

Peer Review File

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Reviewer A

Comment 1: Authors did comprehensive analysis using bioinformatics online databases. The description in the result section is not clear and confusing (Fig.1-10). Please give a clear description in the result section.

Reply 1: I am very grateful to the reviewers for their valuable comments. I have described the results section of Figures 1-10 further. Including results and legend.

Changes in the text: In the Results section, I further describe the results of the study in Figures 1-10. (See Line 229-232, 234-235, 250-252, 255-257, 269-271, 279-286, 651-652, 658.)

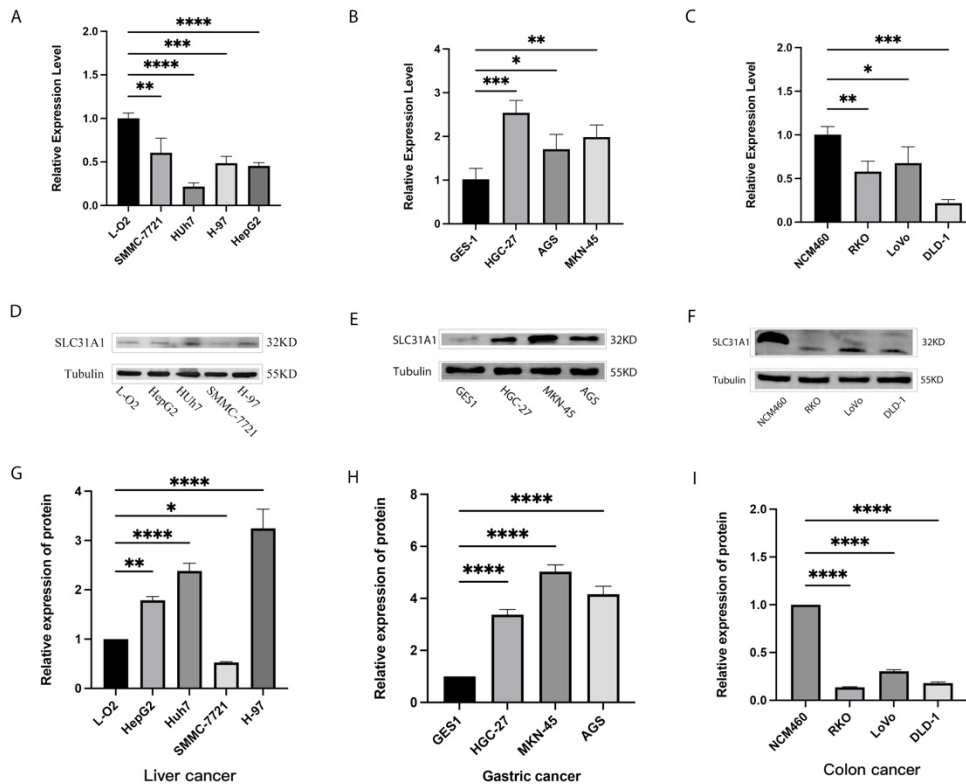
Comment 2: What is the aim of this manuscript? Please provide description in introduction section.

Reply 2: I am very grateful to the reviewers for their valuable comments. In order to enhance the clarity and coherence of the introduction section, the purpose of this study will be explicitly stated as follows: The objective of this study was to examine potential targets for cancer therapy by analyzing the expression prognosis and immunity of SLC31A1 in pan-cancer, building upon previous research. The findings aim to offer novel insights into the molecular mechanisms of cancer and facilitate personalized treatment for pan-cancer patients.

Changes in the text: I have provided the purpose of this study in the introductory section (see Lines 96-102).

Comment 3: The western blot showed that colon cancer cells decreased CTR1 expression (Fig 13F) but it was not reflected in quantification data (Fig. 13I). Clarify the controversy.

Reply 3: I am very grateful to the reviewers for their valuable comments. I apologize for the error in quantifying the colon cancer results due to my negligence. I have re-quantified the colon cancer results as shown below:



Changes in the text: As shown above, the manuscript is located at (line681-682)

Comment 4: In liver cancer, the CTR1 mRNA expression is decreased in cells (Fig. 13A) but the protein expression is increased in cells (Fig. 13G). What is the mechanism of opposite result and discuss.

Reply 4: I. I am very grateful to the reviewers for their valuable comments. In light of this study's purpose of validating bioinformatics analysis, it is possible that inconsistencies may arise between gene expression at the transcript and protein levels. II. Notably, these inconsistencies could be attributed to various modifications occurring at the transcriptome or protein level, which will be the focus of future research in this study. III. Considering the absence of evidence regarding the expression of the SLC31A1 gene in hepatocellular carcinoma from previous relevant literature, further investigation into this result is warranted. IV. Despite the low mRNA level, the protein level adequately reflects the clinical significance of SLC31A1, and its elevated expression is strongly associated with the clinical prognosis.

Changes in the text: See line 471-481

Comment 5: Antibody information is not available. Please provide a catalog number etc.

Correct "CTR1" instead of "CRT1" (line 70)

Reply 5: SLC31A1 antibody was purchased from Wuhan Three Eagles Biotechnology Co. Catalog number is Cat No. 67221-1-1g. I have changed CRT1 to CTR1.

Changes in the text: I've made changes, (line 82-83).

Reviewer B

Comment 1: For abbreviations in the text, it is suggested to place the full names **before the abbreviations**. For example, **kidney renal clear cell carcinoma (KIRC)**. Please check through your article and unify them.

Reply 1: Thanks to the editor's valuable comments, I have defined all the abbreviations in the full text as per the requirements of your journal. All changes have been marked with revision tracking.

Comment 2: Figures

- (1) Please recheck the **capitalization** used in your figures. For example, use “Glioblastoma Multiforme” or “glioblastoma multiforme” in **Figure 2B**. Please check all your figures for this issue.

Glioblastoma multiforme

Thanks for the valuable comments, which have been revised.

Protein expression of SLC31A1 in glioblastoma multiforme

- (2) Would it be better to use **liver hepatocellular carcinoma** in **Figure 2B**?

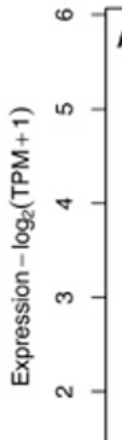
Protein expression of SLC31A1 in Hepatocellular carcinoma

Thanks for the valuable comments, I have revised "hepatocellular carcinoma" to "liver hepatocellular carcinoma".

Protein expression of SLC31A1 in liver hepatocellular carcinoma

- (3) Please add a description for the **y-axis of Figure 2D**.

Thanks for the valuable comments, I have added a description for the Y-axis.



(4) Please supplement the explanation for “*****” in the legend of **Figure 2**.

Thanks for the valuable comments, which have been added.

682 endometrioid cancer; UCS, uterine carcinosarcoma; UVM, ocular melanoma. (****p < 0.0001, ***p <
683 0.001, **p<0.01, *p<0.05).↵

(5) Note: For each image/cell map from the **HPA database**, please indicate **the source and the URLs in the legends**.

Thanks for the valuable comments, which have been added.

686 Figure 3: SLC31A1 expression in the UACLAN and HPA databases, Immunohistochemistry 40×
687 images were presented. BRCA, breast invasive carcinoma(Normal
688 Breast:<https://www.proteinatlas.org/ENSG00000136868-SLC31A1/tissue/breast#img>, BRCA:
689 <https://www.proteinatlas.org/ENSG00000136868-SLC31A1/pathology/breast+cancer#img>); LIHC,
690 liver cancer(Normal Liver:
692 [SLC31A1/pathology/liver+cancer#img](https://www.proteinatlas.org/ENSG00000136868-SLC31A1/pathology/liver+cancer#img)); STAD, stomach cancer(Normal stomach
693 <https://www.proteinatlas.org/ENSG00000136868-SLC31A1/tissue/stomach#img>, STAD:
694 <https://www.proteinatlas.org/ENSG00000136868-SLC31A1/pathology/stomach+cancer#img>); UCEC,
695 endometrioid cancer(Normal endometrioid:
697 [SLC31A1/pathology/endometrial+cancer#img](https://www.proteinatlas.org/ENSG00000136868-SLC31A1/pathology/endometrial+cancer#img)). (****p < 0.0001, ***p < 0.001, **p<0.01, *p<0.05).↵

(6) Please supplement the explanations for “*” and “****” in the legend of **Figure 3**.

Thanks for the valuable comments, which have been added.

697 [SLC31A1/pathology/endometrial+cancer#img](https://www.proteinatlas.org/ENSG00000136868-SLC31A1/pathology/endometrial+cancer#img)). (****p < 0.0001, ***p < 0.001, **p<0.01, *p<0.05).↵

(7) The **staining methods** and the **magnification** for all cell maps are needed. Please supplement it in their legends respectively.

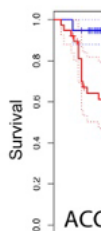
Thanks for the valuable comments, which have been added.

6 Figure 3: SLC31A1 expression in the UACLAN and HPA databases, Immunohistochemistry 40x

7 images were presented. BRCA, breast invasive carcinoma(Normal

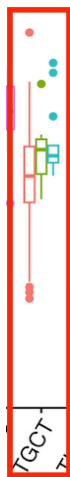
(8) Please remove the “percent” in the y-axis of **Figure 4** since it ranges from 0 to 1 (without %).

Thanks for the valuable comments, which have been removed.



(9) Please recheck if the **TGCT** is proper in the following sentence:

- “As shown in Figure 5A, SLC31A1 was highly expressed in ACC and **TGCT** stage III-IV patients and low in stage I-II patients.”



Thanks for the valuable comments, I have rewritten the sentence.

276 As shown in Figure 5A, SLC31A1 was highly expressed in ACC stage III-IV patients and low in stage

277 I-II patients. SLC31A1 was highly expressed in TGCT stage II-III patients and low in stage I patients.

(10) Please recheck the highlighted content in the following sentence.

- Of these, SLC31A1 was highly expressed in OV and UCEC patients up to and including 65 years of age, while it was highly expressed in ESCA, SARC and STAD **up to and including 65 years of age.**

Thanks for the valuable comments, I have rewritten the sentence.

280 OV, Sarcoma (SARC), STAD, and UCEC patients. Of these, SLC31A1 was highly expressed in OV and
 281 UCEC patients up to and including 65 years of age, while it was highly expressed in ESCA, SARC and
 282 STAD patients over 65 years of age. Finally, we found that SLC31A1 is highly expressed in female

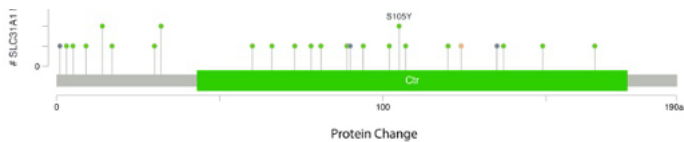
(11) Please supplement the explanation for “*”, “**”, and “***” in the legend of Figure 5.

Thanks for the valuable comments, which have been added.

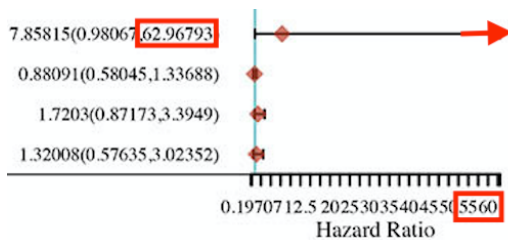
725 thymoma; UCEC, endometrioid cancer; UCS, uterine carcinosarcoma; UVM, ocular melanoma. (***p
 726 < 0.0001, ***p < 0.001, **p<0.01, *p<0.05).

(12) Please confirm if any description is needed for the x-axis of Figure 6B.

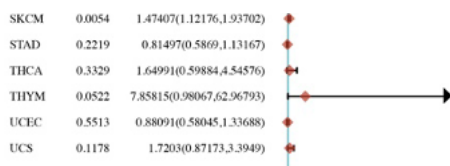
Thanks for the valuable comments, I have added a description for the x-axis.



(13) To standardize the results, the part that exceeds the horizontal coordinates should be indicated by the **arrow** below. Or you can add a **scale bar** of 65 on the x-axis. **Please check all your figures for it.**



Thanks for the valuable comments, which have been revised.



(14) It is suggested to change “pvalue” to “p value” in **Figure 7**.

Thanks for the valuable comments, which have been revised.

Cancer P value Hazard Ratio(95% CI)

(15) The figures are too close to distinguish in **Figure 7**. Please revise.

0.1970712.5 202530354045505560

Thanks for the valuable comments, which have been revised.



(16) Please supplement the explanations for “*”, “**”, and “***” in the legend of **Figure 8**.

Thanks for the their valuable comments, which have been added.

759 carcinosarcoma; UVM, ocular melanoma. (****p < 0.0001, ***p < 0.001, **p < 0.01, *p < 0.05).

(17) The following two words (**Figure 8C**) are too close. Please slightly modify for it if possible.

LUADLUSC***

Thanks for the valuable comments, which have been revised.

LUAD LUSC***

(18) Please supplement the explanation for “***” in the legend of **Figure 9**.

Thanks for the valuable comments, which have been added.

764 endometrioid cancer. (****p < 0.0001, ***p < 0.001, **p < 0.01, *p < 0.05).

(19) Please check if it should be “p ≤ 0.05” in **Figures 10 and 11**.

■ p ... 0.05

Thanks for the valuable comments, which have been revised.

■ p ≤ 0.05

(20) Please supplement the explanation for “***” and “****” in the legend of **Figure 11**.

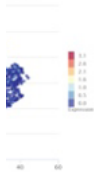
Thanks for the valuable comments, which have been added.

788 cancer; MEL, mouse erythro leukemia; RB, retinoblastoma; UM, uveal melanoma. (****p < 0.0001, ***p
789 < 0.001, **p<0.01, *p<0.05).↵

(21) If the following content in **Figure 11** is complete?



Thanks for the valuable comments, which have been revised.



(22) Please **unify** the “p-value” and “p value” **in all your figures**.

Thanks for the valuable comments, which have been revised.

(23) Please supplement the explanations for “***” and “****” in the legend of **supplementary figure 1**.

Thanks for the valuable comments, which have been added.

databases. (****p < 0.0001, ***p < 0.001, **p < 0.01, *p < 0.05).↵