

Peer Review File

Article information: <https://dx.doi.org/10.21037/jgo-23-198>

Reviewer A

Comment 1: Page 3-line 56 PDAC or PC- be specific and consistent.

Reply 1: Thank you very much for your suggestion, we have modified it.

Changes in the text: we have modified our text as advised (see Page 3, line 60).

Comment 2: Line 65- Messenger RNAs (mRNAs) and MicroRNAs (miRNAs) – use small letters not capital.

Reply 2: Thank you very much for your suggestion, we have modified it.

Changes in the text: we have modified our text as advised (see Page 3, line 70).

Comment 3: APC is not a novel gene.

Reply 3: Thank you very much for your suggestion, we have modified it.

Changes in the text: we have modified our text as advised (see Page 4, line 90).

Comment 4: little/no info on miR125b-add more info-justify the relevance.

Reply 4: Thank you very much for your suggestion, we have modified it.

Changes in the text: we have modified our text as advised (see Page 3 and 4, line 72-85).

Comment 5: Add the ethics number.

Reply 5: Thank you very much for your suggestion, we have modified it.

Changes in the text: we have modified our text as advised (see Page 5 and 6, line 135 and 172).

Comment 6: CAF-NO info on cell culture? Where do they come from?

Reply 6: Thank you very much for your suggestion, we have modified it.

Changes in the text: we have modified our text as advised (see Page 5, line 128-130).

Comment 7: WB- Which WNTs? Which abs?

Reply 7: Thank you very much for your suggestion, we have modified it.

Changes in the text: we have modified our text as advised (see Page 5, line 149).

Comment 8: qPCR- include the primer seqs.

Reply 8: Thank you very much for your suggestion, we have modified it.

Changes in the text: we have modified our text as advised (see Page 6 and 24, line 162 and 623).

Comment 9: imaged with which magnification, microscope?

Reply 9: Thank you very much for your suggestion. The previous manuscript, as well as some of the content included, we have further added.

Changes in the text: we have modified our text as advised (see Page 7, line 195-196).

Comment 10: it was mentioned bioinformatics analysis in the abstract, but not in methods

Reply 10: Thank you very much for your suggestion, we have modified it.

Changes in the text: we have modified our text as advised (see Page 7, line 202-206).

Comment 11: Transwell/motility- how many cells, how long?

Reply 11: This was included in the previous manuscript (see Page 7, line 189).

Comment 12: Fig legends need more info as general.

Reply 12: We have written the legend at the bottom of the diagram.

Comment 13: Fig 1C, why there are 2pics but 3 paired bars in the chart?

Reply 13: The statistical graphs count the results of the experiment for three different samples of tissues. We use the image to show the results of one sample.

Comment 14: Line 200: which miRs.

Reply 14: Thank you very much for your suggestion, we have modified it.

Changes in the text: we have modified our text as advised (see Page 8, line 235-237).

Comment 15: Fig 3a refers to different PDAC cell lines, but in the methods only for Miapaca, panc1

Reply 15: Thank you very much for your suggestion, we have modified it.

Changes in the text: we have modified our text as advised (see Page 5, line 125-126).

Comment 16: why in subscript?

Reply 16: Thank you very much for your suggestion, We have made changes.

Changes in the text: we have modified our text as advised (see Page 11, line 316).

Comment 17: date after the ref.

Reply 17: Thank you very much for your suggestion, We have made changes.

Changes in the text: we have modified our text as advised (see Page 11, line 325).

Comment 18: again, more specific for bioinformatics.

Reply 18: Thank you very much for your suggestion, we have modified it.

Changes in the text: we have modified our text as advised (see Page 11, line 330-334).

Reviewer B

Comment: Increasing evidence has shown that cancer-associated fibroblasts (CAFs) assist tumor development, invasion, and metastasis in PDAC. However, CAFs-derived molecular determinants that regulate the molecular mechanisms of PDAC have not been fully characterized. In this manuscript the authors the authors have shown that CAFs release exosomes into PDAC cells, which significantly increase the level of miR-125b-5p in those cells. They have shown that 125b-5p binds to the 3'-untranslated region (3'-UTR) of the adenomatous

polyposis coli (APC), thereby limiting the progression of pancreatic cancer. They suggest that Exosomal miR-125b-5p inhibition offers an alternate strategy for combating PDAC. The authors have provided sufficient that which supports the conclusion. The reviewer believes the manuscript is worthy enough of its publication JGO.

Reply: Thank you for your affirmation and support.

Reviewer C

1. APC functions as a tumor suppressor in literature. The present work also indicates the same. Yet, the author called APC as oncogene (line 72).

Reply: We have modified it accordingly.

Changes in the text: we have modified our text as advised (see Page 4, line 90).

2. Overall articulation in English is very poor. For example, the meaning is not clear at line 116-18.

Reply: We have modified it accordingly.

Changes in the text: we have modified our text as advised (see Page 5, line 149-154).

3. Methodology is very superficial. Particularly no information is available on miR-mimic used in the work.

Reply: We have made the necessary additions.

Changes in the text: we have modified our text as advised (see Page 5, line 142-145).

4. Fig 4 B which one is mimic or control, not mentioned.

Reply: We have made the changes and provided the modified image. The legend has also been modified.

Changes in the text: we have modified our text as advised (see Page 22, line 608).

5. Fig 3 C-F is completely contradicting to the text (line 213). Although in figure, miR mimic sets show less proliferation/migration, in the text it is claimed that overexpression system indicates more proliferation (line 213). With this, I do not know how to proceed further.

Reply: We have made the changes and provided the modified image. The legend has also been modified.

Changes in the text: we have modified our text as advised (see Page 21, line 587-600).

Reviewer D

1. ARRIVE Checklist

Item 5: If no blinding was used in your study, please just fill "N/A" for this item in the checklist.

Blinding	5	Describe who was aware of the group allocation at the different stages of the experiment (during the allocation, the conduct of the experiment, the outcome assessment, and the data analysis).	All authors
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Reply: We have revised the Item 5 and 19 according to the Editorial Comments

2. MDAR checklist: They were reported in your paper, please do not fill “N/A”. Please check and revise.

Ethics	Yes (indicate where provided: section/paragraph)	n/a
Studies involving human participants: State details of authority granting ethics approval (IRB or equivalent committee(s), provide reference number for approval.		n/a
Studies involving experimental animals: State details of authority granting ethics approval (IRB or equivalent committee(s), provide reference number for approval.		n/a
Studies involving specimen and field samples: State if relevant permits obtained, provide details of authority approving study; if none were required, explain why.		n/a

Reply: We have modified it according to your suggestion.

3. Legends of Figure 2D are missing, please check and supplement.

Reply: We have modified it according to your suggestion.

Changes in the text: we have modified our text as advised (see Page 8 and 20, line 240 and 563).

4. “*” was not included in your Figure 3, please check the legends.

752 Zeiss lens, qRT-PCR, quantitative real-time polymerase chain reaction; CCK8, cell
 753 counting kit 8. *p < 0.05, **p < 0.01 and ***p < 0.001.

Reply: We have modified it according to your suggestion.

Changes in the text: we have modified our text as advised (see Page 22, line 583).

5. All those images in Figure 3 are in 400X magnification? Please check.

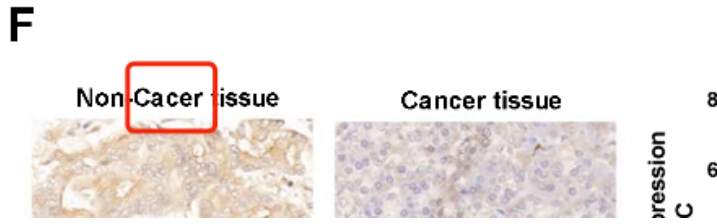
The figure consists of two main parts, E and F. Part E shows two line graphs of Absorbance vs Days (0-6) for Mia Paca2 cells. The top graph compares miR-Mimic (red line) and miR-125b-5p (blue line), showing a significant increase in absorbance for the miR-Mimic group. The bottom graph compares miR-Mimic (red line) and miR-125b-5p (blue line), showing a significant increase in absorbance for the miR-Mimic group. Part F shows micrographs of cell migration and scratch assays. The top row shows invasion and migration assays for Mia Paca2 cells, comparing miR-Mimic and miR-125b-5p groups. The bottom row shows plate cloning assays for PANC-1 and Mia Paca2 cells, comparing miR-Mimic and miR-125b-5p groups. Red arrows point from the text in the document to the corresponding parts of the figure.

Reply: We have modified it according to your suggestion.

Changes in the text: we have modified our text as advised (see Page 22, line 578).

6. Figure 4

a. Please check and revise the typo.



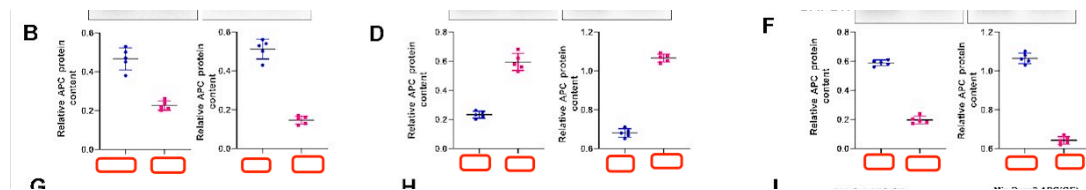
b. Please define “ns” in figure legends.

Reply: We have modified it according to your suggestion.

Changes in the text: We have modified the text (see page 23, line 597) and the picture as suggested.

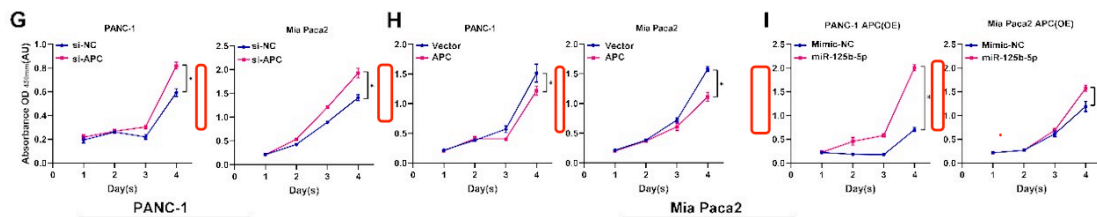
7. Figure 5

a. Please add description of X-axis in panels B, D, F.



Reply: We have added description of X-axis in panels B, D, F. And we provide the modified images with image replacement in the manuscript.

b. Please add description of Y-axis in panels G, H, I.



Reply: We have added description of Y-axis in panels G, H, I. And we provide the modified images with image replacement in the manuscript.

c. “**” was not included in your Figure 5, please check the legends.

612 assay in APC-overexpressed cells. Stained with 0.1% crystal violet. Images were taken
613 at a 400x zoom using a Zeiss lens, APC, adenomatous polyposis coli; CCK8, cell
614 counting kit 8. *p < 0.05, **p < 0.01 and ***p < 0.001

Reply: Thank you very much for your suggestions, we commented on the changes.

8. Please define APC and PCR in Table 1 footnote.

Reply: We have modified it according to your suggestion.

Changes in the text: we have modified our text as advised (see Page 24, line 624).

9. Please check the citation, it seems wrong.

268 informatics. According to the PCR (Figure 4A) and western blotting (Figure 4B)
269 results, both the protein and mRNA levels of APC were down-regulated in miR-125b-
270 5p-overexpressing cells. To further confirm the underlying mechanism, a luciferase
271 activity assay was performed. As shown in Figure 2C,2D, wild-type APC reporter
272 gene luciferase activity was reduced when miR-125b-5p was overexpressed in both
273 pancreatic cancer cell lines. In addition, 36 fresh pancreatic cancer tissue samples
274 were used to investigate the underlying relationship between miR-125b-5p and APC.
275 Figure 4E shows that miR-125b-5p was over-expressed while APC was

Reply: We have modified it according to your suggestion.

Changes in the text: we have modified our text as advised (see Page 9, line 261).