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Reviewer A

ABCB1 protein (also known as PGP1) is the first member of the ABC transporter superfamily associated with response to chemotherapy and prognosis in solid cancer and in leukemia.

Less defined is the role of the other ABCB members. In this paper the authors performed a comprehensive analysis of the prognostic significance of ABCB genes across a spectrum of cancers. Moreover the authors investigated the association between expression levels, clinical characteristics, immune cells in tumor microenvironment, drug sensitivity.

The paper contains many interesting information, but there are also many points to be clarified.

Comment 1: The section on methods should be implemented and methods better described. For example: how overexpressing cases are defined? At what expression level? And so one.. The characteristics of the different immune subgroups detailed in this section, to facilitate results comprehension.

Reply 1: Thank you for your careful review and constructive comments.

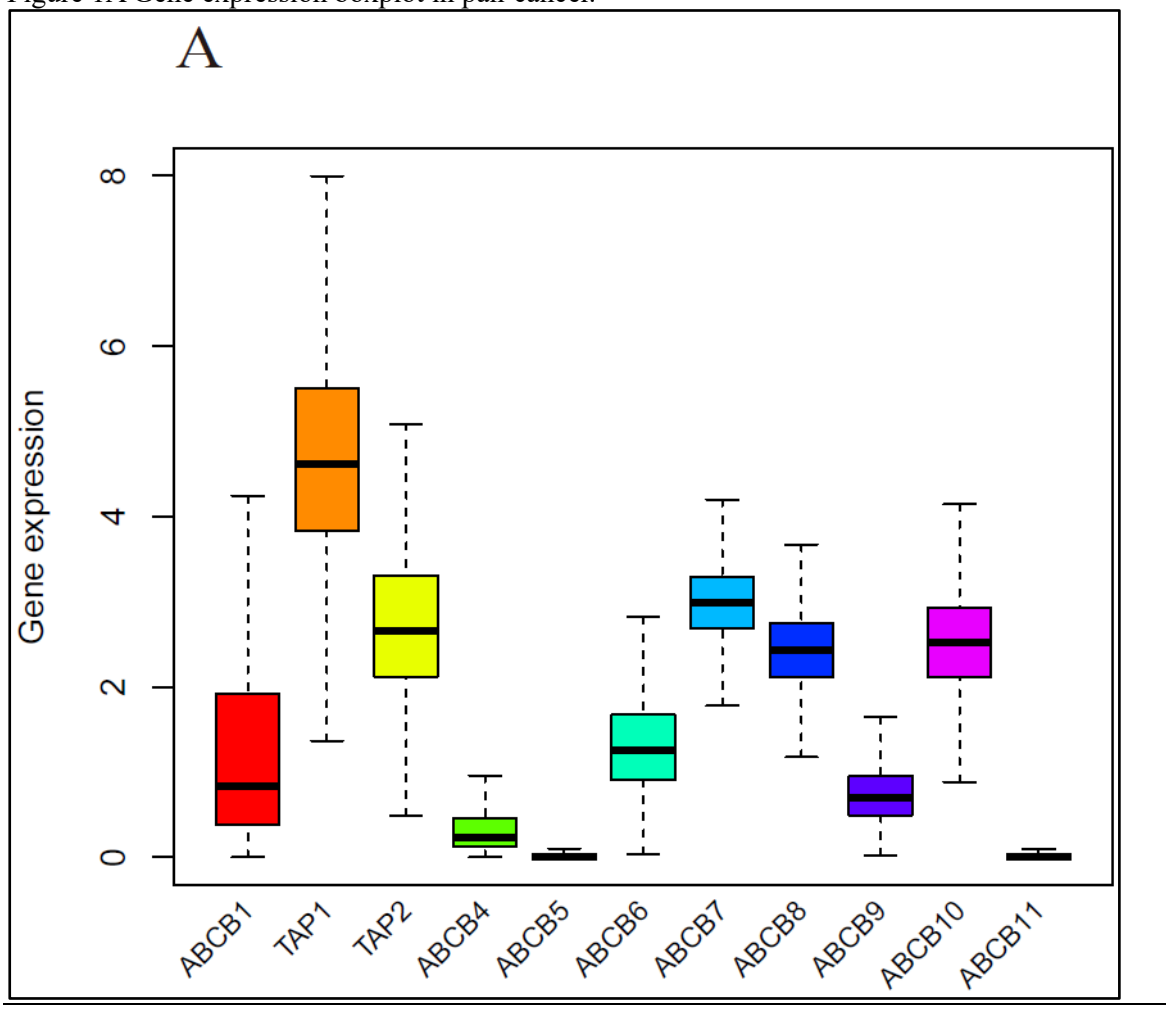
In addressing this suggestion, we have revised the methods section to provide a more detailed description of the techniques employed, aiming to enhance result interpretation. Additionally, I will now briefly address specific concerns raised by the reviewer.

1.How are overexpressed cases defined and what is their expression level?

The description of over-expression is mainly concentrated in three places in the article:

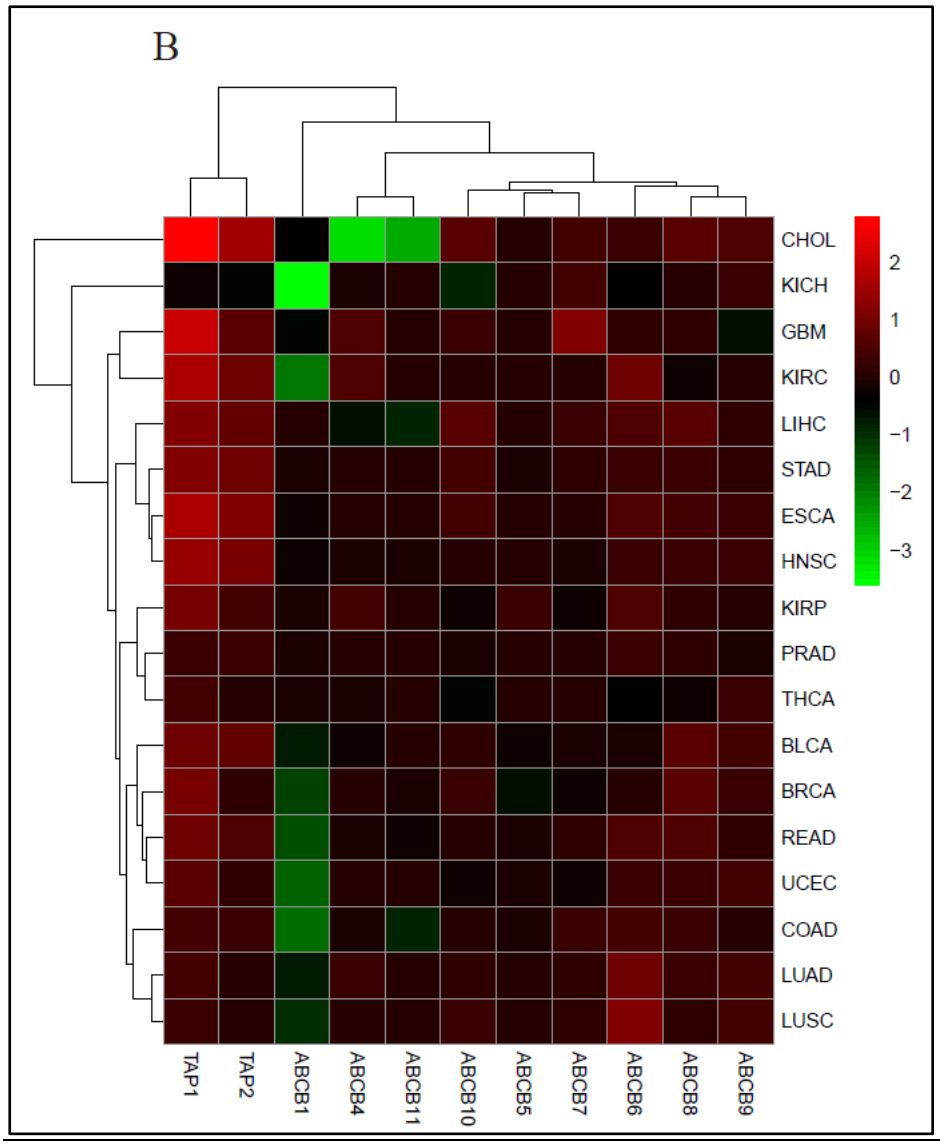
The first description (Figure 1A. boxplot): the expression level of each member of the ABCB family genes (the description of high expression instead of over-expressed is used here). The generation of this result is based on standardized and uniform background conditions of gene expression levels. By using the absolute value to describe the gene level, a high or low expression group of the gene is generated. This overexpression situation is obtained by comparing the absolute value of the gene expression. We can call it a relatively high expression group (such as TAP1 versus ABCB5 for the high expression group). The expression level of TAP1 gene exceeds 4 and other ABCB family genes are under 4.

Figure 1A Gene expression boxplot in pan-cancer.



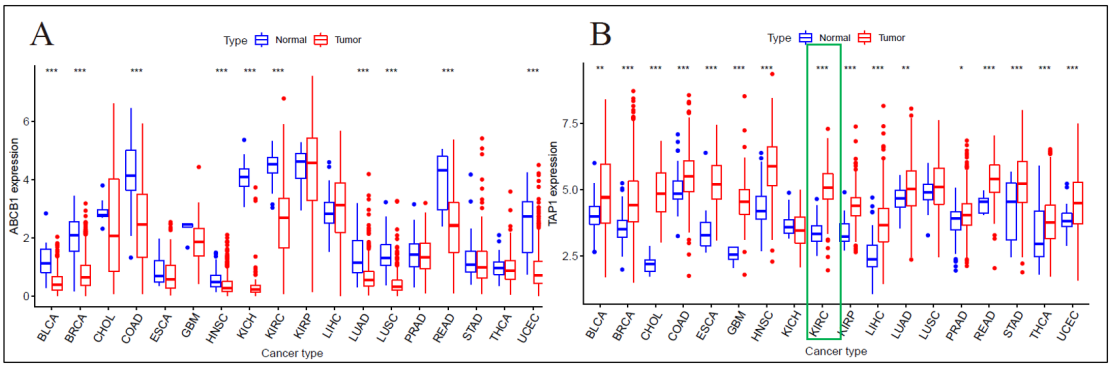
In the second place (Figure 1B, heatmap), the judgment principle is similar to that of a histogram. However, for enhanced visualization, red signifies high gene expression, while green represents low expression. Typically, we compute the arithmetic average of all gene expression values within the same cancer type as the cutoff value. Those below this threshold exhibit low expression, whereas those above it demonstrate high expression (overexpression). Next, we calculate the difference between the gene expression value and the cutoff value across different tumors. When the gene is highly expressed, the difference is positive; conversely, when the gene has low expression, the difference is negative. From a visualization standpoint, when the expression value precisely matches the cutoff value, the color appears black. As expression deviates further from the cutoff value, the color shifts towards darker red. Conversely, lower expression levels result in progressively greener shades.

Figure 1B Gene expression heatmap.



The third point pertains to gene expression levels in tumor tissues compared to adjacent normal tissues, often described as overexpression (Figure 2). In essence, overexpression signifies that the gene is more abundantly expressed in tumor tissue than in its normal counterpart. This difference is typically quantified as a fold change. For instance, consider TAP1 gene in kidney renal cell carcinoma (KIRC) tumor tissue, which is about 2 times more abundant than in normal tissue. In this scenario, we label TAP1 gene as overexpressed in tumors. To precisely define and compare gene expression levels between tumor and normal tissues, various methods are employed. One commonly used approach is the t-test. This statistical test assumes that the gene expression values in tumor tissues and adjacent tissues are equal. By applying scientific testing methods, we assess the likelihood that this assumption holds true. If the probability (p-value) is less than 5%, we infer that the expression of gene differs significantly between tumor and adjacent tissue. Specifically, when the average expression value in tumor tissue exceeds that in normal tissue, we conclude that gene is overexpressed in the tumor. The magnitude of overexpression is determined by the multiple of the average expression values.

Figure 2 Differences of gene expression between cancer and normal tissue.



2.What are characteristics of different immune subtypes?

The immune subtypes discussed in this article were proposed by Thorsson et al. (PMID: 29628290). They conducted immunogenomic analysis on over 10,000 tumor samples across 33 cancer types, resulting in the classification of tumors into six distinct immune subtypes:

C1 (Wound Healing): Associated with tissue repair processes.

C2 (IFN-γ Dominant): Characterized by an abundance of interferon-gamma signaling.

C3 (Inflammatory): Linked to inflammatory responses.

C4 (Lymphocyte Depleted): Exhibits low lymphocyte presence.

C5 (Immunologically Quiet): Displays minimal immune activity.

C6 (TGF-β Dominant): Influenced by transforming growth factor-beta.

This immunological grouping transcends histological type and tumor site, providing a more comprehensive reflection of tumor immunogenicity. Each immune subtype possesses distinct features, including variations in macrophage and lymphocyte characteristics, Th1:Th2 cell ratios, tumor heterogeneity, aneuploidy levels, neoantigen loads, cell proliferation rates, and expression of immune regulatory genes. These differences impact prognosis and other clinical indicators.

C1 (Wound Healing) exhibited elevated angiogenic gene expression, high cellular proliferation, and a Th2 cell-biased adaptive immune infiltrate.

C2 (IFN-γ Dominant) demonstrated the most pronounced M1/M2 macrophage polarization and CD8 signal, sharing the highest T cell receptor (TCR) diversity with C6. This subtype also presented a high proliferation rate, potentially overriding an emerging Type I immune response.

C3 (Inflammatory) was characterized by increased Th17 and Th1 gene levels, low to moderate tumor proliferation, and, in conjunction with C5, the least aneuploidy and somatic copy number alterations among the subtypes.

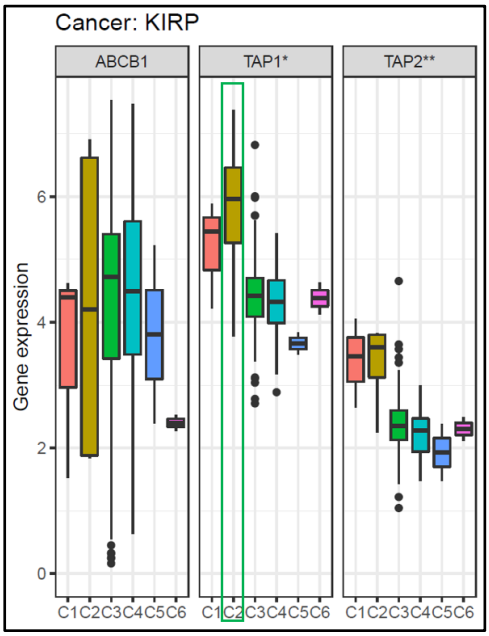
C4 (Lymphocyte Depleted) showed a significant macrophage presence, subdued Th1 activity, and an elevated M2 response.

C5 (Immunologically Quiet) had the minimal lymphocyte and maximal macrophage responses, predominantly M2 macrophages.

Lastly, C6 (TGF-β Dominant), a diverse group of mixed tumors not specifically categorized in any TCGA subtype, had the strongest TGF-β signature and a balanced distribution of Type I and II T cells (Thorsson et al. 2019).

The essence of immune subtypes lies in the combination of various indicators at different levels. Consequently, responses to immunotherapy and prognoses differ significantly among these subtypes. In our article, we specifically analyze the main immune subtypes associated with varying expression states of the ABCB gene. This knowledge enables more precise treatment guidance. For instance, in Figure 6A, if the TAP1 gene exhibits high expression in KIRC, it is likely associated with the C2 immune subtype, allowing for targeted selection of IFN-γ dominant immunotherapy.

Figure 6A TAP1 gene exhibits high expression in C2 immune subtype in KIRC



3.Other key issues in the methods section:

Survival Analysis plays a pivotal role in our research. We employed both the Kaplan-Meier (K-M) method and the Cox proportional hazards model to corroborate each other and comprehensively assess survival outcomes. One critical aspect in survival analysis is the selection of the expression value cutoff value. In our study, we chose the median expression value as the cutoff. Specifically, if a sample's expression value exceeded the median, it was categorized as the overexpression group, and vice versa for the low expression group.

Here's how the process unfolds:

Data Preparation: We merge survival data with the grouping information based on expression levels.

Statistical Testing: We rigorously analyze whether a relationship exists between gene expression values and survival time.

In Kaplan-Meier (K-M) analysis, the p-value determines the significance of any observed differences in survival curves.

In Cox analysis, the hazard ratio (HR) quantifies the degree of risk associated with gene expression levels.

By employing both tests simultaneously, we enhance the reliability of our research findings. This comprehensive approach ensures robust conclusions regarding the impact of gene expression on survival outcomes.

Regarding the immune microenvironment, stemness score, and drug sensitivity:

Immune Microenvironment:

We employed the ESTIMATE algorithm to assess the immune microenvironment within tumor samples. This algorithm calculates two key components:

Stromal Score: Reflects the presence of stromal cells (such as fibroblasts) in the tumor microenvironment.

Immune Score: Quantifies the abundance of immune cells infiltrating the tumor.

Additionally, we determined tumor purity, which indicates the proportion of cancer cells relative to non-cancerous cells. A higher tumor purity suggests a more homogenous tumor cell population.

Stemness Score:

Stemness scoring provides insights into cancer stem cell activity. These specialized cells possess the ability to self-renew and differentiate into various cell types.

A stronger stemness score indicates heightened malignant behavior, as cancer stem cells contribute to tumor growth, metastasis, and therapy resistance.

Drug Sensitivity Testing:

Leveraging the CellMiner database, we assessed gene expression levels in 60 tumor cell lines (NCI-60 cancer cell set).

We then evaluated the sensitivity of these cell lines to over 20,000 compounds designed to target tumor cells.

By correlating these key indicators (gene expression, drug sensitivity) with ABCB gene expression values with spearman method, we gained insights into potential therapeutic strategies.

Changes in the text:

We have modified our method section as advised.

Specifically,

description of expression levels is supplemented in Page 6-7, line 193-214.

description of survival analysis is supplemented in Page 7, line 219-222.

description of tumor stemness is supplemented in Page 7, line 232-235.

description of tumor purity and immune infiltration is supplemented in Page 7-8, line 237-240.

description of drug sensitivity analysis is supplemented in Page 8, line 247-254.

description of immune subtype is supplemented in Page 8, line 258-264.

Comment 2: Again, results should be better described and not simple refer to the figures

Reply 2: Thank you for your professional advice.

Description of results is crucial to the quality of the article. We have rewritten the results section so that its content better corresponds to the full text and is more comprehensively explained and easier to understand.

After careful inspection and checking the description of each picture result, we found that there are indeed some results that only have legends but no detailed explanations. Specifically located in: Figure S2, Figure S3, Figure S4, and the descriptions of Figure 4 and Figure 9. We have effectively explained and supplemented these shortcomings.

Specifically:

Figure S2: Testing prognosis using COX method. Correlation analysis of the Regarding this figure, we also describe in detail the results in the figure and what they represent. Results manifested that ABCB1 take a protective role in PAAD,COAD,HNSC,KIRC,LUAD,SKCM and SARC. TAP1 had a detrimental role in LGG,UVM,PAAD, but take a protective role in SKCM and OV. TAP2 take a detrimental role in LGG,LAML,KIRP,PAAD but have a protective in SKCM. ABCB6 displayed a detrimental role in SKCM,UVM,ACC,KIRP,LIHC and PAAD but own a protective role in LGG.

Figure S3: Testing prognosis in GEPIA database using K-M method. Correlation analysis of the expression levels of ABCB family gene and prognosis by GEPIA database in pan-cancers. We supplement the results depicted in figure. With regard to GEPIA analysis, ABCB1 manifested a protective role in CESC, KIRC, LUAD and PAAD . Meanwhile, TAP1 exhibited a detrimental role in LGG,OV, SKCM and UVM . TAP2 also played a detrimental role in LAML,LGG, KIRP and PAAD. ABCB6 have a detrimental role in ACC, KIRP, LIHC, SKCM, and UVM.

Figure S4: Using the K-M method to test prognosis in the K-M-P database. Correlation analysis of the expression levels of ABCB family gene and prognosis by Kaplan-Meier Plotter database in pan-cancers. We provide detailed explanations of the genes we focus on. In Kaplan-Meier plotter database, ABCB1 played a detrimental role in LIHC and TAP2 manifested a detrimental role in KIRP and PAAD. Meanwhile, ABCB6 displayed a detrimental role in KIRC, KIRP and LIHC.

Figure 4: Relationship between ABCB gene expression and tumor microenvironment and stemness score in pan-cancer. A, B, C, and D represent RNA stemness score, DNA stemness score, matrix score and immune score respectively. In each score, there is a matrix composed of cancer types and ABCB family genes. The content of the matrix is the correlation between the two (positive or negative correlation and the magnitude of the correlation). Since the matrix contains too much information and it would be too lengthy to express each one in text, we do not state the correlation between specific cancer types and specific genes one by one here. In Figure 5, we focus on showing in detail the correlation of ABCB family genes in three selected

tumors with strong prognostic consistency (scatter plots, fitting curves and Spearman analysis can be seen). Detailed results can be found in Table 2.

Figure 9: Detailed expression differences of TAP1, TAP2 and ABCB6 between tumors and paired normal tissues. TAP1, TAP2 and ABCB6 protein expression statuses in tumor and paired normal tissues in KIRP. (A) TAP1 protein status in normal renal tissue. (B) TAP1 protein status in tumor renal tissue. (C) TAP2 protein status in normal renal tissue. (D) TAP2 protein status in tumor renal tissue. (E) ABCB6 protein status in normal renal tissue. (F) ABCB6 protein status in tumor renal tissue.

Changes in the text:

We have modified our result section as advised.

Specifically,

description of Figure S2, Figure S3, Figure S4 is supplemented in Page 10-11, line 341-352.

description of Figure 4 is supplemented in Page 11, line 362-366.

description of Table 2 is supplemented in Page 12, line 401-403.

description of Figure 9 is supplemented in Page 13, line 454-456.

Comment 3: In the evaluation of ABCB member expression the authors reported a co-overexpression in many tumors. Which its impact on survival and response to therapy?

Reply 3: We are very grateful for your objective evaluation of our article.

As shown in the heat map in Figure 1B, red squares occupy the majority, ABCB family genes indeed show a common high expression status in many tumors. So, what impact this overexpression will have on prognosis and drug response is indeed a question worthy of further exploration.

Specifically, first of all, it is closely related to the function of the ABCB family. The ABCB superfamily, also known as the ATP-binding cassette subfamily B, is a group of genes that encode ATP-binding cassette transporters. These transporters are membrane proteins involved in the active transport of a variety of substrates across cellular membranes. The ABCB superfamily includes several members, each with distinct substrate specificities and functions. **Structural Features:** Members of the ABCB superfamily share a common structural organization. They typically consist of two transmembrane domains (TMDs) that form the substrate-binding site and two nucleotide-binding domains (NBDs) responsible for ATP binding and hydrolysis. This structural arrangement allows these transporters to pump substrates across cellular membranes.

Multidrug Resistance: Certain members of the ABCB superfamily, such as ABCB1, also known as multidrug resistance 1 (MDR1), are associated with multidrug resistance in cancer cells. These transporters actively pump out chemotherapeutic drugs, reducing their effectiveness. Some well-known members of ABC subfamily B include MDR1 (ABCB1), MDR2 (ABCB4). These transporters contribute to the efflux of a broad spectrum of substrates, and their overexpression can impact the efficacy of therapeutic drugs. Understanding the function and regulation of ABC subfamily B transporters is important in the fields of

pharmacology and cancer research, as it can provide insights into drug resistance mechanisms and potential therapeutic targets.

Antigen Presentation: The TAP1 protein assembles with another protein called TAP2 (produced from the TAP2 gene) to form a protein complex known as the transporter associated with antigen processing (TAP) complex. This complex is involved in antigen processing within cells. It pumps degraded cytosolic peptides from the cytoplasm through the endoplasmic reticulum (ER) membrane into the membrane-bound compartment where class I molecules are located. MHC-I molecules then present these peptides on the cell surface, allowing the immune system to recognize and respond to infected or abnormal cells.

However, due to the large number of ABCB family gene members, the specific distribution in tissues, and the heterogeneity of many tumors, the impact of ABCB overexpression status on survival and drug response needs to be analyzed on a case-by-case basis. The specific functions of ABCB family genes are summarized as follows.

Table R1. Function of ABCB family genes.

Gene	Function and Role	Associated Diseases/Conditions	Regulation and Clinical Significance	Research and Implications	Prognosis	Response to Cancer Treatment	Cancer Types/Implications
ABCB1	Involved in the efflux of various substrates, contributing to multidrug resistance in cancer cells.	Multidrug resistance in cancer cells	Regulated at transcriptional and post-transcriptional levels; Clinical relevance in drug resistance	Drug development and overcoming multidrug resistance	Influences prognosis in some cancers	Resistance to chemotherapy, poor prognosis	Various cancers, including breast, colon, lung, leukemia
TAP1	Translocates antigenic peptides from cytoplasm to endoplasmic reticulum for MHC class I antigen presentation.	Bare Lymphocyte Syndrome (BLS)	Regulated by interferon-gamma; Critical for MHC class I antigen presentation; Key role in immunity	Understanding immune responses; Therapeutic targeting for immune-related diseases	Limited evidence; Potential immunotherapy target	Response associated with immune contexture	Relevant in immune surveillance; Impact on immunotherapy
TAP2	Forms TAP complex with TAP1; Facilitates transport of peptides into	Bare Lymphocyte Syndrome (BLS)	Regulated by interferon-gamma; Key role in MHC class I antigen presentation;	Investigating immune responses; Therapeutic targeting for immune-	Limited evidence; Potential immunotherapy target	Response associated with immune contexture	Relevant in immune surveillance; Impact on immunotherapy

	endoplasmic reticulum for MHC class I presentation.		Immunological significance	related diseases			
ABCB4	Functions as a phospholipid floppase; Translocates phospholipids from inner to outer leaflet of canalicular membrane in hepatocytes.	Progressive Familial Intrahepatic Cholestasis type 3 (PFIC3), Intrahepatic Cholestasis of Pregnancy (ICP)	Regulated by bile acids, hormones; Implications in bile formation; Clinical relevance in liver diseases	Understanding liver physiology; Implications for cholestatic liver disorders	Relevant in cholestatic liver diseases	Not well-established	Cholestatic liver diseases
ABCB5	Associated with stem cell properties; Expression linked to drug resistance in melanoma cells.	Melanoma, drug resistance, stem cell properties	Role in drug resistance; Potential therapeutic target in melanoma	Investigating cancer stem cells; Therapeutic strategies for drug-resistant cancers	Impacts prognosis in melanoma	Drug resistance, poor prognosis	Melanoma, potential role in cancer stem cells
ABCB6	Involved in translocation of porphyrins; Implicated in heme biosynthesis.	Implications in cancers, role in heme biosynthesis	Regulated at transcriptional and post-transcriptional levels; Potential role in cancer and heme synthesis	Understanding heme biosynthesis; Implications for cancer research	Limited evidence; Potential role in cancer	Limited evidence	Limited evidence
ABCB7	Exporter of iron-sulfur clusters from mitochondria to cytoplasm; Crucial for	X-Linked Sideroblastic Anemia with Ataxia (XLSA/A), mitochondrial disorders	Regulated; Implications in iron metabolism and mitochondrial function;	Understanding mitochondrial iron homeostasis; Therapeutic targets for	Influences prognosis in mitochondrial disorders	Limited evidence; Potential role in iron-related cancers	Limited evidence

	mitochondrial function and erythropoiesis.		Associated with XLSA/A	mitochondrial disorders			
ABCB8	Less studied member; Potential role in mitochondrial processes.	Limited information available	Limited information available	Limited information available	Limited evidence; Potential mitochondrial role	Limited evidence; Potential mitochondrial role	Limited evidence
ABCB9	Less studied member; Potential role in mitochondrial processes.	Limited information available	Limited information available	Limited information available	Limited evidence; Potential mitochondrial role	Limited evidence; Potential mitochondrial role	Limited evidence
ABCB10	Implicated in heme synthesis and iron metabolism within mitochondria, particularly associated with erythropoiesis.	Limited information available	Limited information available	Limited information available	Limited evidence; Potential role in cancer	Limited evidence	Limited evidence
ABCB11	Mediates active transport of bile salts from hepatocytes into bile canaliculi; Crucial for bile formation and digestion of fats. Mutations	Cholestasis, Progressive Familial Intrahepatic Cholestasis type 2 (PFIC2), drug-induced liver injury	Regulated; Clinical relevance in liver diseases; Key role in bile formation	Understanding liver physiology; Implications for cholestatic liver disorders; Drug-induced liver injury	Influences prognosis in cholestatic liver diseases	Limited evidence; Response to bile acid-based therapy	Cholestatic liver diseases

	associated with cholestatic liver diseases.						

In the subsequent discussion, we direct our attention to the implications of gene overexpression on both patient survival and treatment response.

Gene overexpression and patient survival were summarized in Table 1.

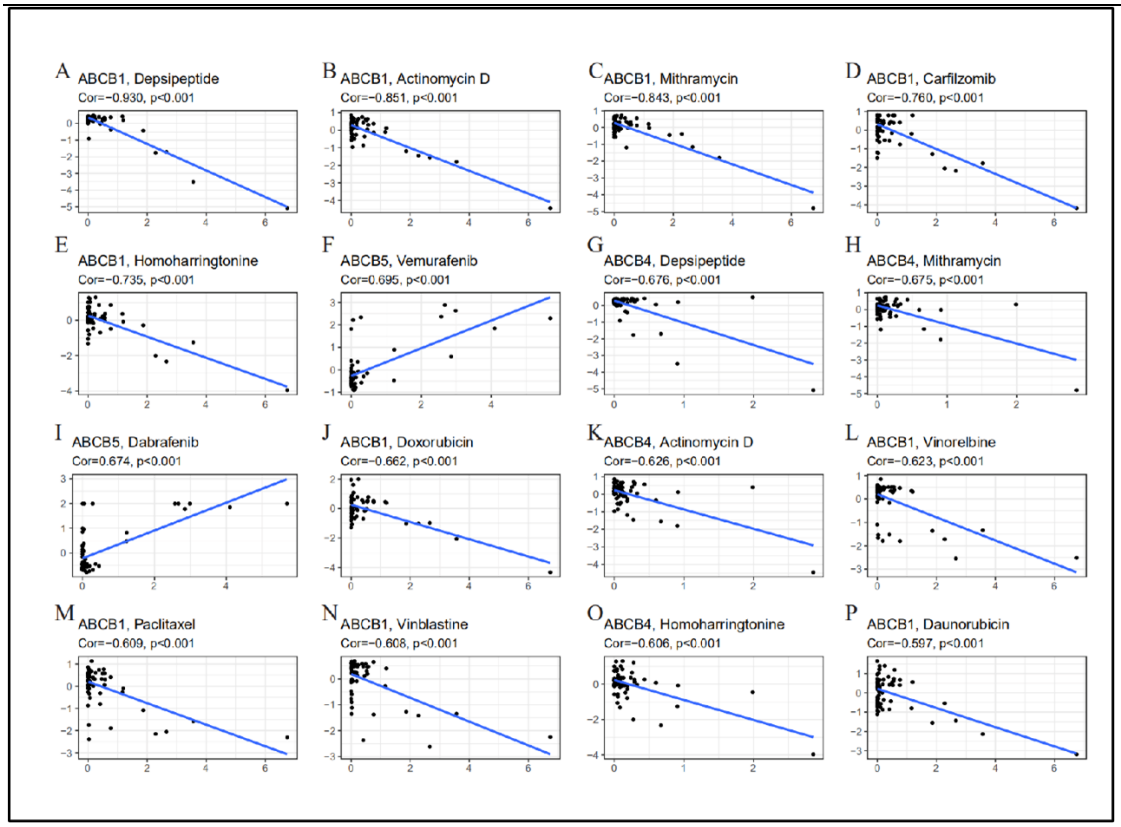
Table 1. Correlation analysis of the expression levels of ABCB family gene and prognosis by multiple method in pan-cancers.

Gene	Role	TCGA（Kaplan-Meier）	TCGA(COX)	Kaplan-Meier Plotter	GEPIA
		OS	OS	OS	OS
ABCB1	Detrimental	/	/	LIHC	/
	Protective	PAAD,COAD,HNSC,KIRC, LUAD,SKCM,SARC	SKCM,KIRC,HNSC,PAAD,MESO,LUAD	/	CESC,KIRC,LUAD, PAAD
TAP1	Detrimental	LGG,UVM,PAAD	LUAD,UVM,KIRP,PAAD, LGG,THYM	/	LGG,OV,SKCM,UVM
	Protective	OV,SKCM	REA, SKCM,STAD,OV	/	/
TAP2	Detrimental	LGG,LAML,**KIRP, PAAD**	LUAD,LAML,UVM,LGG, ACC,**KIRP,PAAD**	**KIRP,PAAD**	LAML,LGG,**KIRP,PAAD**
	Protective	SKCM	SKCM	/	/
ABCB4	Detrimental	UVM,ACC,LGG,READ,STAD	LGG,UVM,ACC,KICH, THCA,DLBC	KIRP,STAD,THCA	ACC,LGG
	Protective	KIRC,PAAD,SKCM	KIRC,PAAD,SKCM,HNSC,SARC	/	HNSC,PAAD
ABCB6	Detrimental	SKCM,UVM,ACC, **KIRP,LIHC**,PAAD	ACC,**KIRP,LIHC**,UVM,SKCM,KIRC,COAD,KICH	KIRC, **KIRP,LIHC**	ACC,**KIRP,LIHC**,SKCM,UVM
	Protective	LGG	PAAD,LGG	/	/
ABCB7	Detrimental	UCEC	LGG,ESCA,UCEC,SARC,BRCA	BRCA,HNSC, PAAD,SARC,UCEC	/
	Protective	GBM,KIRC	KIRC,MESO,GBM,SKCM	/	GBM,KIRC,MESO
ABCB8	Detrimental	GBM,KIRC,LGG,SKCM,THCA,UVM	UVM,KIRC,LGG,KICH,GBM,SKCM	KIRC,LIHC,THCA	ACC,KICH,LGG,SKCM,UVM
	Protective	DLBC	DLBC,HNSC,KIRP,PAAD	/	DLBC
ABCB9	Detrimental	KIRC,LGG,THCA, THYM,UVM	KIRC,LGG,THYM,KIRP, UVM,DLBC,BLCA,KICH	BLCA,BRCA,KIRC, LIHC,SARC,THCA	BLCA,BRCA,DLBC,LGG,THCA,UVM

	Protective	CESC	/	/	/
ABCB10	Detrimental	MESO,ACC,ESCA	PCPG,ACC,MESO	BLCA,CESC,PCPG	ACC,CESC,LUAD, MESO,PCPG
	Protective	HNSC,KIRC	KIRC	/	/

Gene overexpression and treatment response were summarized in Figure 7.

Figure 7. Correlation analysis of the expression levels of ABCB family gene and drug sensitivity in pan-cancers.



ABCB1 (MDR1):

The expression level of ABCB1 gene was negatively correlated with the drug sensitivity of depsipeptide, actinomycin D, mithramycin, carfilzomib, homoharringtonine, doxorubicin, vinorelbine, paclitaxel, vinblastine, and daunorubicin.

ABCB4 (MDR2):

The expression of ABCB4 gene showed a negative correlation with depsipeptide, mithramycin, actinomycin D, and homoharringtonine.

Changes in the text:

We have supplemented the description about ABCB family gene co-overexpression impact on survival and response to therapy in Result section.

Specifically,

description of co-overexpression impact on survival is supplemented in Page 10, line 315-353.

description of co-overexpression impact on response to therapy is supplemented in Page 13, line 434-443.

Comment 4: How ABCB expression and stemness scores impact on survival?

Reply 4: Thank you for your careful review.

Tumor cell stemness signifies the capacity to sustain critical biological behaviors, including proliferation, differentiation, and invasion. Notably, heightened tumor stemness correlates with increased malignancy. In this context, the DNA stemness and RNA stemness discussed in this article correspond to epigenetic and transcriptomic stemness, respectively. As tumor stemness reflects a tumor's ability to maintain malignant behavior and shape the neoplastic environment, it profoundly influences the behavior of tumor cells. Stronger tumor stemness equates to enhanced abilities of tumor stem cells. These cells give rise to a larger pool of differentiated tumor cells, each endowed with potent functions. Consequently, tumor stemness directly impacts disease progression. Specifically, our article highlights the significance of RNA and DNA stemness. Elevated RNA and DNA stemness levels correspond to reduced epigenetic and transcriptional variation in cancer stem cells. This stability reinforces the transmission of malignant behavior within the tumor, ultimately leading to a worse prognosis.

A detailed analysis hinges on the specific roles of ABCB family genes. For instance, the TAP functional complex (composed of TAP1 and TAP2) plays a crucial role in immune presentation and cellular drug resistance. High expression of this complex enhances MHC I assembly, increases drug efflux, and decreases drug sensitivity, ultimately contributing to drug resistance. Simultaneously, it also elevates tumor stemness, impacting patient survival. As for ABCB1, its main function is to participate in the extracellular and intracellular transport of many substances. Our analysis results show that its high expression is beneficial to survival in various tumors. Obviously, this is contrary to its ability to reduce drug concentrations in cancer cells, but the overall survival rate is affected by many aspects. We must consider that when ABCB1 is highly expressed, the stemness of tumor cells is reduced. The impact of the level of cell stemness on the overall survival rate exceeds its impact on drug resistance. For ABCB5, its impact on melanoma is crucial and affects drug sensitivity. When its expression is up-regulated, the prognosis is reduced, and its mechanism involves cell stemness and drug resistance. When ABCB5 is upregulated, cell stemness increases and prognosis decreases.

Changes in the text:

We have modified our result section as advised.

Specifically,

description of stemness scores impact on survival is supplemented in Page 15, line 503-506.

description of ABCB expression impact on survival is supplemented in Page 10-11, line 315-353 and Page 14, line 491-493.

Comment 5: How the immune microenvironment and the different analyzed immune subgroups impact on response to therapy and survival?

Reply 5: Your questions are important for us to improve the quality of our articles.

Below, I will address the immune microenvironment and the six immune subtypes discussed in the article, along with the function and expression of the ABCB gene itself. I will explore their impact on drug treatment response and patient survival.

The influence of the immune microenvironment on treatment outcomes and patient survival is readily apparent. This microenvironment encompasses infiltrating immune cells, diverse immune regulatory factors, and stromal cells surrounding tumor sites. Theoretically, tumor cells will create an immunosuppressive microenvironment and inhibit immune cells from playing the role of immune surveillance and immune clearance, thereby conducive to tumor proliferation and metastasis. But at the same time, the activation of immune microenvironment can also enhance the function of immune cells to kill tumor cells, including stimulating and upregulating immune presentation functions by immune regulatory factors, increasing the differentiation of cytotoxic T cells, etc. Under such an immune background, drug treatments that modulate immune function are poised to amplify the tumor-killing effect. An immune-promoting microenvironment correlates with improved response to drug therapy and a more favorable prognosis. In conjunction with the findings in our article, the ABCB gene—encoding a transport protein on the cell membrane—merits attention. As a highly conserved housekeeping gene, ABCB governs material transport functions critical to the immune microenvironment. Our analysis reveals a close association between ABCB gene expression, DNA/RNA stemness, tumor purity, and immune profiling. Thus, ABCB gene expression significantly influences the immune context, treatment response, and overall prognosis.

The association between immune subtypes and treatment outcomes and prognosis is notably pronounced. According to Thorsson's theory, immune subtypes can be categorized into six distinct types, namely, C1-wound healing type, C2-IFN-γ dominant type, C3-inflammatory type, C4-lymphocyte depleted type, C5- immunologically quiet type, C6—TGF-β dominant type.

Each immune subtype exerts varying effects on treatment response and survival. Prognostically, immune subtypes are closely linked to overall survival (OS) and progression-free interval (PFI). Notably, the C3 inflammatory subtype demonstrates the most favorable prognosis. Surprisingly, despite containing a substantial number of immune components, both C1 and C2 exhibit worse prognoses than C3. Conversely, the more intricate immune subtypes—C4 and C6—display the poorest OS prognosis. The essence of immune subtype classification lies in its reliance on several distinct immune parameters (variations in macrophage or lymphocyte signatures, Th1 to Th2 cell ratios, proliferation rates, levels of intratumor heterogeneity). These parameters intricately shape the immune microenvironment, ultimately influencing patient outcomes. We refer to these influential factors as "components affecting prognosis." In subtypes C1 and C2, an increased proportion of lymphocytes correlates with improved prognosis. However, within the C3 subtype, any alteration in the five characteristics leads to a worsened prognosis. This phenomenon may arise because the C3 subtype maintains a balanced proportion of various immune components, optimizing immune surveillance and clearance function, albeit resulting in a poorer prognosis. Notably, elevated levels of Th17 and Th1 are consistently associated with worse prognoses across most immune subtypes (Thorsson et al. 2019).

Delving further into the analysis, we investigated the correlation between the expression of ABCB family genes and immune subtypes in KIRP, PAAD, and LIHC. The findings revealed that, whether across pan-cancers or within the specified three tumors, the genes TAP1 and TAP2 exhibited higher expression in the C2 immune subtype. The C2 immune subtype is characterized by IFN-aggregation, marked by increased infiltration of CD8+ lymphocytes and a higher polarization ratio of M1 macrophages, indicating heightened immune activity. These results imply the potential development of targeted drugs based on TAP1 and TAP2, which could effectively operate through the C2 immune subtype.

The discussion below delves into the intricate connection between immune subtypes and treatment outcomes. Notably, the establishment of immune subtypes transcends histological variations and tumor locations, constituting a novel classification system grounded in immune characteristics. Each unique immune profile is expected to yield distinct responses to treatment. Immune subtypes C1 and C2, characterized by a richer array of immune components, are anticipated to exhibit more favorable responses to treatments that rely on immune cells for their cytotoxic effects. These subtypes may benefit significantly from immunotherapies that harness immune cell activity. Conversely, the immune-deficient C5 subtype, lacking essential effector cells, faces substantial limitations. Even if immune preparations fully permeate the body, their impact remains greatly reduced due to the absence of target cells. Beyond immune-based therapies, other treatment modalities prioritize enhancing or weakening specific immune components. Consider immune cells with low-expression ABCB—a transport protein responsible for effluxing chemotherapy drugs. In this scenario, compromised ABCB function leads to drug accumulation within immune cells, reducing the therapeutic concentration available to tumor cells. Consequently, tumor chemotherapy sensitivity is compromised.

Changes in the text:

We have added the discussion about how the immune microenvironment and the different analyzed immune subgroups impact on response to therapy and survival in Discussion section.

Specifically,

description of immune microenvironment impact on response to therapy and survival is supplemented in Page 15-16, line 521-536.

description of immune subgroups impact on response to therapy and survival is supplemented in Page 16, line 542-549.

Comment 6: How did the authors choose drugs for sensitivity tests? Many of them are less used in solid tumors.

Reply 6: Thank you for your question.

Our selection of drugs for sensitivity testing is predicated upon the medications identified within the CellMiner database. The underlying methodology of the CellMiner database involves subjecting 60 cancer cell lines (NCI-60 cell line set) to treatment with 20503 analyzed compounds, followed by the assessment of genetic changes in more than 20,000 genes within these cell lines. Given the diverse array of compounds within this database, some of which may not yet be established in clinical practice or are in various stages of clinical or cellular trials.

So that, our approach encompasses a comprehensive range of pharmaceutical agents. This process enables the elucidation of drug sensitivity profiles.

It is noteworthy that the identified drugs associated with sensitivity to the ABCB gene may not necessarily align with those commonly employed in clinical settings. In light of valuable suggestions from reviewers, and for a better clinical practice, we screened the drugs in CellMiner, leaving only those drugs that have been approved by the FDA or have entered the clinical trial stage.

The specific drugs are listed in the following Table R2.

Table R2. FDA approved or clinical trial stage drugs in drug sensitivity testing.

Drug Name	FDA Approval Status	Clinical Trial Status
Depsipeptide	Not FDA approved	Orphan drug for multiple myeloma and peripheral T-cell lymphoma
Actinomycin D	FDA approved	/
Mithramycin	Not FDA approved	Orphan drug for chronic myeloid leukemia
Carfilzomib	FDA approved	/
Homoharringtonine	Not FDA approved	Orphan drug for chronic myeloid leukemia
Doxorubicin	FDA approved	/
Vinorelbine	FDA approved	/
Paclitaxel	FDA approved	/
Vinblastine	FDA approved	Palliative treatment for breast cancer, choriocarcinoma, Hodgkin lymphoma, and testicular cancer
Daunorubicin	FDA approved	Treatment for acute lymphoblastic leukemia and acute myeloid leukemia
Vemurafenib	FDA approved	Treatment for metastatic melanoma with BRAF V600E mutation
Dabrafenib	FDA approved	Treatment for metastatic melanoma with BRAF V600E mutation, non-small cell lung cancer, and anaplastic thyroid cancer

Nonetheless, it is crucial to acknowledge the inherent limitations of this analysis. The Cellminer database may not encompass all drugs routinely utilized in clinical practice, leading to potential gaps in the obtained results.

Based on the valuable suggestions of the reviewers, our future studies will incorporate drugs commonly employed in clinical trials. The sensitivity relationships between these drugs and ABCB family genes will be systematically verified across various tumor types.

Changes in the text:

We have added principles for selecting drugs for drug sensitivity analysis in the Discussion section. See specifically in Page 16, line 552-558.

Reviewer B

1. The Abstract of the Orginal Article should be structured as Background, Methods, Results, and Conclusions. Please modify the "**objectives**" for it.

   Reply:

   Many thanks for your kindness, I have modified my abstract section to meet the standard (line 62-74).

2. **Figures**

(1) It is suggested to use **capital "P"** for p value in all your figures.

Reply:

I have adjusted to **capital "P"** in all figures. Thank you.

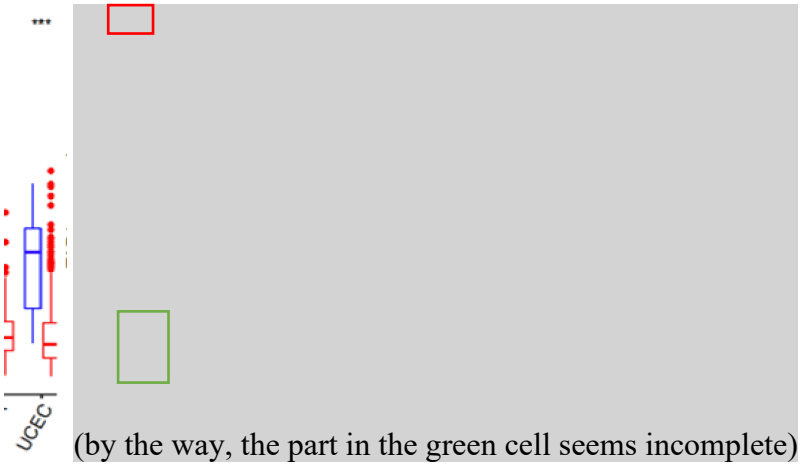
(2) **All abbreviations** in figures/tables and legends should be explained. Please check all your figures and tables.

Reply:

Thank you for your warm prompt. I have supplemented the detailed explanations for all abbreviations appeared in the figures and tables in the corresponding figure/table legends. But for some figures and tables, the content includes almost all cancer types. Therefore, in order to simplify the length, moreover there is a table that specifically details the cancer types (Supplementary Materials Table S1), here we use a sentence "The abbreviations of the cancer name can be found in Supplementary Materials Table S1." to guide reading. If there is anything inappropriate, please feel free to contact me and I will correct it immediately.

(3) Please check if **UCEC** should be added to the following sentence.

database. Our investigation unveiled that ABCB1 exhibited lower expression in tumor tissues compared to adjacent tissues across multiple cancer types, including bladder urothelial carcinoma (BLCA), breast invasive carcinoma (BRCA), colon adenocarcinoma (COAD), head and neck squamous cell carcinoma (HNSC), kidney chromophobe (KICH), kidney renal clear cell carcinoma (KIRC), lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), and rectum adenocarcinoma (READ) (**Figure 2A**). Simultaneously, our analysis indicated that TAP1 exhibited



(by the way, the part in the green cell seems incomplete)

Reply:

Thank you for your kind reminder. I have added UCEC in the sentence (line332-333). Moreover, I have adjusted the Figure 2A, please see below.

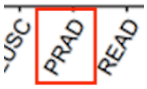
(4) Please check if **KIRP** should be added to the following sentence.

adenocarcinoma (READ) (**Figure 2A**). Simultaneously, our analysis indicated that TAP1 exhibited a state of elevated expression in several cancer types, including esophageal carcinoma (ESCA), prostate adenocarcinoma (PRAD), stomach adenocarcinoma (STAD), thyroid carcinoma (THCA), and uterine corpus endometrial carcinoma (UCEC), BLCA, BRCA, CHOL, COAD, GBM, HNSC, KIRC, LIHC, LUAD and READ (**Figure 2B**). The examination further revealed that TAP2

Reply:

I appreciate it for your kindness. KIRP should be added to the above sentence (line336).

(5) Please check and confirm whether it should be **PRAD** in the following sentence.



demonstrated higher expression levels in KICH, BLCA, CHOL, COAD, ESCA, GBM, HNSC, KIRC, KIRP, LIHC, PAAD, READ, and STAD. However, it exhibited lower expression specifically in KICH (**Figure 2C**). Our analysis indicated that ABCB4 exhibited elevated expression levels in

Reply :

Many thanks for your kindness. It should be **PRAD** instead of PAAD (line340).

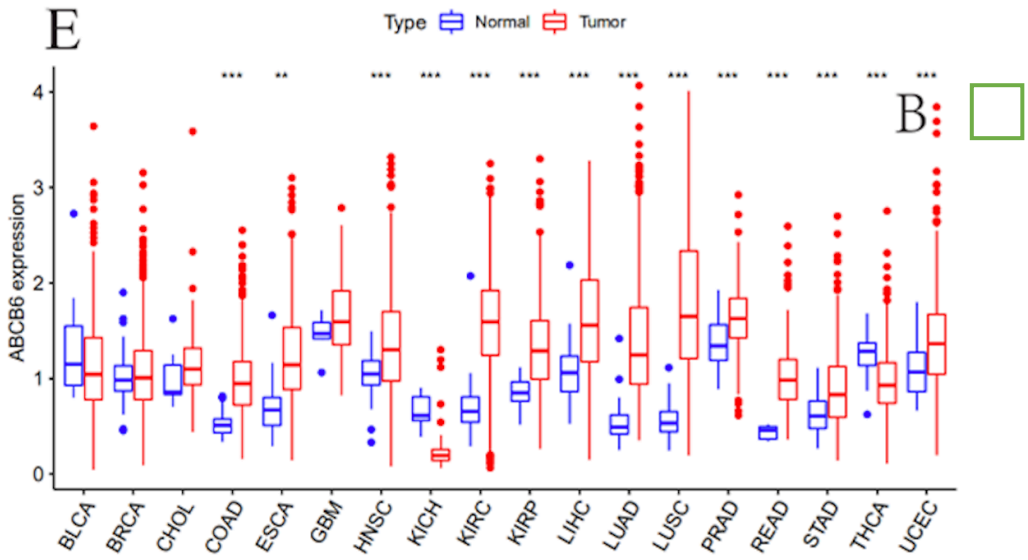
(6) Please check if **READ** and **UCEC** should be added to the following sentence.

BRCA, GBM, KIRC, KIRP, LUAD, and LUSC. Conversely, a lower expression of ABCB4 was observed in BLCA, CHOL, COAD, HNSC, KICH, and LIHC **(Figure 2D)**. The examination

Reply:

Yes, I have added READ and UCEC in the sentence (line343).

(7) Please remove the extra "**B**" from **Figure 2E**.



Reply:

I have removed the extra "B" from Figure 2E. Thank you so much.

(8) Please remove the highlighted content from the following sentence.

demonstrated lower expression in BRCA, KIRP, and UCEC **(Figure 2F)**. Our investigation found that ABCB8 displayed higher expression levels in BLCA, BRCA, CHOL, COAD, ESCA, HNSC, KIRP, LIHC, LUAD, LUSC, PRAD, READ, STAD, THCA, and UCEC. Conversely, it

Reply:

I have removed the highlighted content from the sentence. Thank you (line351).

(9) Please remove the highlighted content from the following sentence.

ABCB9 exhibited higher expression levels in BLCA, BRCA, CHOL, ESCA, HNSC, KICH, KIRC, LIHC, LUAD, LUSC, READ, STAD, THCA, and UCEC. Conversely, it displayed lower expression

Reply:

I have removed the highlighted content from the sentence. Thank you (line353).

(10) Please supplement proper **spaces** to the following words in the figures.

StromalScore ImmuneScore ESTIMATEScore

Reply 10:

I have supplemented proper spaces to the above words in the figures.

(11) There is no figure of **KIRP** in the **J-K part in Figure 8**. Please recheck the legend of Figure 8.

genders in KIRP, LIHC, PAAD, respectively. (J-K) The association between the expression level of ABCB family genes and patients' tumor grades in KIRP, LIHC, PAAD, respectively. * P<0.05;

Reply:

I have deleted KIRP in this sentence (line 778). Thank you so much.

(12) Please supplement **units** for the "Age" in **Figure 8**.

Reply:

Thank you so much. I have added the unit "year" for the "Age" in Figure 8.

(13) It is suggested to specify which group that "**Age=60**" belongs to in Figure 8.

Reply:

Thank you so much. I have specified "**Age=60**" group in Figure 8.

(14) Please indicate the observation methods and the magnification/scale bars in the legend of **Figure 9**.

Reply:

I have mentioned the observation methods and the magnification/scale bars in the legend of Figure 9 (line785-786).

3. Supplementary

(1) The following highlighted content has not been shown in Supplementary Materials Figure S1. Will an additional figure be added for it?

protective role in LGG **(Figures 3R-X)**. ABCB7 played a detrimental role in UCEC, while having a protective role in GBM and KIRC **(Supplementary Materials Figure S1)**. ABCB8 exhibited a

Reply:

Thanks for checking carefully and pointing out errors. I am wrong. I have deleted it (line 373).

(2) Some content in Figure S2, and Figure S4 is not clear enough. Please update it with a higher resolution one.

Reply:

I have supplied the higher quality version of Figure S2 and Figure S4. Additionally, I have replaced all the figures with high-quality one. I am so sorry for the trouble I caused before.

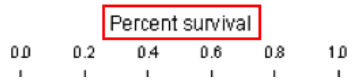
(3) Please check if **COAD** is correct in the following sentence.

(**Table 1**). In **Figure S2**, correlation analysis was done between the expression levels of ABCB family gene and prognosis by the COX method in pan-cancers. Results manifested that ABCB1 take a protective role in PAAD,COAD,HNSC,KIRC,LUAD,SKCM and SARC. TAP1 had a detrimental

Reply:

Thanks for checking carefully and pointing out errors. It is wrong. I have deleted it (line388).

(4) It is suggested to remove the "**percent**" in the y-axes in **Figure S3**.



Reply:

I have removed the "percent" in the y-axes in Figure S3. Thank you.

(5) Please unify the citations of your supplementary material to "Figure Sxxx" in your text.

Reply:

I have unified the citations of my supplementary material to "Figure Sxxx" in my text. They have been unified to the format of "Supplementary Materials Figure Sxxx."