

## Peer Review File

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### Reviewer A

The manuscript describes the function of the PIMREG molecule in melanoma. Bioinformatic analysis tools and databases are used.

Since the manuscript is very identical to the article: Zhao W, Chang Y, Wu Z, Jiang X, Li Y, Xie R, Fu D, Sun C, Gao J. 2023. Identification of PIMREG as a novel prognostic signature in breast cancer via integrated bioinformatics analysis and experimental validation. PeerJ 11:e15703 <http://doi.org/10.7717/peerj.15703>, the article feels somewhat arbitrary to the reviewer.

Reply: The same gene, such as PIMREG, may have different effects in different tumors. Breast cancer is a common cancer and a research hotspots, there are many databases about breast cancer. However, melanoma is often difficult to collect data due to the difficulty in obtaining adjacent tissue (normal skin). We chose melanoma for PIMREG research, which might has certain clinical significance for the prevention and treatment of melanoma.

I have also cited the above article. It is [3].

The basis for designating PIMREG as a diagnostic and prognostic marker for melanoma, as was done in the conclusion in lane 29, is based only on bioinformatics analyses. The reviewer therefore considers this to be an overstatement. In the conclusion lane 306 this was correctly relativized.

Reply: We have modified the text description in conclusion (see Page 3, line 48) and other lines (Page 4, line 74) (Page 8, line 141) (Page 11, line 210) (Page 13, line 266). Changes in the text: PIMREG is a promising diagnostic and prognostic biomarker and may be regarded as a possible therapeutic target for SKCM.

Details: In line 248 and the following, a link is also very quickly developed between the analysis in cholangiocarcinoma relevant cells and skin cancer, which the reviewer also sees as an exaggeration.

Reply: We have modified our text as advised (see Page 11, line 215).

Changes in the text: The bioinformatics analyses results above are similar to in vitro experimental results obtained from glioma cells (23). Altogether, these results suggest that PIMREG may have a significant impact on the malignant phenotype of SKCM.

In principle, the approach of using bioinformatic analyses to obtain ideas about the function of a molecule in cancer is good. But these are ideas for generating hypotheses and cannot yet be linked to diagnostics and prognostics for cancer patients. This should also be mentioned in the manuscript.

Reply: We added some text description in discussion (see Page 13, line 260).

Changes in the text: The limitation of this study was that it was primarily based on

bioinformatics analysis. The data was collected from online platform databases and still needs to be confirmed by in vitro and in vivo experimental validation.

What is particularly interesting, however, are all the analysis tools that were used by the working group and these are also solid and sensibly applied.

Reply: Thank you.

## Reviewer B

### 1. Abstract

- 1) It is needed to describe the aim/objective of this study in the Background.
- 2) Please provide the full name of PIMREG, TCGA, GTEX, GEO, ROC, GO, KEGG, GSEA, ssGSEA, AUC, EMT in the Abstract.

Answer: We have revised them as suggested.

### 2. Main text

- 1) Please check all abbreviations in the main text, such as TCGA, GEO, MSigDB, TIMER, AUC, HR, EMT, TISCH etc. All abbreviated terms should be full when they first appear.
- 2) Please provide website link/URL for TCGA, GTEX and GEO database.
- 3) Please revise the Subheading “Data analysis” to “Statistical analysis”.
- 4) Should it be “has shown”?

364 Recent study also **shown** that PIMREG phosphorylation at S16 activates NF-kappaB and promotes

Answer: We have revised them as suggested.

### 3. Figures

- 1) Figure 1: Please provide an **editable version** as a stand-alone WORD/PPT file, so that the editor can slightly and properly adjust the lines and structures, and text during the editing.

Answer: We used the drawing software that comes with the Windows operating system to complete Figure 1, it could not meet the request for modification by editor. So, now, we draw it again as PPT. There might be a little differences between the current Figure 1 and the original one.

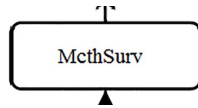
- 2) Figure 1: Please define IHC, SKCM, PIMREG, TIMER, ROC, STRING, GSEA, GO, KEGG in the legend.

Answer: We have revised them as suggested. STRING is the name of the database (<https://cn.string-db.org>), it's not an abbreviation.

- 3) Figure 1: please unify the following information.

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2.5 **MethSurv** Analysis



Answer: Sorry for it. We have unified them to MethSurv.

- 4) Figure 2: Please define SKCM, PIMREG, TCGA, GTEx, ROC, AUC, CI, FPR, TPR, HPA, OS, DSS, PFI, HR, TPM in the legend.

Answer: We have revised them as suggested.

- 5) Figure 2D: please unify the following data.

41 correlated with poor survival outcome in melanoma patients. [ROC analysis also revealed that PIMREG](#)

42 [had high diagnostic potential, with AUC values of 0.895](#). Multivariate regression [also](#) identified

167 high diagnostic potential, with AUC values of 0.895 (see Fig. 2D). It was observed that the protein

	FAM64A
	AUC: 0.874
	CI: 0.854-0.894

Answer: Sorry for it. We have revised them as suggested.

- 6) Figure 2D: please revise “CI” to “95%CI”.

	AUC: 0.874
CI	0.854-0.894

Answer: We have revised them as suggested.

- 7) Figure 2E: For cell maps from HPA database, it's needed to indicate the source and the URL for each image.

Answer: We have revised them as suggested.

In Figure 2 legend, it is added: “Image credit goes to the HPA, the staining method is immunohistochemistry. The links to the normal and SKCM tissues of PIMREG protein are provided ([https://images.proteinatlas.org/43783/97662\\_B\\_5\\_8.jpg](https://images.proteinatlas.org/43783/97662_B_5_8.jpg); [https://images.proteinatlas.org/43783/97660\\_B\\_4\\_8.jpg](https://images.proteinatlas.org/43783/97660_B_4_8.jpg)).”

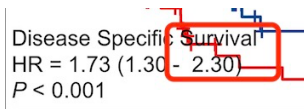
- 8) Figure 2E: Please indicate the staining method.

Answer: We have revised them as suggested. The staining method is immunohistochemistry.

- 9) Figure 2E: Please provide the scale bar or magnification.

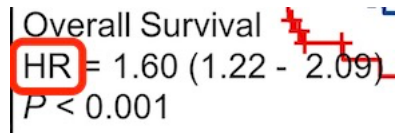
Answer: Image credit goes to the HPA (<https://www.proteinatlas.org/>). There is no scale bar or magnification displayed in the image. Perhaps I could not provide it.

- 10) Figure 2F-H: the words are overlapped. Please check and revise.



Answer: We have revised them as suggested.

11) Figure 2F-H: please revise “HR” to “HR (95% CI)”.



Answer: We have revised them as suggested.

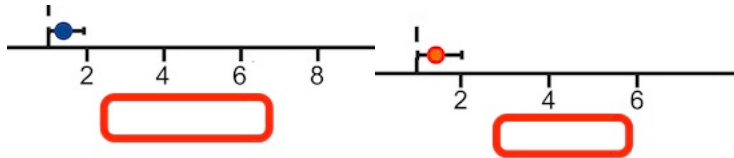
12) Figure 3: Please define SKCM, PIMREG, OS, DSS, HR, CI in the legend.

Answer: We have revised them as suggested.

13) Figure 3A-B: Please add unit for Age.

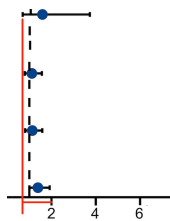
We have revised them as suggested

14) Figure 3A-B: Please provide description for x-axis.



Answer: We have revised them as suggested.

15) Figure 3A-B: please extend the a-axis.



Answer: The dashed line represents 1. We have changed the number in Figure 3A-B as suggested.

16) Figure 4-6 is not clear. Please provide Figure 4-6 in higher resolution.

Answer: We have changed them to as higher resolution as possible.

17) Figure 4: Here is a typo.



Answer: We have changed negative to negative.

Figure 4: Please define SKCM, PIMREG, EMT in the legend.

Answer: We have revised them as suggested.

18) Figure 4: Please indicate the meaning of \*\*, \*\*\* in the legend.

Answer: We have added the meaning of \*\*, \*\*\* in the legend as suggested.

The correlation between PIMREG and functional status: \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ .

19) Figure 5: Please define SKCM, PIMREG, STRING, KEGG, GSEA, BP, CC, MF, NES, FDR, TPM in the legend.

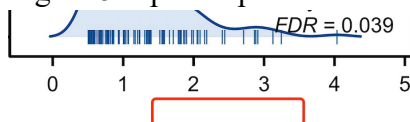
Answer: We have revised them as suggested. In addition, STRING is not an abbreviation.

20) Figure 5C: the words are overlapped. Please check and revise.



Answer: The figures were downloaded from the website TIMER (<https://cistrome.shinyapps.io/timer/>) and could not be revised. We have tried our best to make them better.

21) Figure 5F: please provide description for x-axis.



Answer: We have added “Log2FC” for x-axis as suggested.

22) Figure 5F: please revise “Pi3kakt” to “PI3K/Akt”.

Pi3kakt  
Signaling Pathway -

Answer: We have revised them as suggested.

23) Figure 5G: please revise “G2m” to “G2/M”.



Answer: We have revised them as suggested.

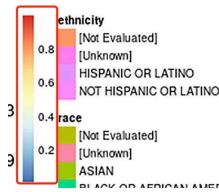
24) Figure 6: Please define SKCM, PIMREG, BMI in the legend.

Answer: We have revised them as suggested.

25) Figure 6: please add unit for BMI, Age.

Answer: We have revised them as suggested.

26) Figure 6: please add description for the bar.



Answer: It means the correlation between PIMREG mRNA expression level and methylation level. we have checked the previous paper, and noticed that the description for the bar was in legend. So we revised the legend of Figure 6.

27) Figure 7: Please define SKCM, PIMREG, TPM in the legend.

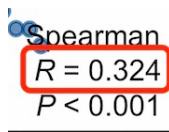
Answer: We have revised them as suggested.

28) Figure 7: please indicate the meaning of ns, \*, \*\*, \*\*\* in the legend.

Answer: We have revised them as suggested.

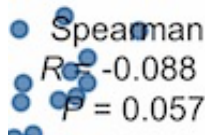
29) Figure 7C: Please unify the following data.

254 of Th2 cells showed a positive association with PIMREG ( $R = 0.321$ ,  $P < 0.001$ ) (Fig. 7C). Additionally,



Answer: We have revised them as suggested.

30) Figure 7C-F: the words are overlapped. Please check and revise.



Answer: We have revised them as suggested.

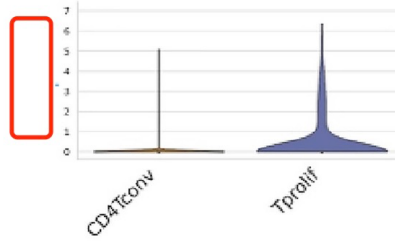
31) Figure 8 is not clear. Please provide Figure 8 in higher resolution.

Answer: We have changed them to as higher resolution as possible.

32) Figure 8: Please define SKCM, PIMREG, TPM in the legend.

Answer: We have revised them as suggested.

33) Figure 8B-C: please provide description for y-axis.



Answer: We have added “Expression level” for y-axis as suggested.

34) Figure 9: Please define PIMREG, GDSC, IC50 in the legend.

Answer: We have revised them as suggested.

35) Figure 9: please use lowercased letter as “Dacarbazine”.

FAM64A **DACARBAZINE**  
 Cor=-0.341, p=0.008

Answer: We have revised them as suggested.