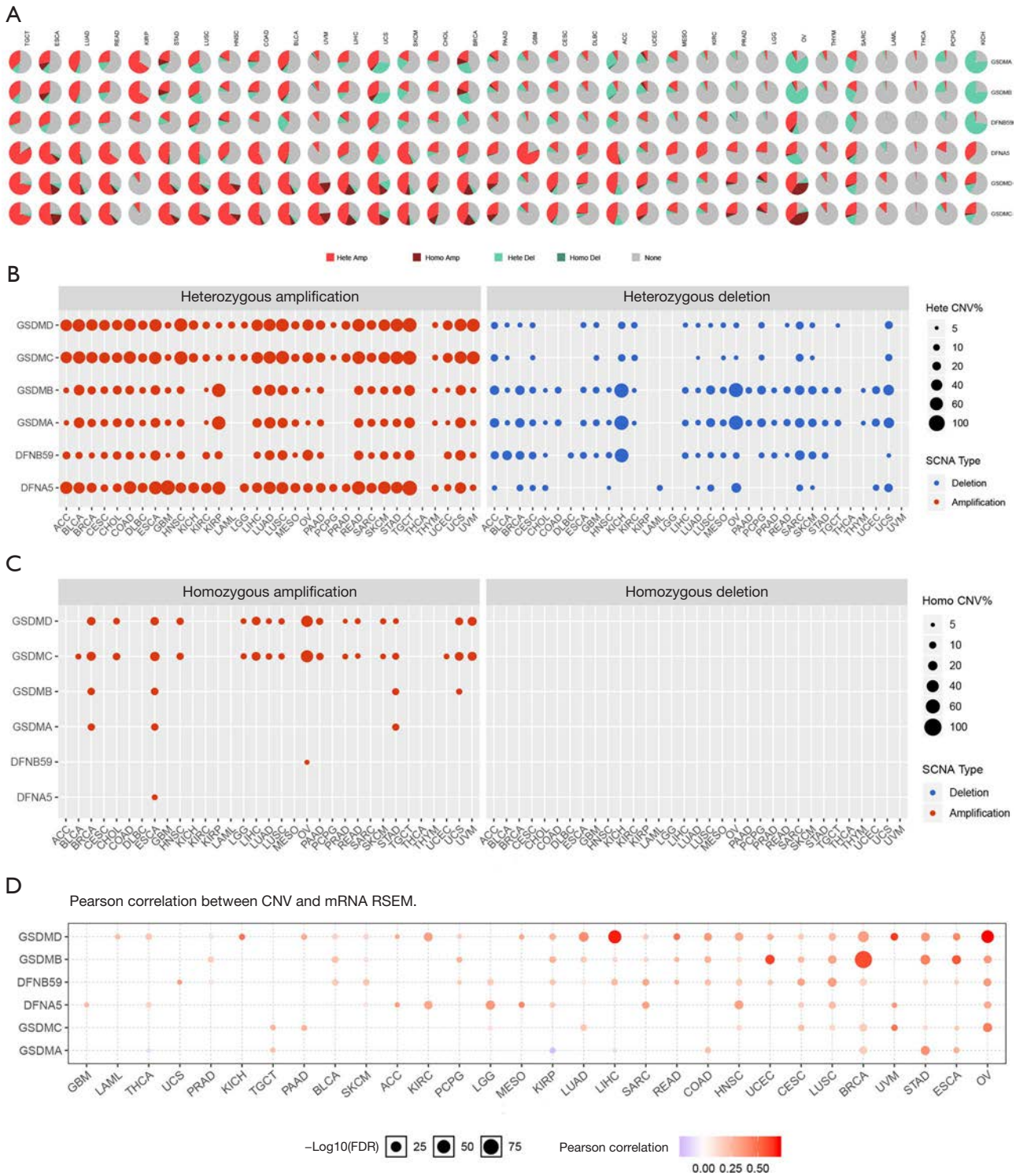


Figure 3 The CNV characteristics of the genes of GSDMs in tumors. (A) The constitute of heterozygous/homozygous CNV of each gene in each cancer. (B) The amplification and deletion percentage of heterozygous CNV about each gene in each cancer. (C) The amplification and deletion percentage of homozygous CNV about each gene in each cancer. (D) The association between paired mRNA expression and CNV percentage. CNV, copy number variation; GSDMs, gasdermins.

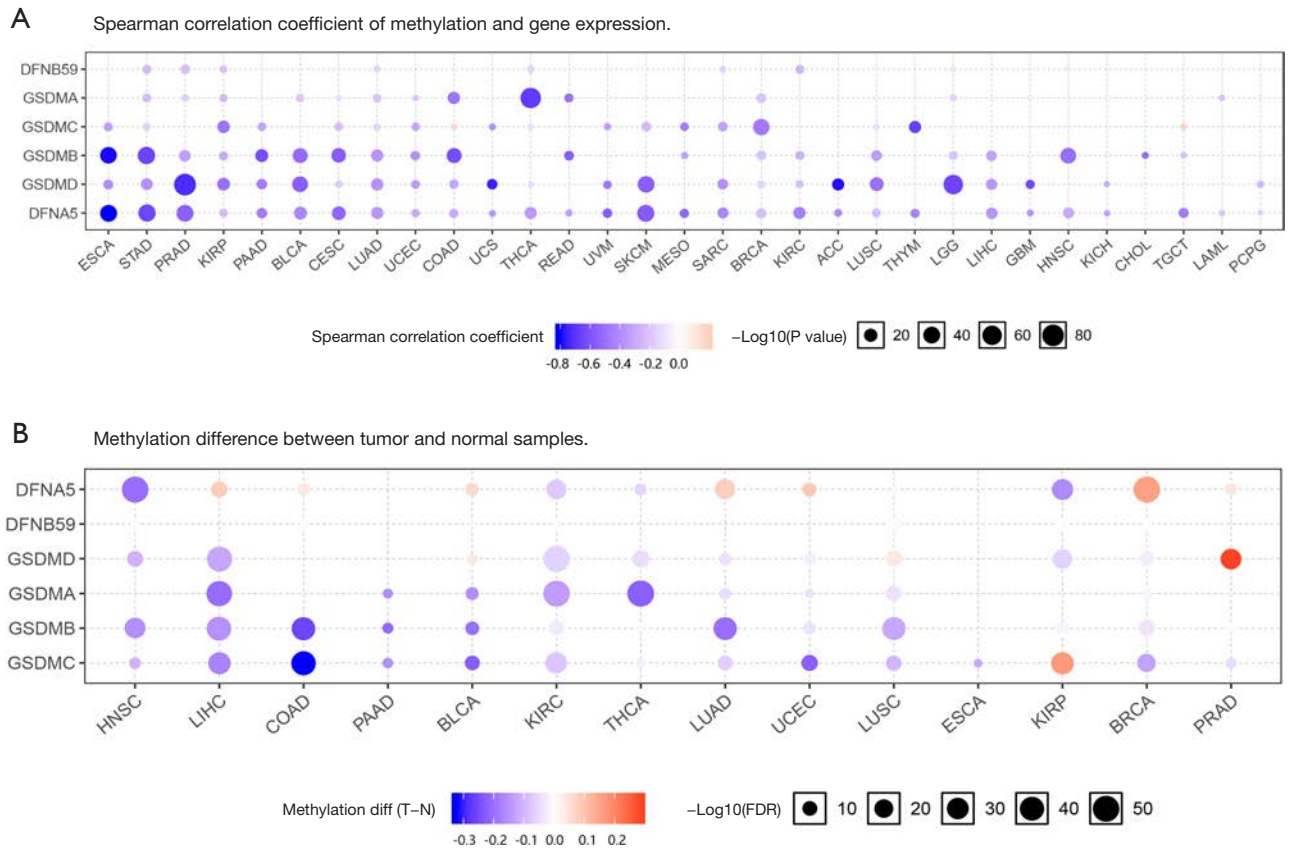
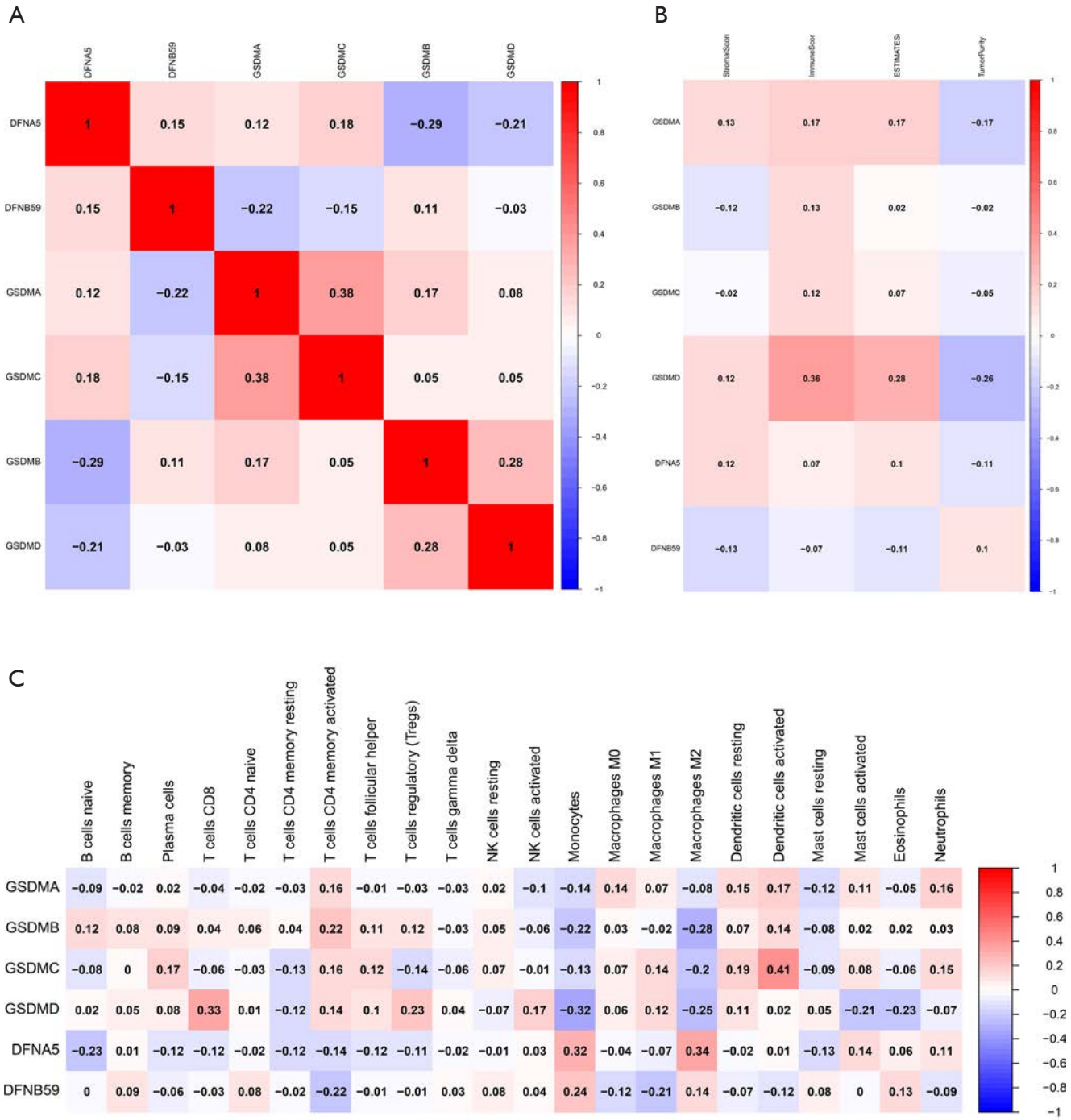


Figure 4 The methylation characteristics of the genes of GSDMs in tumors. (A) The correlation between methylation and mRNA gene expression. (B) The genes' methylation change between tumor and normal samples in each cancers. GSDMs, gasdermins.

GSDMA, GSDMB, GSDMC, and GSDMD, and a strong positive correlation with DFNA5 and DFNB59. The specific correlations are shown in the figure below (*Figure 5C*).

However, there were also specific differences in the relationship between each gene and each type of immune cell. GSDMA had the strongest positive correlation with activated dendritic cells, with a correlation coefficient of 0.17. GSDMB showed the strongest positive correlation with activated memory CD4 T cells, with a correlation coefficient of 0.22. GSDMC had the strongest positive correlation with activated dendritic cells, with a corelation coefficient of 0.41. GSDMD had the strongest positive correlation with CD8 T cells and regulatory T cells (Tregs), with correlation coefficients of 0.33 and 0.23, respectively. DFNA5 had the strongest positive correlation with monocytes and M2 macrophages, with correlation coefficients of 0.32 and 0.34, respectively. DFNB59 also had the strongest positive correlation with monocytes and M2 macrophages, with correlation coefficients of 0.24 and 0.14, respectively (*Figure 5C* and Figure S4).

Gene expression analysis in different immune subtypes showed that the expression characteristics of GSDMA, GSDMB, GSDMC, and GSDMD were similar among varying immune subtypes of tumors. They all had higher expression in C2 and C6, and lower expression in C1, C3, C4, and C5. The expression of GSDMD was slightly different, and its expression in C5 was significantly reduced. The expression of DFNA5 and DFNB59 in different immune subtypes was similar. Their expression in C1, C2, C3, C4, and C5 gradually increased, with the highest expression in C5, which then decreased in C6 (*Figure 5D*). The overall analysis across cancer species showed that GSDMA, GSDMB, GSDMC, and GSDMD had similar immune microenvironment characteristics, and DFNA5 and DFNB59 had similar immune microenvironment characteristics. However, each had its own characteristics.



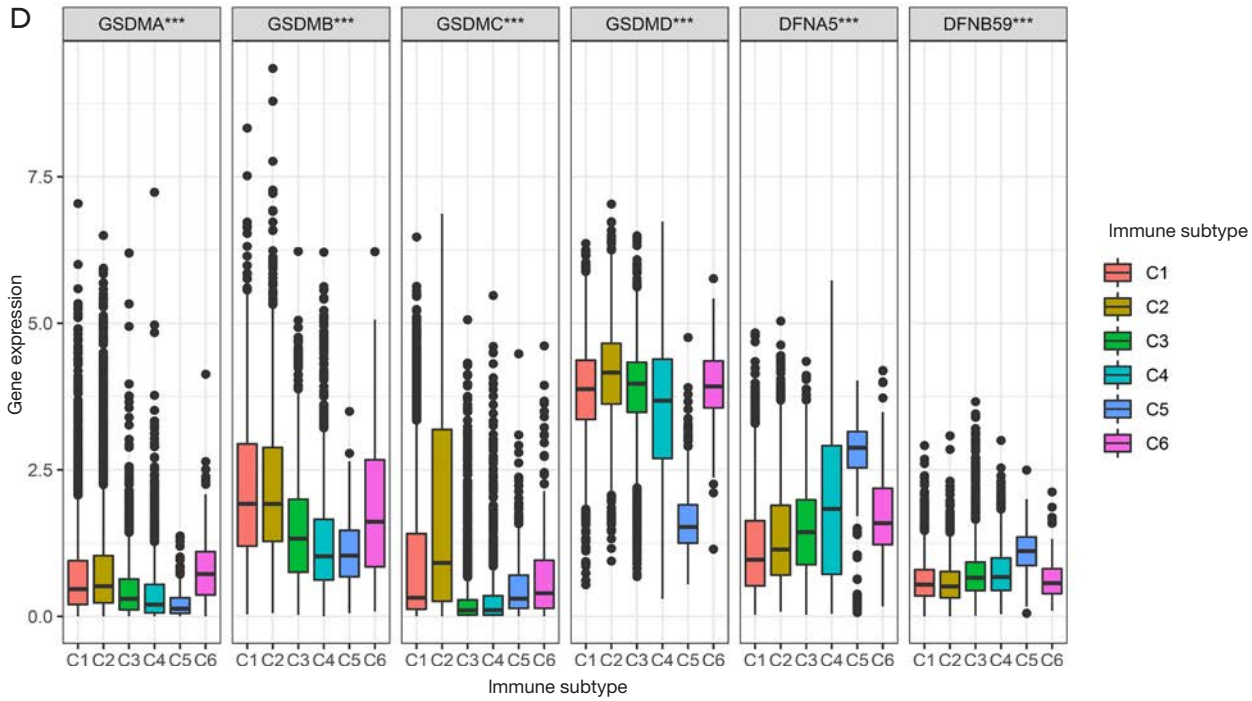


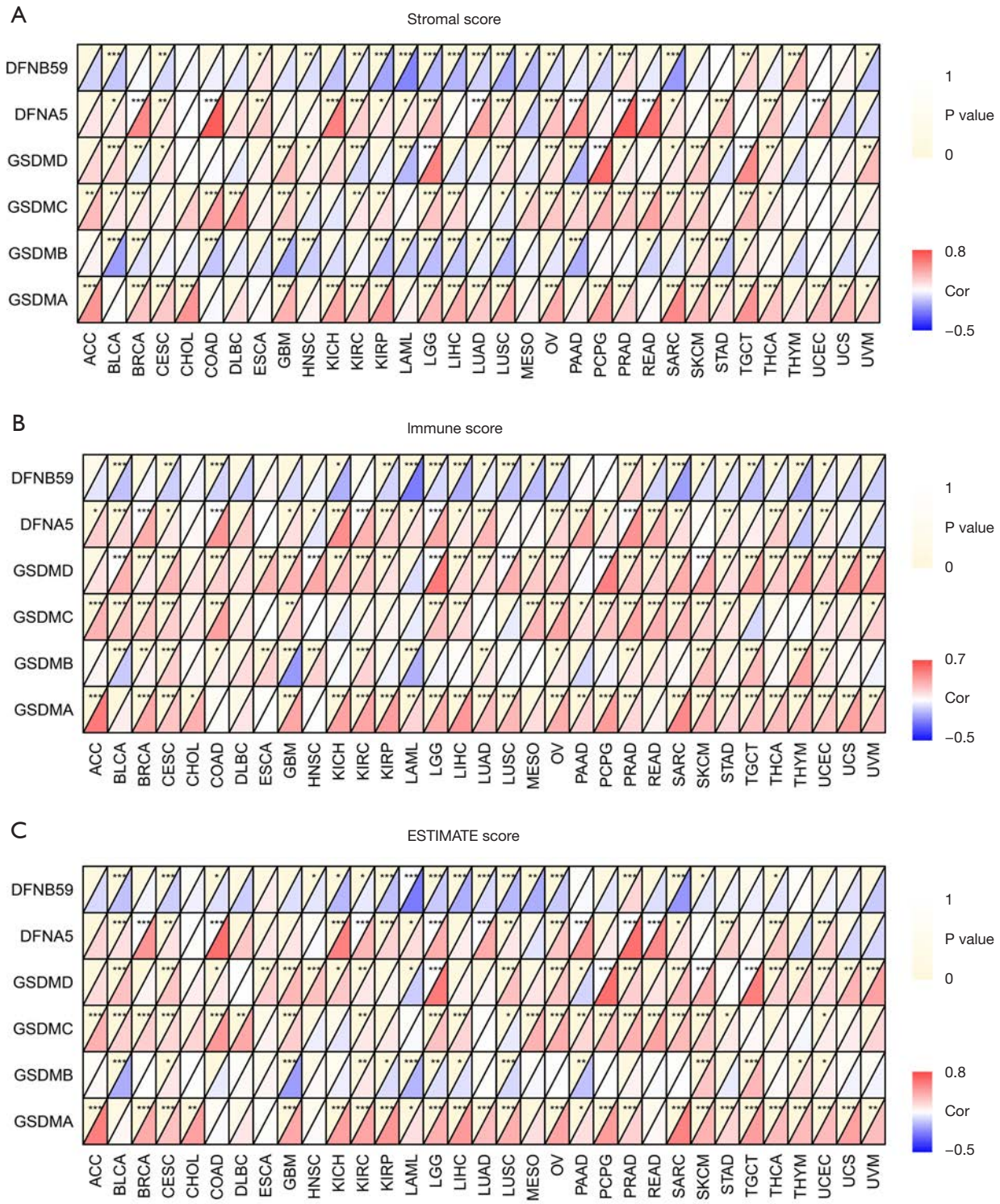
Figure 5 Pan-cancer analysis of the relationship between the genes of GSDMs and immune microenvironment. (A) The correlation of the genes expression of GSDMs based on all cancer species. (B) The correlation analysis of genes expression of GSDMs and immune and stromal scores based on all cancer species. (C) The correlation analysis of genes expression of GSDMs and immune cell infiltration based on all cancer species. (D) The relationship between the genes expression of GSDMs and immune subtypes based on all cancer species. ***, P<0.001. GSDMs, gasdermins.

Correlation between GSDM genes and immune and stromal scores in each cancer

GSDMA had a positive correlation with the stromal score in most tumors. Moreover, GSDMC, GSDMD, and DFNA5 were positively correlated with stromal score in some tumors, whereas GSDMB and DFNB59 showed a negative correlation in most tumors (*Figure 6A*). GSDMA and GSDMD showed a positive correlation with immune score in most tumors. GSDMB, GSDMC, and DFNA5 showed a positive correlation with immune score in some tumors, but no correlation was found in other tumors. DFNB59 showed a negative correlation with immune score in most tumors (*Figure 6B*). The ESTIMATE score is a combination of the stromal score and the immune score. GSDMA, GSDMC, GSDMD, and DFNA5 were positively correlated with the ESTIMATE score of most tumors, whereas GSDMB was negatively correlated with the ESTIMATE score of some tumors. DFNB59 had a positive correlation with the ESTIMATE score of most tumors (*Figure 6C*). GSDMA, GSDMC, GSDMD, and DFNA5 were negatively correlated with tumor purity in most tumors. GSDMB had a negative correlation with purity in some tumors, whereas DFNB59 was positively correlated with purity in most tumors (*Figure 6D*).

Correlation between the GSDM genes and immune cell infiltration in each type of cancer

GSDMA expression was positively correlated with the infiltration of activated memory CD4 T cells and negatively correlated with the infiltration of activated natural killer (NK) cells, monocytes, and resting mast cells in most tumors (*Figure 7A*). GSDMB expression was positively correlated with the infiltration of CD8 T cells, follicular helper T cells, and Tregs in some tumors, but negatively correlated with the infiltration of monocytes, M0 macrophages, M2 macrophages, and resting dendritic cells in some tumors (*Figure 7B*). GSDMC expression was positively correlated with activated memory CD4 T cells, M1 macrophages, activated dendritic cells, and neutrophil



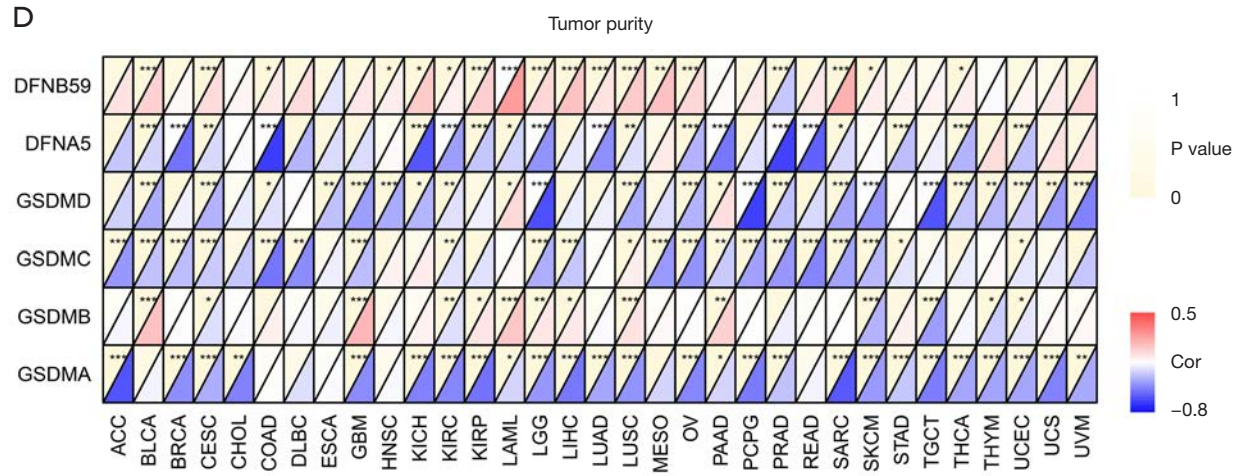


Figure 6 The correlation between the genes of GSDMs and immune and stromal scores in each cancer. (A) The correlation between the genes of GSDMs and immune score in each cancer. (B) The correlation between the genes of GSDMs and stromal score in each cancer. (C) The correlation between the genes of GSDMs and ESTIMATE Score in each cancer. (D) The correlation between the genes of GSDMs and Tumor Purity in each cancer. *, P<0.05; **, P<0.01; ***, P<0.001. GSDMs, gasdermins.

infiltration in most tumors, but negatively correlated with the infiltration of naïve B cells, CD8 T cells, resting memory CD4 T cells, and Tregs in most tumors (*Figure 7C*). GSDMD expression was positively correlated with the infiltration of CD8 T cells, activated memory CD4 T cells, follicular helper T cells, Tregs, activated NK cells, and M1 macrophages in most tumors. However, it was negatively correlated with the infiltration of resting memory CD4 T cells, M0 macrophages, M2 macrophages, resting mast cells, eosinophils, and neutrophils in most tumors (*Figure 7D*). DFNA5 expression was positively correlated with the infiltration of monocytes, M0 macrophages, M2 macrophages, and neutrophils in some tumors and negatively correlated with the infiltration of naïve B cells, plasma cells, CD8 T cells, resting memory CD4 T cells, and resting mast cells in some tumors (*Figure 7E*). DFNB59 expression was positively correlated with the infiltration of M2 macrophages and resting mast cells in some tumors. No correlation was found with the other immune cells (*Figure 7F*).

Correlation between the GSDM genes and immune checkpoint-related gene expression in each cancer

GSDMA was positively correlated with the expression of immune checkpoint-related genes in ACC, BRCA, CESC, CHOL, GBM, KICH, KIRC, KIRP, LAML, LGG, LIHC, LUAD, LUSC, OV, PCPG, PRAD, SARC, TGCT, THCA, THYM, UCEC, UCS, and UVM. No correlation was detected in BLCA, COAD, DLBC, ESCA, HNSC, MESO, PAAD, READ, SKCM, nor STAD (*Figure 8A*). GSDMB was negatively correlated with most immune checkpoint genes in BLCA and GBM and some immune checkpoint genes in LAML and THYM. A strong positive correlation was found with most immune checkpoint genes in HNSC, KIRC, SKCM, and TGCT and some immune checkpoint genes in THYM. In other tumors, a weak positive correlation was found with certain immune checkpoint genes. In addition, it exhibited a strong positive correlation with TNFRSF25 in most tumors, and a strong negative correlation was found with CD276 in most tumors (*Figure 8B*). GSDMC was positively correlated with some or most immune checkpoint genes in ACC, BLCA, BRCA, CESC, CHOL, COAD, DLBC, LGG, LIHC, MESO, OV, PAAD, PCPG, PRAD, READ, SARC, THYM, UCEC, and UVM. In ESCA, GBM, HNSC, KICH, KIRC, KIRP, LAML, LUAD, LUSC, SKCM, STAD, TGCT, THCA, and UCS, no correlation or negative correlation was found with most immune checkpoint-related genes (*Figure 8C*). GSDMD was negatively correlated with the expression of most immune checkpoint genes in LAML. In PAAD, a negative correlation was found with some immune checkpoint-related genes, whereas some immune checkpoint genes showed a positive correlation. In the

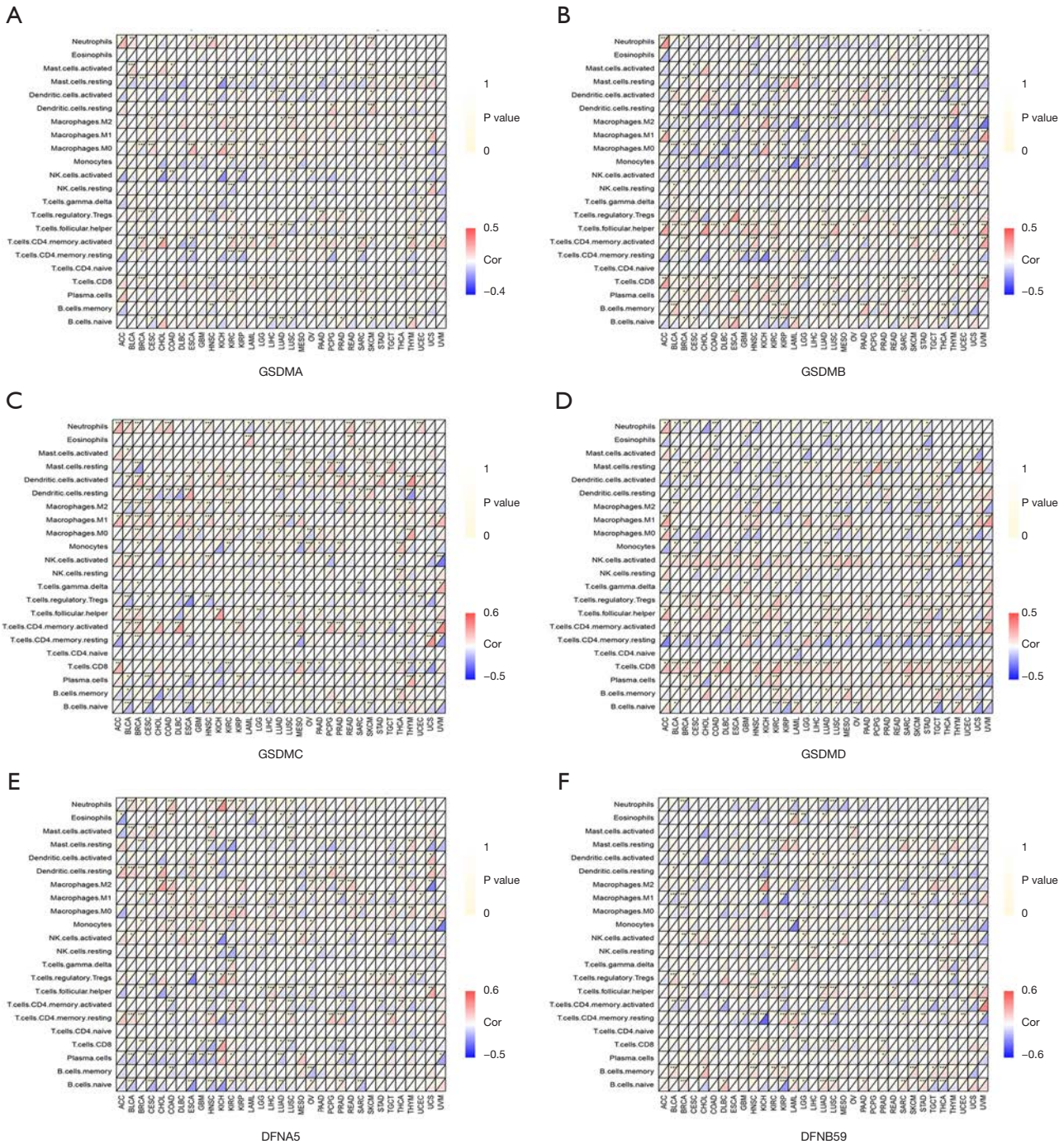


Figure 7 The correlation between the genes of GSDMs and immune cell infiltration in each cancer. (A) The correlation between the genes of GSDMA and immune cell infiltration in each cancer. (B) The correlation between the genes of GSDMB and immune cell infiltration in each cancer. (C) The correlation between the genes of GSDMC and immune cell infiltration in each cancer. (D) The correlation between the genes of GSDMD and immune cell infiltration in each cancer. (E) The correlation between the genes of DFNA5 and immune cell infiltration in each cancer. (F) The correlation between the genes of DFNB59 and immune cell infiltration in each cancer. *, P<0.05; **, P<0.01; ***, P<0.001. GSDMs, gasdermins.

remaining tumors, a significant positive correlation was found with most immune checkpoint-related genes. However, a significant negative correlation was found with NRP1 in some tumors. The correlation with BTNL2 and TNFSF18 in most tumors was not strong and even negative in some tumors (*Figure 8D*). DFNA5 showed a positive correlation with most immune checkpoint-related genes in ACC, BLCA, BRCA, COAD, KICH, KIRC, KIRP, LAML, LGG, LIHC, LUAD, OV, PAAD, PCPG, PRAD, READ, and THCA. In CESC, CHOL, DLBC, ESCA, GBM, HNSC, LUSC, MESO, SARC, SKCM, STAD, TGCT, THYM, UCEC, UCS, and UVM. A weak positive or negative correlation was found with some immune checkpoint-related genes (*Figure 8E*). DFNB59 was positively correlated with the expression of most immune checkpoint genes in PRAD. In ESCA and HNSC, a positive correlation was found with some immune checkpoint-related genes. In most other tumors, a negative correlation was found with most immune checkpoint-related genes. However, a significant positive correlation with TNFRSF25 was found in most tumors (*Figure 8F*).

Correlation between the GSDM genes and MHC-related gene expression in each cancer

Similar to the correlation with the immune checkpoint-related gene expression, an analysis of the relationship between the GSDM genes and MHC molecules demonstrated that GSDMA, GSDMB, GSDMC, GSDMD, and DFNA5 were positively correlated with the expression of MHC molecule-related genes in tumors (*Figure 9A-9E*), while DFNB59 was negatively correlated with the expression of MHC molecule-related genes in most tumors (*Figure 9F*). However, GSDMD had a strong positive correlation with MHC molecules in almost all tumors (*Figure 9D*).

Correlation between the GSDM genes and chemokines and their receptor-related gene expression in each cancer

A similar phenomena were also observed in the relationship between GSDMs and chemokines and chemokines receptor-related gene expression. GSDMA, GSDMB, GSDMC, GSDMD, and DFNA5 were positively correlated with chemokines and chemokines receptor-related gene expression in tumors (*Figure 10A-10E*), while DFNB59 showed negative correlation (*Figure 10F*). GSDMD had a strong positive correlation with chemokines and chemokines receptor-related gene expression in almost all tumors (*Figure 10D*).

Correlation between GSDMD gene methylation and the expression of immune checkpoint-related genes, MHC molecules, chemokines and their receptor-related genes

Since methylation can inhibit GSDM gene expression, the tumor-immune system interactions and drug bank (TISIDB) database was used to analyze the correlation between GSDMD gene methylation and the expression of immune checkpoint-related genes, MHC molecules-related genes, chemokines and receptor-related genes. In most tumors, the methylation of GSDMD was negatively correlated with the expression of immune checkpoint genes (*Figure 11A*,*11B*), chemokines and their receptor genes (*Figure 11C*,*11D*), MHC molecule-related genes (*Figure 11E*).

GO and KEGG pathway enrichment

The results of GO enrichment and KEGG pathway enrichment of different genes varied among the tumors (Figure S5A,S5B). When the GO enrichment results were clustered, the relationship between genes and immune scores was closely related to the function set shown in the black box after cluster analysis (*Figure 12* and Figure S6). When genes were positively correlated with immune scores, genes were more enriched in the function concentration shown in the box. The analysis of the function set shown in the black box revealed that the function set was related to immunity (*Figure 12*). KEGG enrichment analysis found no correlation with immune score (Figure S5B).

Discussion

Our analysis showed that GSDMA was most highly expression in skin tissues. GSDMB was highly expressed in most tissues, but the highest expression was detected in the small intestines. GSDMC was lowly expressed in most tissues, and it was mainly expressed in the skin, spleen, vagina, esophagus, salivary gland, and cervix uteri. GSDMD was highly expressed GSDM in most tissues, with the highest expression detected in the spleen. The expression of DFNA5 in tissues was second only to that of GSDMB, and its expression was highest in the uterus. However, the expression of DFNB59 was generally lower than that of DFNA5. DFNB59 was expressed in various tissues, with the highest expression detected in the testis, followed by

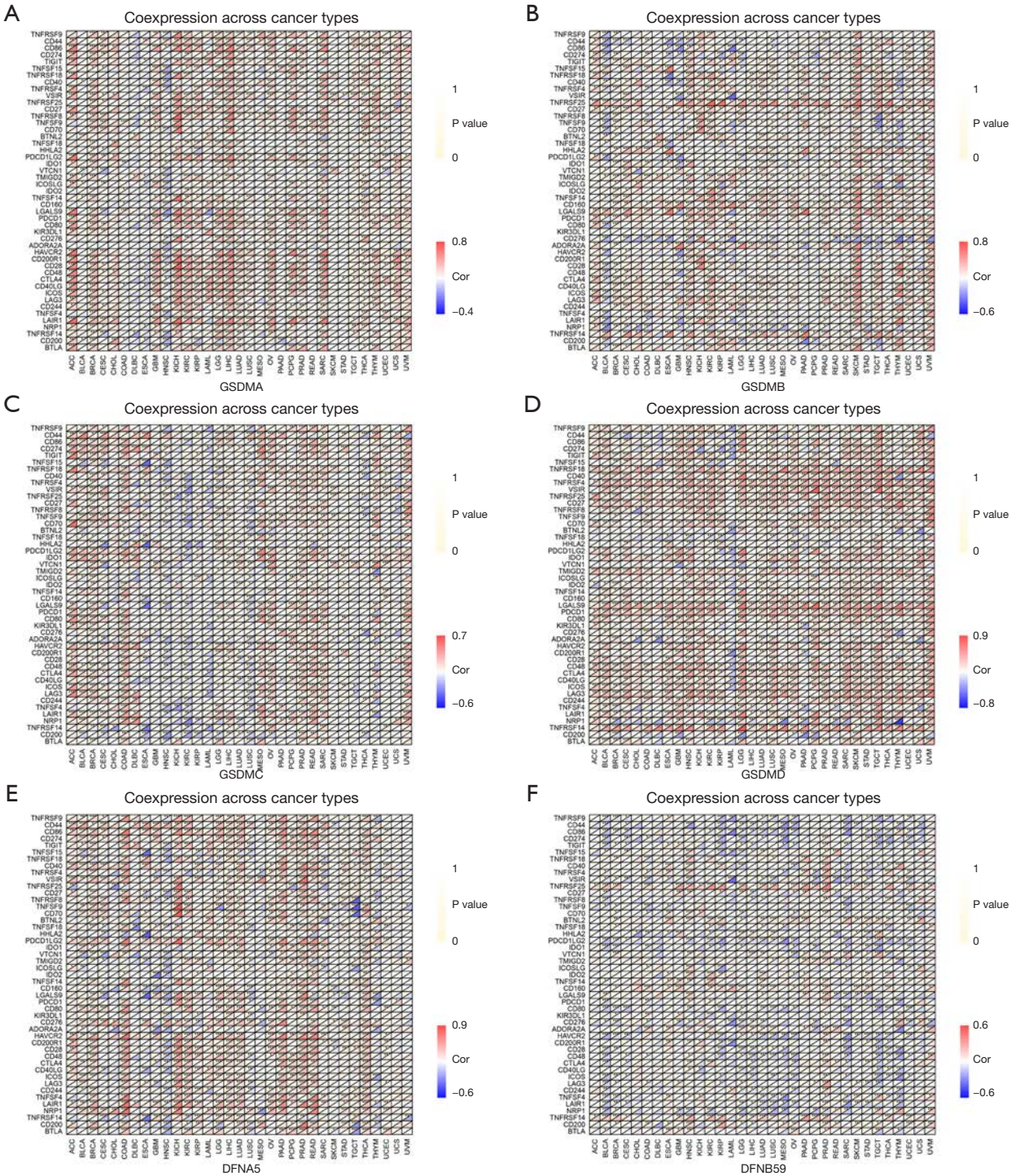


Figure 8 The correlation between the genes of GSDMs and immune checkpoint-related gene expression in each cancer. (A) The correlation between the genes of GSDMA and immune checkpoint-related gene expression in each cancer. (B) The correlation between the genes of GSDMB and immune checkpoint-related gene expression in each cancer. (C) The correlation between the genes of GSDMC and immune checkpoint-related gene expression in each cancer. (D) The correlation between the genes of GSDMD and immune checkpoint-related gene expression in each cancer. (E) The correlation between the genes of DFNA5 and immune checkpoint-related gene expression in each cancer. (F) The correlation between the genes of DFNB59 and immune checkpoint-related gene expression in each cancer. *, P<0.05; **, P<0.01; ***, P<0.001. GSDMs, gasdermins.

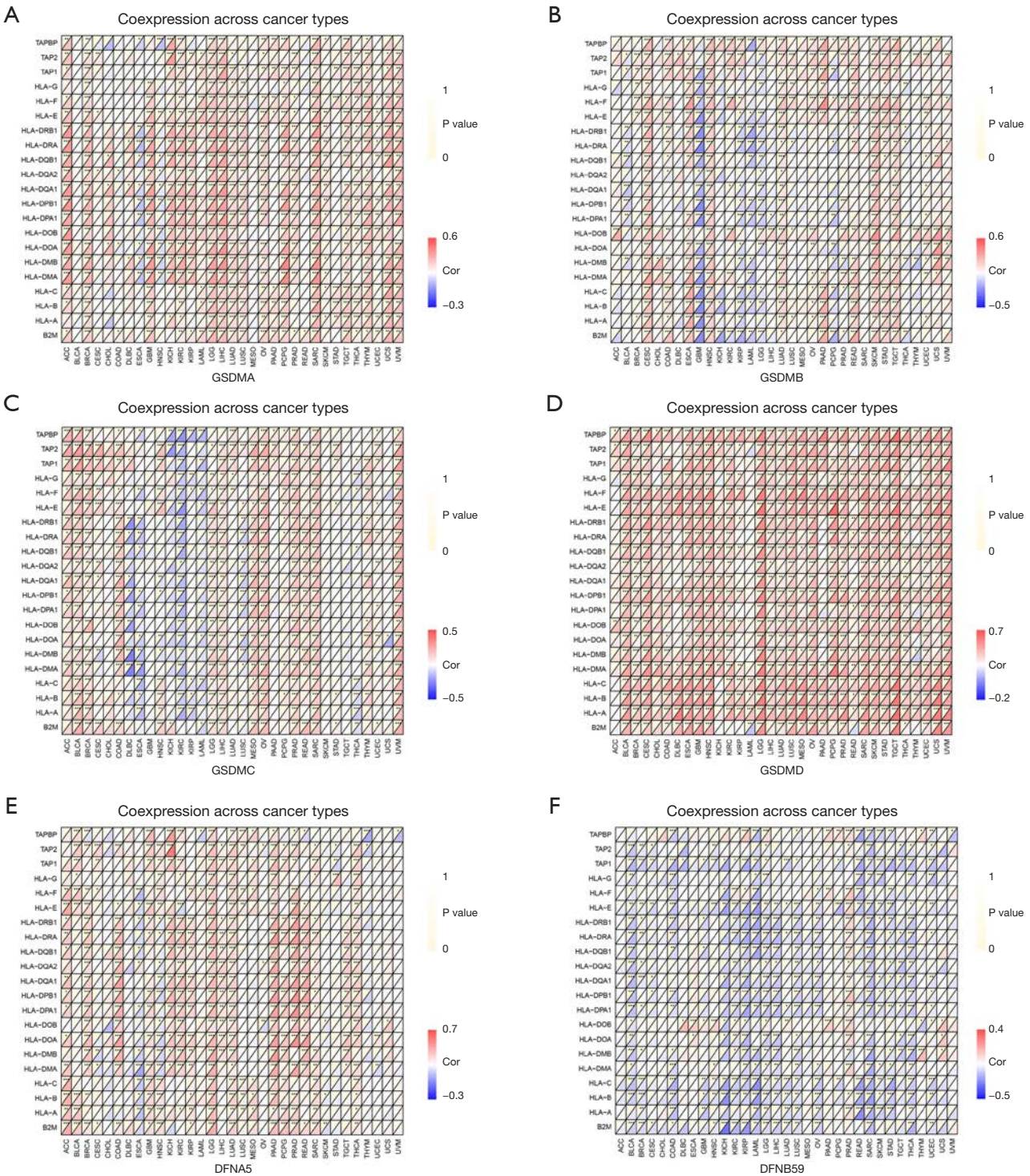


Figure 9 The correlation between the genes of GSDMs and MHC-related gene expression in each cancer. (A) The correlation between the genes of GSDMA and MHC-related gene expression in each cancer. (B) The correlation between the genes of GSDMB and MHC-related gene expression in each cancer. (C) The correlation between the genes of GSDMC and MHC-related gene expression in each cancer. (D) The correlation between the genes of GSDMD and MHC-related gene expression in each cancer. (E) The correlation between the genes of DFNA5 and MHC-related gene expression in each cancer. (F) The correlation between the genes of DFNB59 and MHC-related gene expression in each cancer. *, P<0.05; **, P<0.01; ***, P<0.001. GSDMs, gasdermins; MHC, major histocompatibility complex.

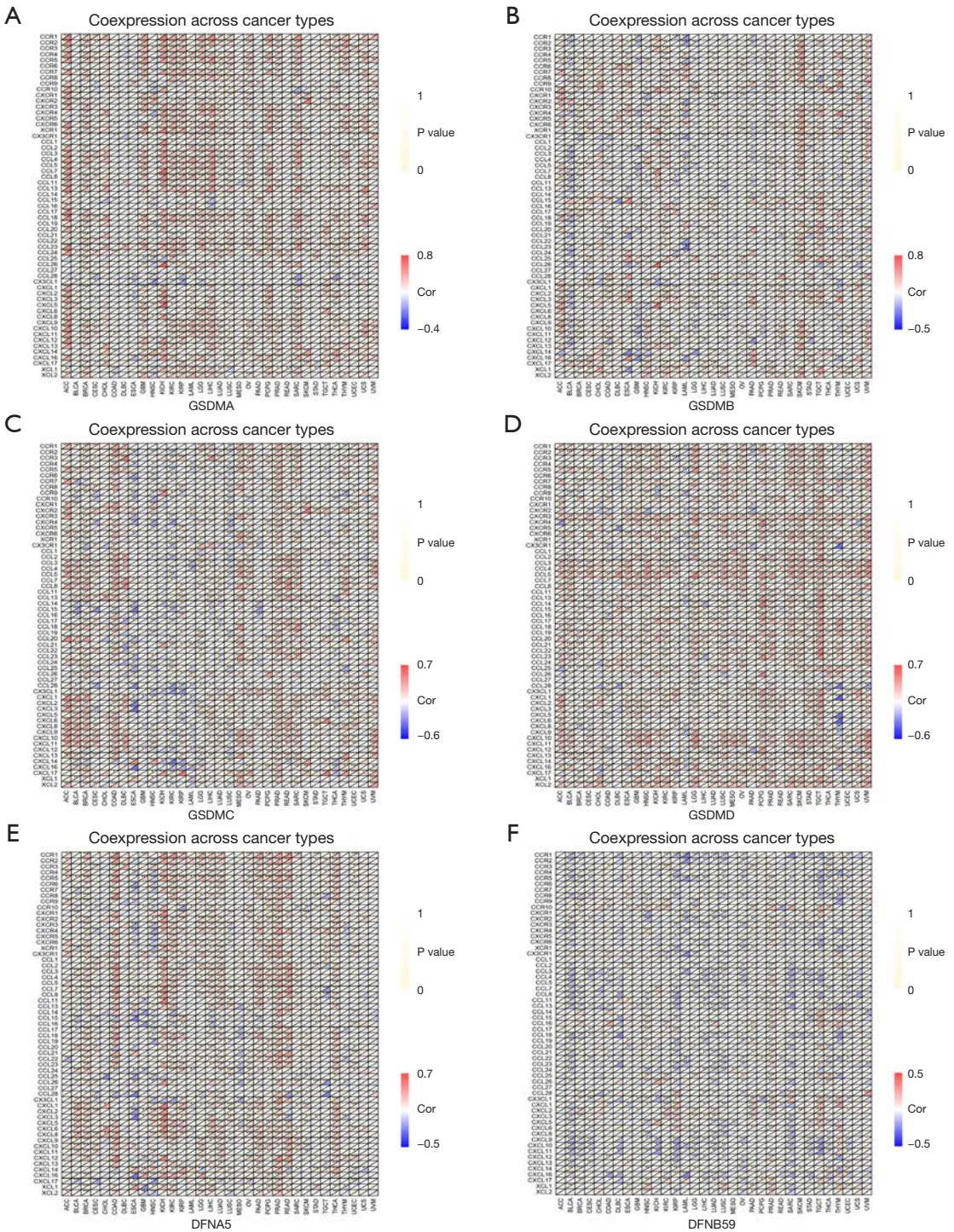


Figure 10 The correlation between the genes of GSDMs and chemokines and their receptor-related gene expression in each cancer. (A) The correlation between the genes of GSDMA and chemokines and their receptor-related gene expression in each cancer. (B) The correlation between the genes of GSDMB and chemokines and their receptor-related gene expression in each cancer. (C) The correlation between the genes of GSDMC and chemokines and their receptor-related gene expression in each cancer. (D) The correlation between the genes of GSDMD and chemokines and their receptor-related gene expression in each cancer. (E) The correlation between the genes of DFNA5 and chemokines and their receptor-related gene expression in each cancer. (F) The correlation between the genes of DFNB59 and chemokines and their receptor-related gene expression in each cancer. *, P<0.05; **, P<0.01; ***, P<0.001. GSDMs, gasdermins.

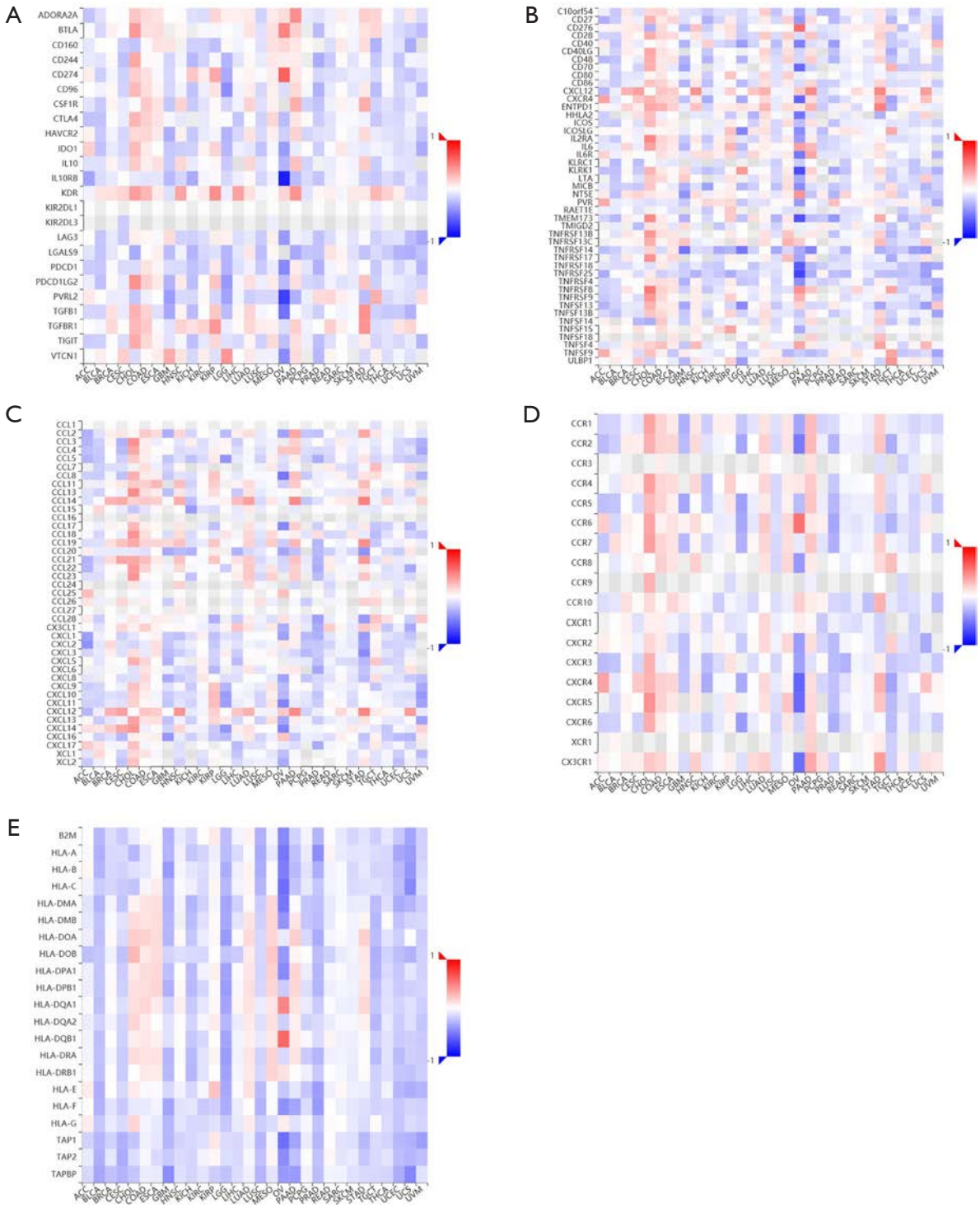


Figure 11 The correlation between GSDMD gene methylation and the expression of immune checkpoint-related genes, MHC molecules, chemokines and their receptor-related genes. (A) The correlation between GSDMD gene methylation and the expression of immunoinhibitory-related genes. (B) The correlation between GSDMD gene methylation and the expression of immunostimulatory-related genes. (C) The correlation between GSDMD gene methylation and the expression of chemokine-related genes. (D) The correlation between GSDMD gene methylation and the expression of chemokine receptor-related genes. (E) The correlation between GSDMD gene methylation and the expression of MHC molecule-related genes. GSDMs, gasdermins; MHC, major histocompatibility complex.

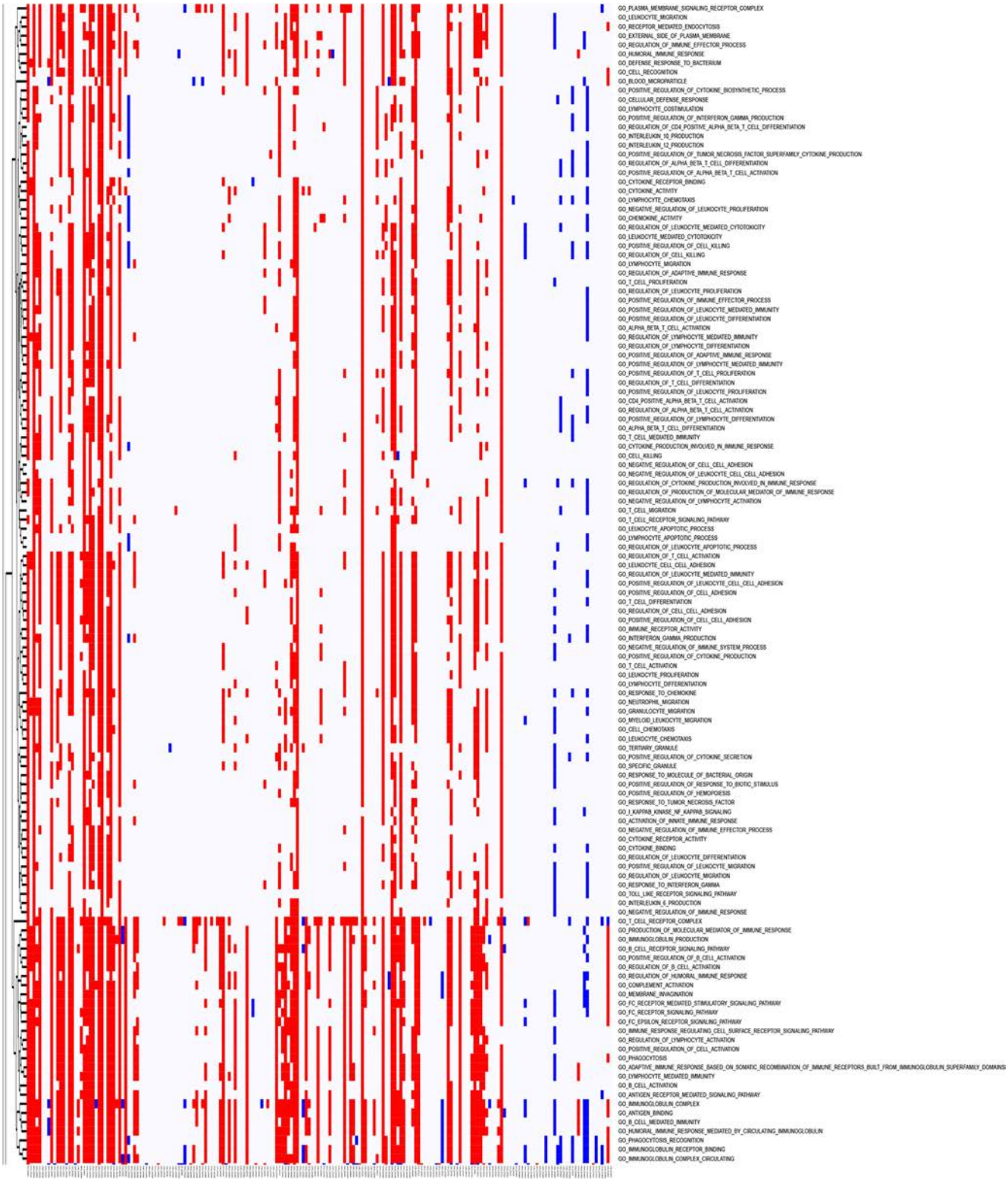


Figure 12 All GO enrichment results after clustering of the genes of GSDMs in each cancer (red represents NES is positive, blue represents NES is negative). GO, Gene Ontology; GSDMs, gasdermins; NES, normalized enrichment scores.

the pituitary and ovaries. The level of gene expression in normal tissue from high to low was as follows: GSDMD, GSDMB, DFNA5, DFNB59, GSDMA, and GSDMC.

GSDMA was highly expressed in COA. GSDMB was highly expressed in HNSC, PAAD, SARC, STAD, and THYM. GSDMC was highly expressed in CESC, KICH, LUAD, and LUSC. GSDMD was highly expressed in CHOL, DLBC, GBM, HNSC, LAML, PAAD, SKCM, and THYM. DFNA5 was highly expressed in ACC, CHOL, DLBC, GBM, HNSC, LGG, PAAD, PCPG, SKCM, and THYM. DFNB59 was highly expressed in DLBC and THYM. GSDMD was expressed in all tumor tissues, and its expression level was the highest, followed by GSDMB, DFNA5, DFNB59, GSDMA, and GSDMC. This finding was consistent with previous research (22).

Gene mutation and methylation can regulate gene expression (23,24). The study demonstrated that the incidence of SNV mutations was very low. CNV and methylation were found to affect gene expression. CNV mutations increased gene expression, and this was observed with GSDMD in most tumors. Methylation decreased gene expression, and this was most significant for DFNA5, GSDMD, and GSDMB. Our analysis revealed that the level of gene methylation in tumor tissues was generally decreased. However, in some tumors, elevation in the level of gene methylation was observed, such as for DFNA5. Furthermore, gene expression was lower in DFNA5-hypermethylated tumors than in normal tissues. Therefore, the differences in GSDM gene expression between tumors and normal tissues may be affected by methylation and gene mutations. Previous studies also found that methylation can regulate DFNA5 expression in breast cancer (25).

The six immune subtypes across cancer types are wound healing, interferon (IFN)-γ dominant, inflammatory, lymphocyte depleted, immunologically quiet, and transforming growth factor (TGF)-β dominant (18). The analysis of the expression of the GSDM genes in different immune subtypes revealed that GSDMA, GSDMB, GSDMC, and GSDMD had similar expression characteristics in different immune subtypes, but that of GSDMD was slightly different. These genes were expressed highly in C2 and C6, whereas low expression was found in C1, C3, C4, and C5. DFNA5 and DFNB59 had similar expression distribution of immune subtypes. The gene expression gradually increased from C1, C2, C3, C4, and C5, with the highest expression in C5 and a significant decrease observed in C6. This finding suggested that GSDMA, GSDMB, GSDMC, and GSDMD may have similar expression characteristics in different immune microenvironments. Moreover, DFNA5 and DFNB59 may have similar expression characteristics in different immune microenvironments. Further analysis of the correlation of GSDM gene expression and immune infiltrating cells revealed that high expression of GSDMA, GSDMB, GSDMC, and GSDMD showed similar characteristics of immune cell infiltration, and GSDMD had the strongest correlation with immune cell infiltration. Their high expression levels were often accompanied by high infiltration of some or all of the CD8 T cells, M1 macrophages, Tregs, and neutrophils in the tumor microenvironment. This finding was consistent with their high expression in C2 and C6, which are strongly lymphocyte infiltrating immune types. C2 has the highest M1/M2 macrophage polarization, a strong CD8 signal and, and, like C6, has the greatest TCR diversity were found (18). C6 displays the highest TGF-β signature and a high lymphocytic infiltrate with an even distribution of Type I and Type II T cells (18).

DFNA5 and DFNB59 showed similar immune cell infiltration characteristics. High expression of DFNA5 and DFNB59 was usually accompanied by high infiltration of monocytes and M2 macrophages, which was consistent with their high expression in C4 and C5. C4 displays a more prominent macrophage signature with suppressed Th1 and a high M2 response (18), while C5 exhibits the lowest lymphocyte and the highest macrophage responses, dominated by M2 macrophages (18).

Immune and stromal scores can predict the fraction of stromal and immune cells in tumor tissues. The current study demonstrated that GSDMA, GSDMB, GSDMC, and GSDMD had a strong positive correlation with immune scores. This correlation was the strongest for GSDMD. The correlation between DFNA5 and DFNB59 and immune score was very weak or even negative. Therefore, the results of pan-cancer analysis of the correlation between the genes of GSDMs and immune subtypes, immune cell infiltration, and immune score showed that GSDMA, GSDMB, GSDMC, and GSDMD had similar immune microenvironment characteristics, whereas DFNA5 and DFNB59 had similar immune microenvironments.

Further analysis in different cancer types revealed that GSDMA and GSDMD were positively correlated with immune scores in most tumors, while GSDMB, GSDMC, and DFNA5 were positively correlated with immune scores in some tumors. DFNB59 was negatively correlated with immune scores in most tumors. In tumors that were

positively correlated with immune scores, the expression of the GSDM genes was often positively correlated with the cell infiltration of some or all of the following cell types: CD8 T cells, M1 macrophages, Tregs, and neutrophils. This is likely because a high immune score is often associated with high infiltration (Figure S7). Studies showed that immune checkpoint genes were expressed in these cells (26). Therefore, genes that are positively associated with immune scores in tumors are often also positively correlated with the expression of immune checkpoint genes. In addition, in tumors where GSDM gene expression is positively correlated with immune scores and immune checkpoint gene expression, high GSDMs gene expression is often accompanied by increased expression of chemokines and their receptors, and MHC-related genes. Notably, GSDMD has a strong positive correlation with these indicators in almost all tumors. High expression of chemokines and their receptors can induce increased immune cell infiltration, and high expression of MHC-related molecules can help immune cells recognize cancer cell antigens and enter the immune microenvironment. DFNB59 was negatively correlated with immune scores in most tumors, and high expression of DFNB59 was often accompanied by low expression of MHC molecules, chemokines and their receptor-related genes.

Therefore, with the exception of DFNB59 , tumors with high expression of GSDMs, especially GSDMD, are often susceptible to treatment with immune checkpoint inhibitors . Combining immune checkpoint inhibitors in this environment is helpful for the body's anti-tumor immunity. In contrast, high expression of DFNB59 is often accompanied by lowered immune scores, lowered immune cell infiltration, lowered expression of chemokines and their receptors, and lowered expression of immune checkpoint-related genes. Tumors with such a microenvironment are often called cold tumors, and the application of immune checkpoint inhibitors may not be effective.

In addition, methylation can inhibit GSDM gene expression. Our analysis demonstrated that with the exception of DFNB59, the methylation of GSDMs-related genes, especially hypermethylation of GSDMD, is accompanied by low immune cell infiltration, low expression of MHC molecule-related genes, low expression of chemokines and receptor-related genes, and low expression of immune checkpoint-related genes. Thus, altering GSDM gene expression through methylation can change the tumor microenvironment.

GO enrichment of the GSDM genes in various cancers showed that tumors with a positive correlation between the GSDM genes and immune scores often presented similar GO enrichment results. Moreover, these genes were mainly enriched in immune-related functions, confirming the role of GSDM genes in the tumor immune microenvironment.

In summary, GSDMD was the most highly expressed GSDM gene in normal and tumor tissues, followed by GSDMB, DFNA5, DFNB59, GSDMA, and GSDMC. GSDM gene expression was affected by CNV and methylation. While DFNB59 tended to show a negative correlation with immune score, immune cell infiltration, and the expression of MHC molecules, chemokines and their receptors, and immune checkpoint genes, GSDMA, GSDMB, GSDMC, GSDMD, and DFNA5 showed positive correlation in some or most tumors. Among them, GSDMD showed the strongest correlation with the immune microenvironment-related indicators in almost all tumors. Tumors with elevated immune cell infiltration and high expression of MHC molecules, chemokines and their receptors, and immune checkpoint-related genes are strongly suitable for treatment with immune checkpoint inhibitors. Therefore, these genes, in particular, GSDMD, may be potential therapeutic targets for changing the tumor microenvironment, and potential biomarkers for predicting therapeutic outcomes, especially with immune checkpoint inhibitor therapy.

The main data of this study comes from TCGA and GEO database. Future work should verify our research results in cell and animal experiments and human tissue samples, as well as examine the therapeutic efficacy of combined checkpoint inhibitor therapy and GSDM gene intervention.

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Footnote

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Conflicts of Interest: All authors have completed the ICMJE

uniform disclosure form (available at https://dx.doi.org/10.21037/tcr-21-1635). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). Institutional ethical approval and informed consent were waived.

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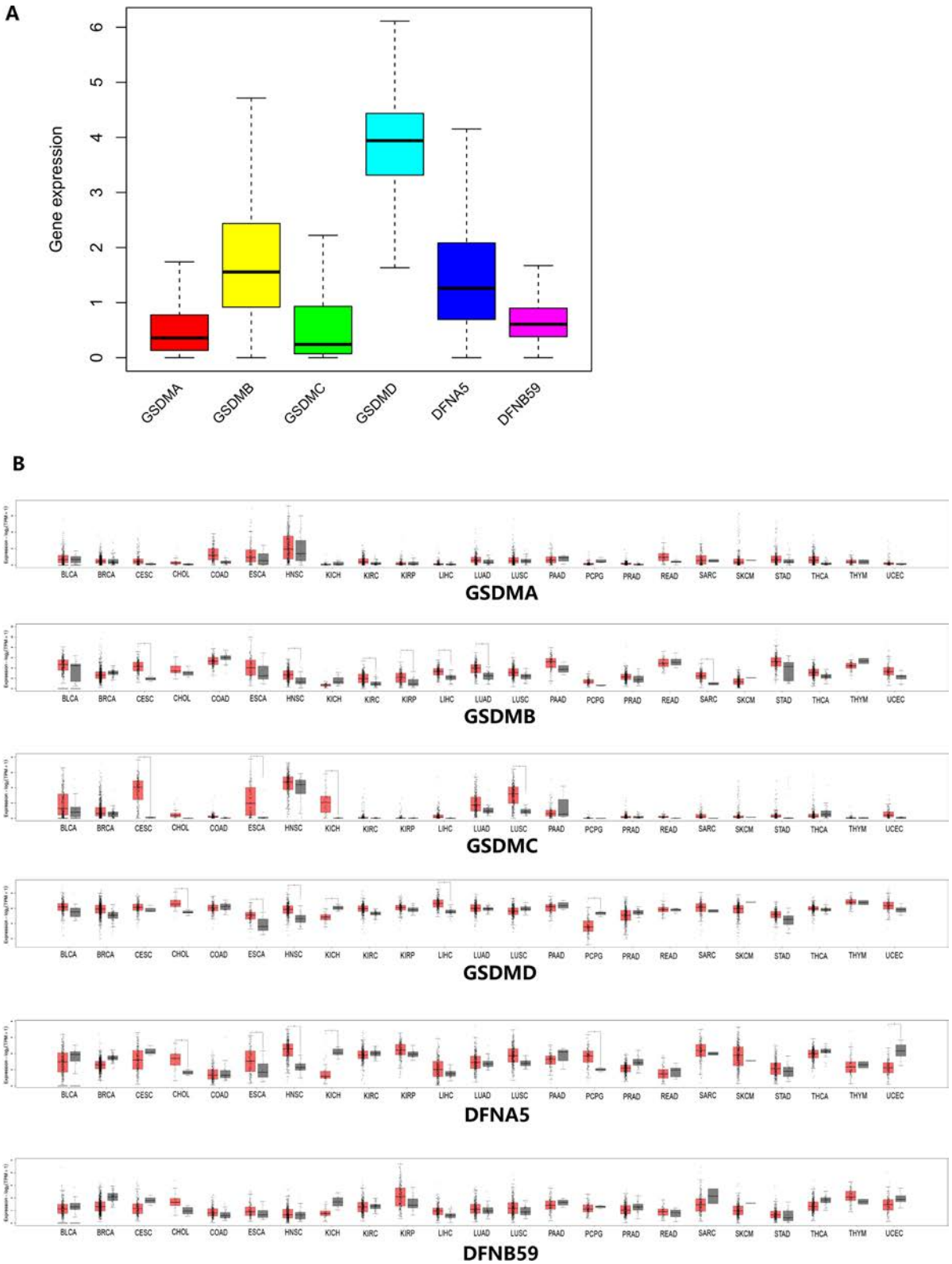

Figure S1 The expression level of the gasdermin (GSDM) genes in tumor tissues and normal tissues derived from the TCGA database. (A) The expression level of the GSDM genes in all tumor tissues. (B) The expression level of the GSDM genes in each tumor tissue. *, P<0.05.

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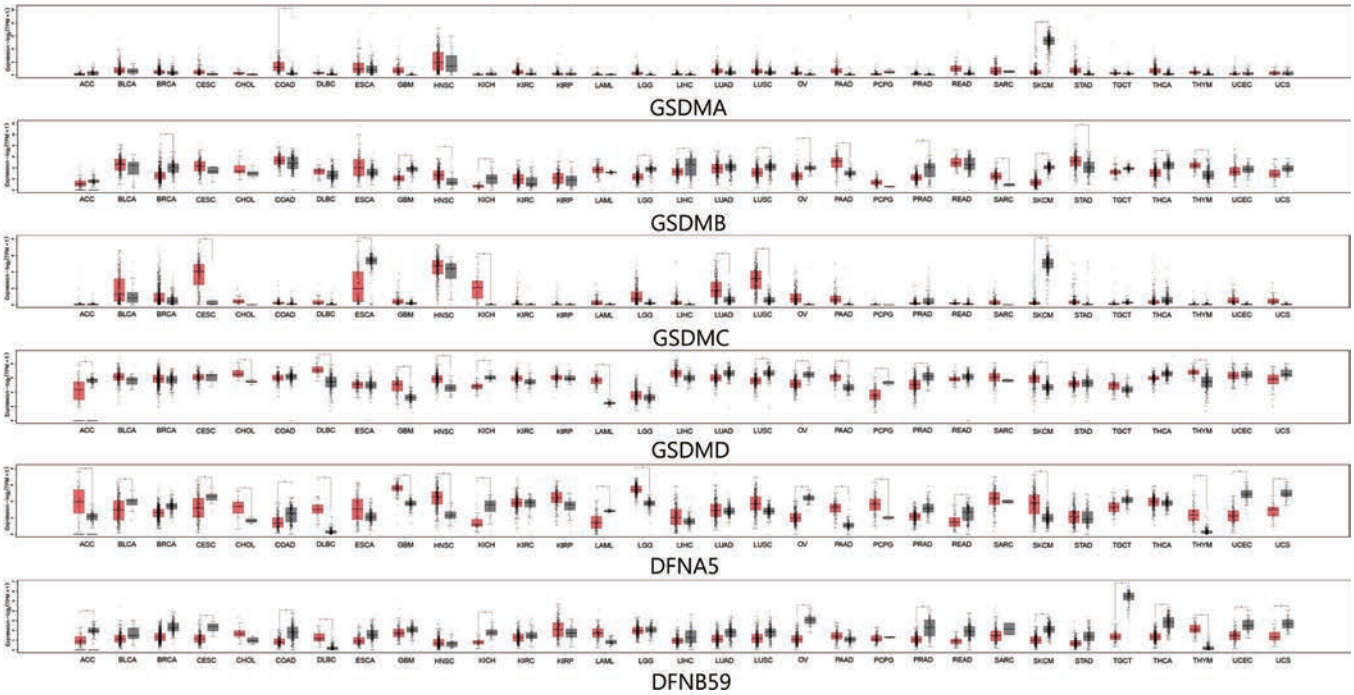

Figure S2 A comparative analysis of the expression of the GSDM genes in tumor tissues and normal tissues based on the TCGA and GTEx databases. *, P<0.05.

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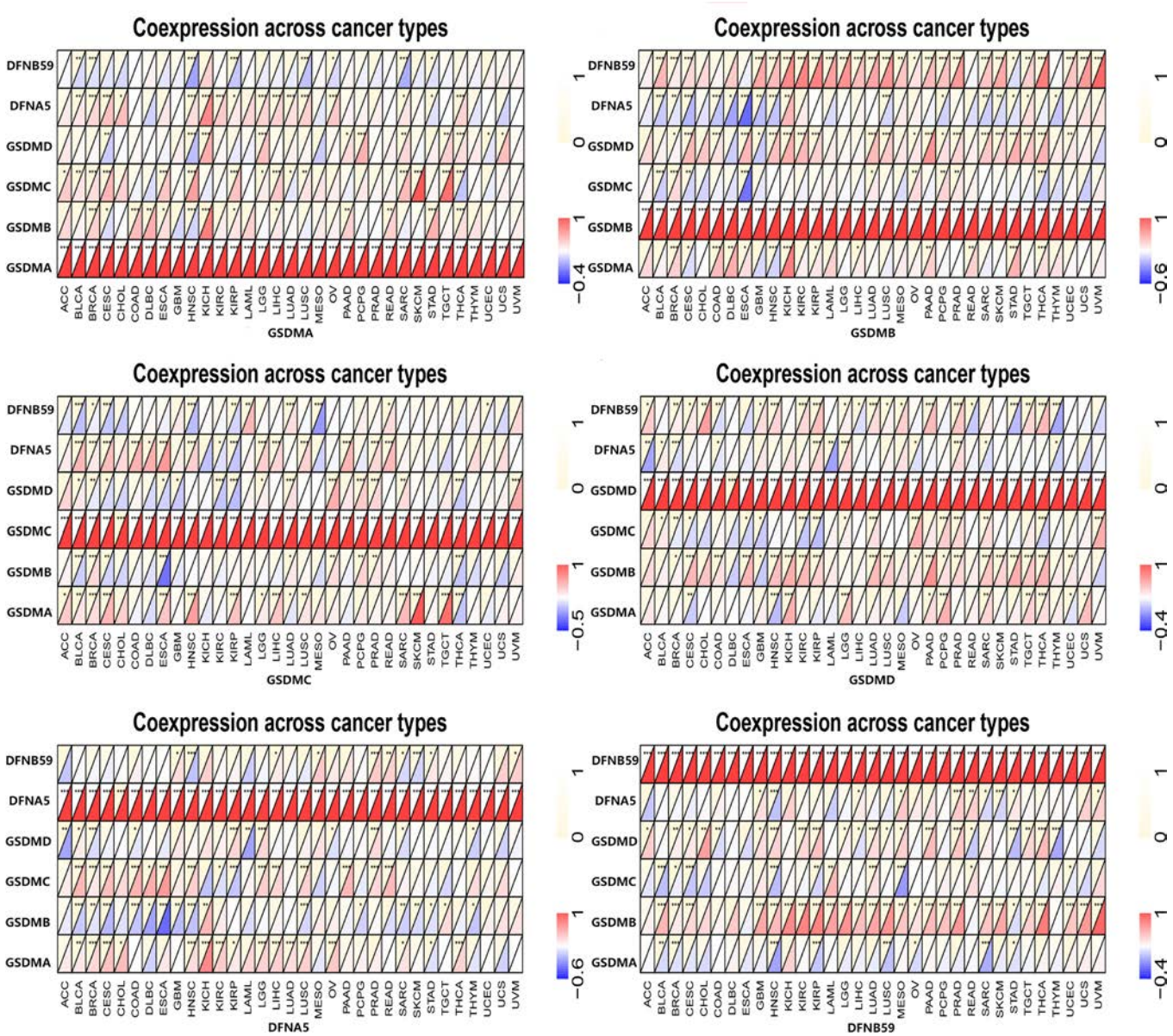

Figure S3 The correlation between GSDM genes and other genes in different tumors. (A) The correlation between GSDMA and other GSDM genes in each tumor. (B) The correlation between GSDMB and other GSDM genes in each tumor. (C) The correlation between GSDMC and other GSDM genes in each tumor. (D) The correlation between GSDMD and other GSDM genes in each tumor. (E) The correlation between DFNA5 and other GSDM genes in each tumor. (F) The correlation between DFNB59 and other GSDM genes in each tumor. *, P<0.05; **, P<0.01; ***, P<0.001.

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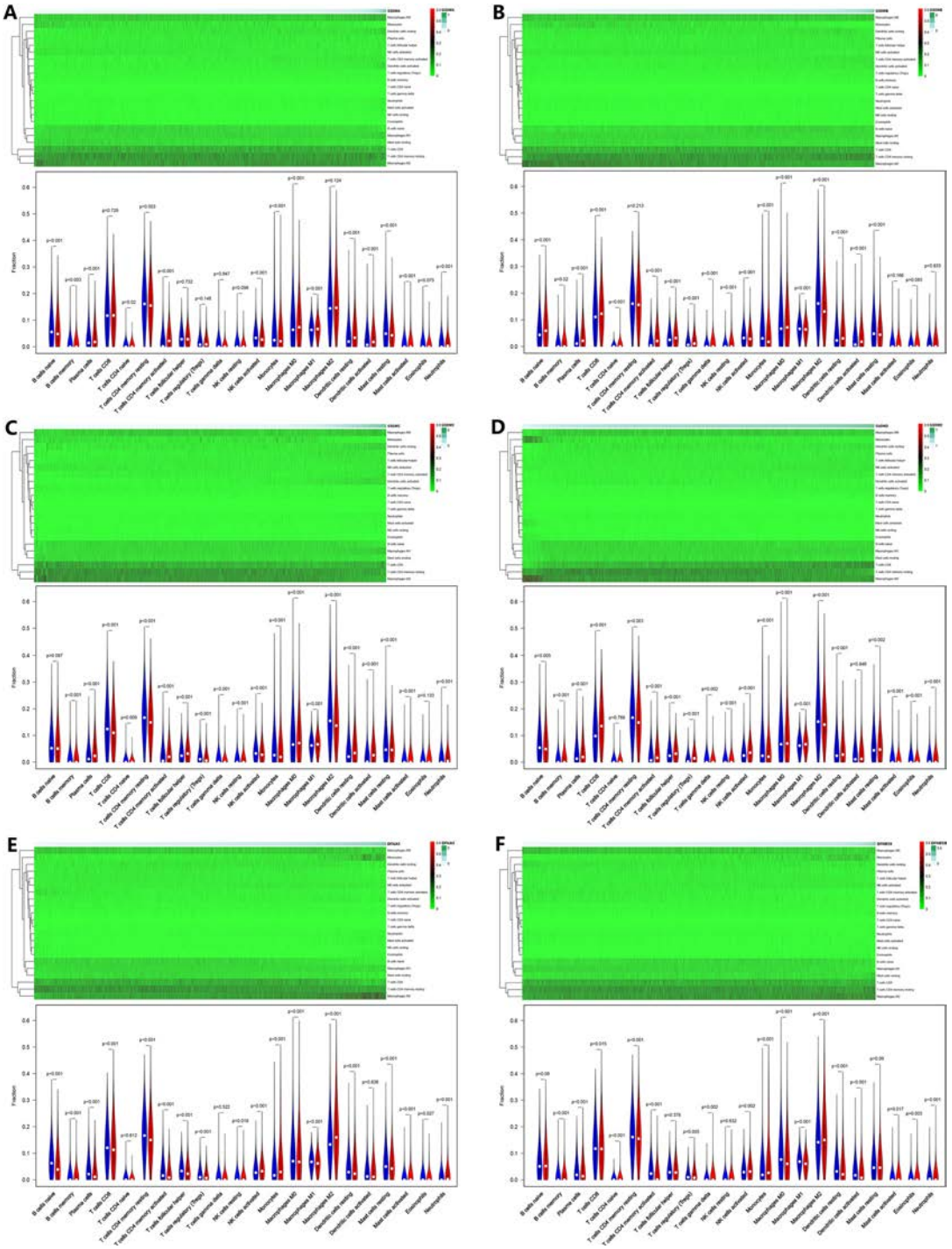

Figure S4 The relationship between the expression of GSDM genes and the infiltration content of various immune cells. (A) The immune cell heat map related to GSDMA expression, and the difference in the infiltration content of various immune cells in the GSDMA high and low expression group. (B) The immune cell heat map related to GSDMB expression, and the difference in the infiltration content of various immune cells in the GSDMB high and low expression group. (C) The immune cell heat map related to GSDMC expression, and the difference in the infiltration content of various immune cells in the GSDMC high and low expression group. (D) The immune cell heat map related to GSDMD expression, and the difference in the infiltration content of various immune cells in the GSDMD high and low expression group. (E) The immune cell heat map related to DFNA5 expression, and the difference in the infiltration content of various immune cells in the DFNA5 high and low expression group. (F) The immune cell heat map related to DFNB59 expression, and the difference in the infiltration content of various immune cells in the DFNB59 high and low expression group.

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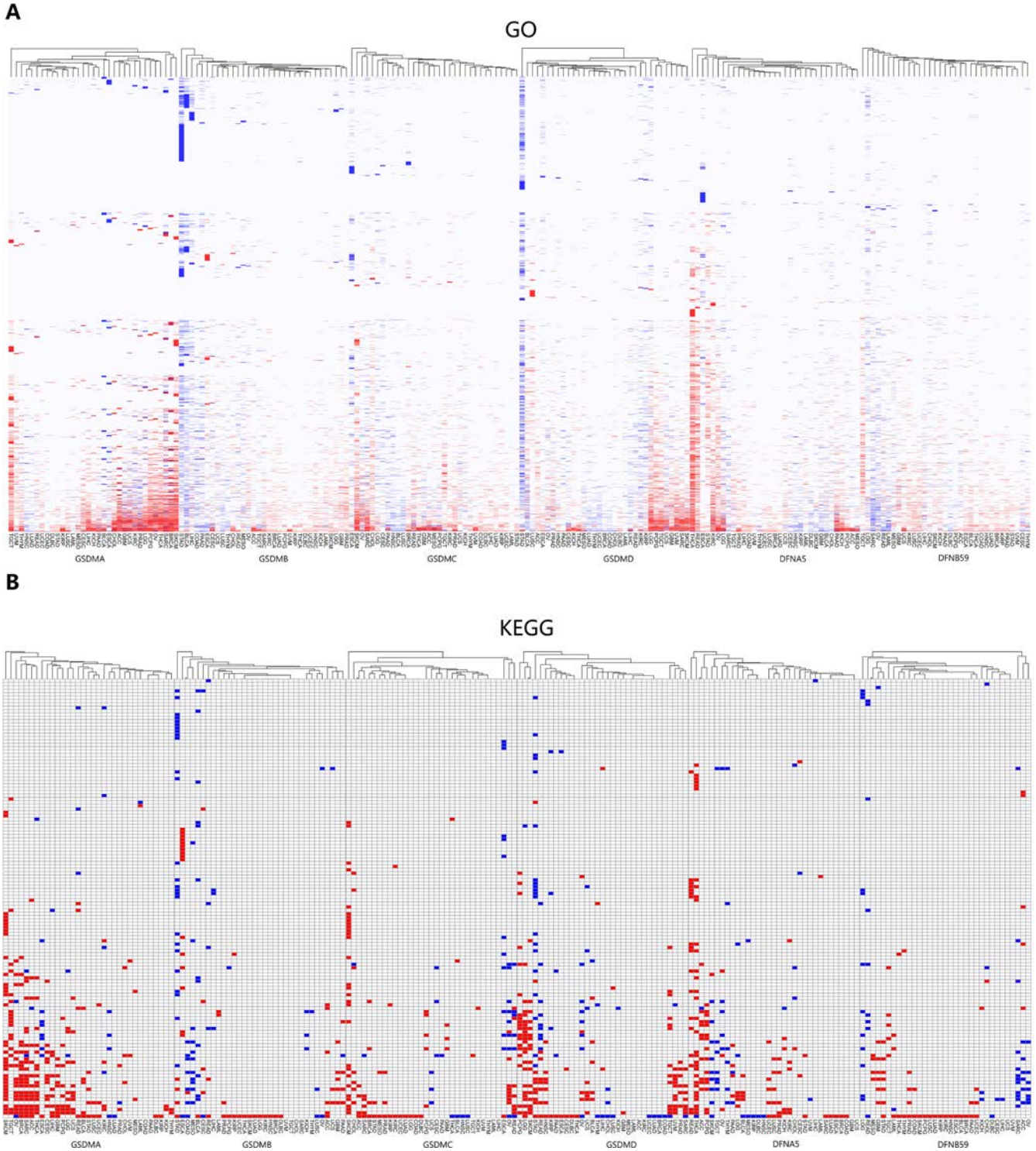

Figure S5 GO (A) and KEGG (B) pathway enrichment results of the GSDM genes in each tumor.

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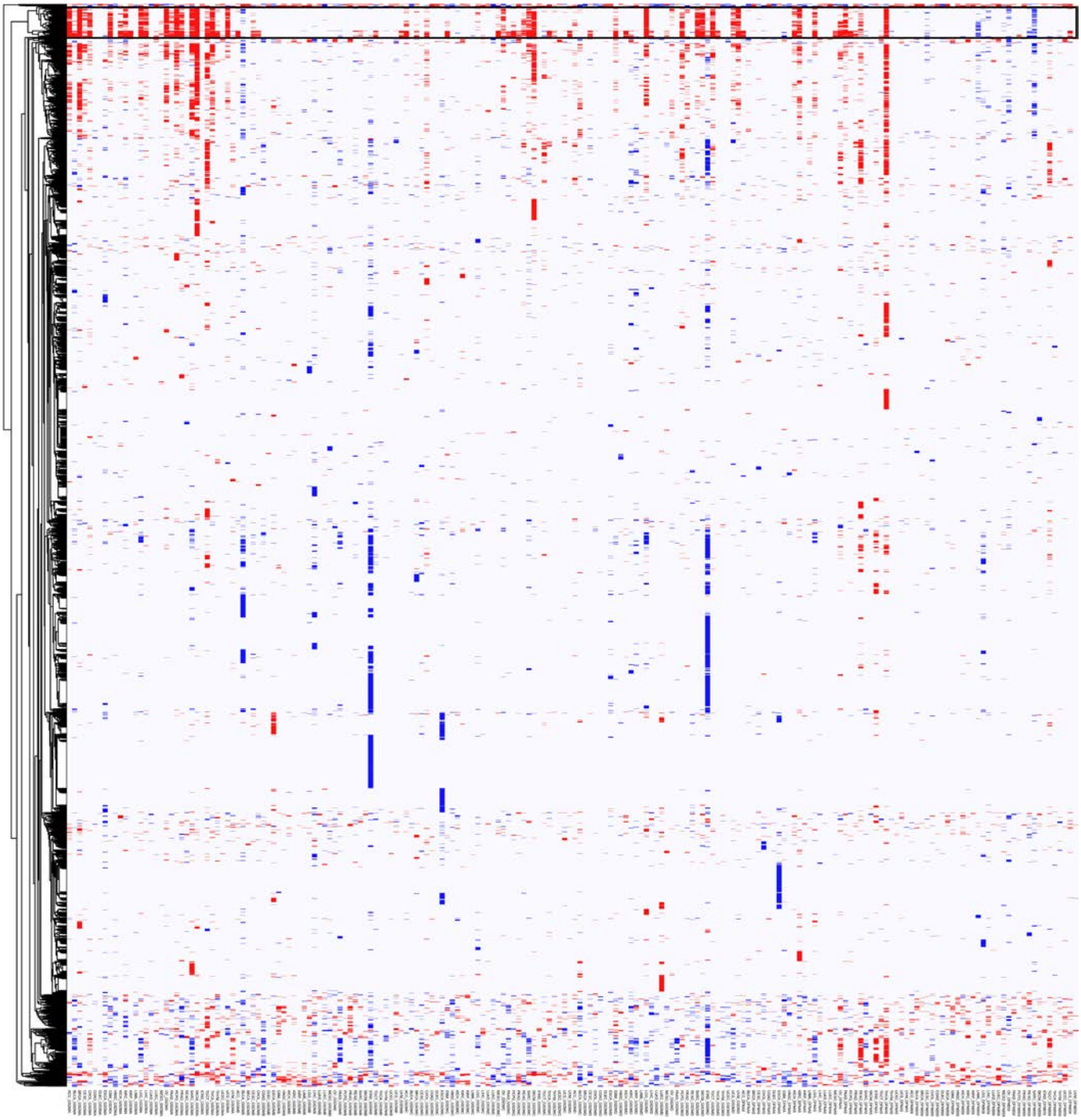

Figure S6 All GO enrichment results after clustering of the GSDM genes in each cancer (red represents NES is positive, blue represents NES is negative).

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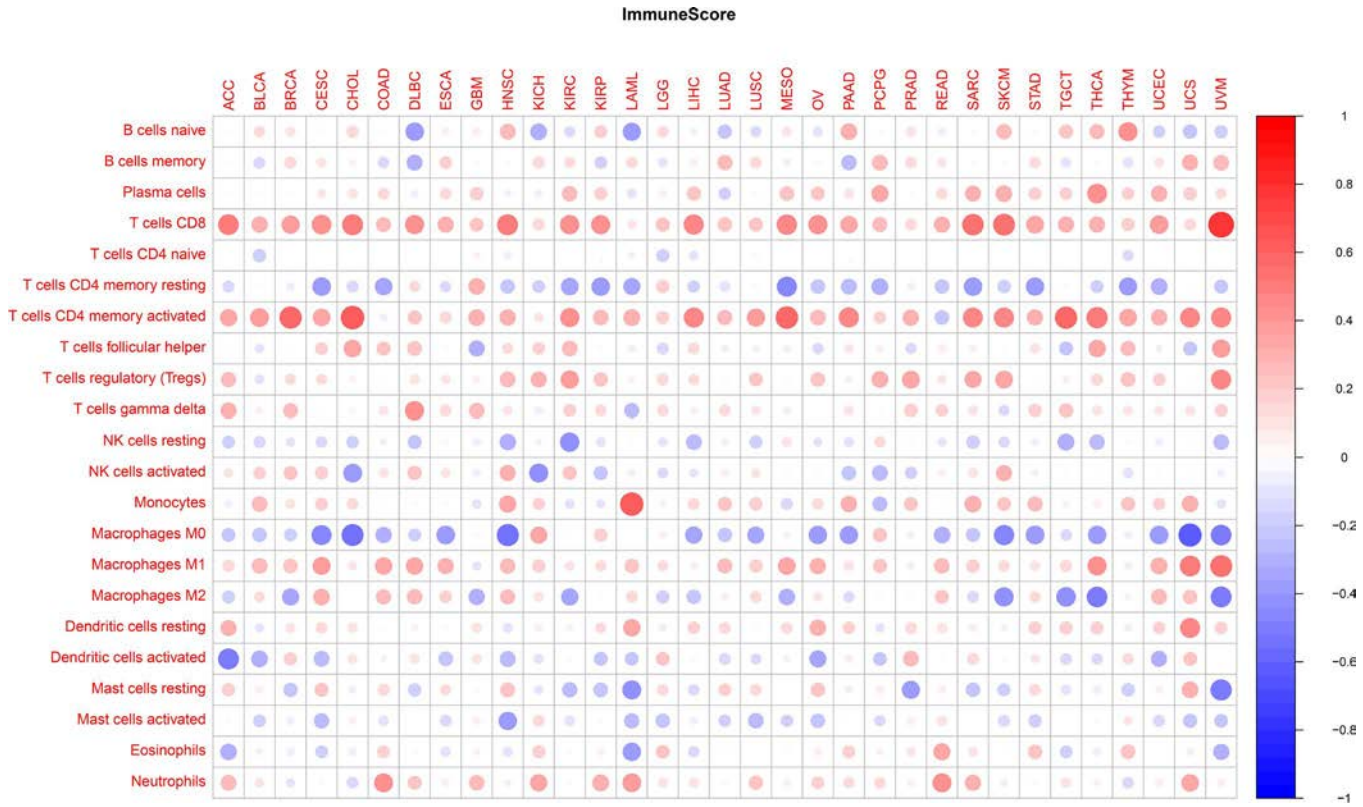

Figure S7 The relationship between immune score and immune cell infiltration in various tumors.

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