**Answers to the reviewers’ specific comments**

**Reviewer: 4**

**General Comments:** In this article the authors showed that myoferlin is overexpressed in highly metastatic pancreatic cells selected through a murine in vivo model. The authors demonstrated that overexpression of myoferlin is associated with both migratory capability and increased mitochondrial activity of selected cancer cells. This manuscript is well written and highlights a potential role of myoferlin in therapeutic strategies targeting pancreatic ductal adenocarcinoma (PDAC). However, this article has some weakness that need to be addressed before publication.

**Specific comments**

1. In the introduction the author should also focus on the pivotal role of tumor microenvironment in cancer metabolic reprogramming (PMID: 29967776, PMID: 27595103).

   *Authors thank the reviewer for his/her relevant suggestion. We have added some information in the introduction section to highlight the importance of the “reverse Warburg effect” and we have cited Arcucci’s reviews as suggested, as well as Sousa’s article describing the alanine feeding of PDAC cells by stellate cells.*

2. In the line 52 the author should add other references, such as PMID: 31052256, that highlight the role of mitochondrial flexibility in cancer.

   *As requested by the reviewer, the review authored by Avagliano and coworkers was cited in the context of the mitochondrial flexibility.*

3. In the figure 1A the authors should add the densitometric ratio of the means of the three experiments, with relative standard errors and \( p \) values.

   *Authors thank the reviewer for his/her sounding remark improving our results. Densitometric analysis was performed in 3 independent biological replicates. Mean±sem, as well as \( p \)-value from one-sample \( T \) test, were added in Fig. 1A. Results confirmed the myoferlin expression difference between Panc-1, Patu8988T and MiaPaCa-2. Correlation between migration speed and mean myoferlin expression was recalculated.*

4. The authors should show the densitometric analysis in Figure 4 C and in the inset of Figure 5 C.

   *Authors thank for the reviewer for his/her sounding suggestion improving the information delivered by our results. Densitometric analysis were included in Fig. 4C and in Fig 5C insets.*

5. In the line 98 the authors should change "of" with "on".

   *We thank the reviewer for his/her careful proofreading. The sentence was corrected as required.*
6. In the subsection 2.2 the authors should demonstrate that the treatments with chain uncoupler (FCCP) and mitochondrial respiratory chain complex 3 inhibitor (antimycin A) mixed with ATP synthese inhibitor (oligomycin) really impair the mitochondrial functions. To this aim oxygen consumption rate should be measured (PMID: 26534958).

We want to thank the reviewer for having suggest us this interesting control. Although, FCCP or Antimycin + Oligomycin treatments were described previously by Allen and coworker [1] in EMBO report for inducing mitochondrial damage leading to mitophagy, we have performed an oxygen consumption rate (OCR) measurement in presence of these compounds. Panc-1 and BxPC-3 showed a sharp decrease of their OCR in presence of Antimycin/Oligomycin, indicating a strong impairment of the respiratory complexes. FCCP alone produced a slow decrease of the OCR, as described by Figarola and coworker with higher concentration of FCCP (25 µM) [2], indicating that the uncoupling activity of the compound is not able anymore to drive mitochondria to maximal OCR. Since this decrease was slower in Panc-1 cells than in BxPC-3, we decide to analyze the mitochondrial potential of Panc-1 after Antimycin + Oligomycin or FCCP treatments. Both conditions reduced the TMRE fluorescence, indicating a loss of the mitochondrial potential and confirming the impairment of the mitochondrial function. These results were added to the manuscript as Fig. 2A.

7. The authors should improve the quality of Figure 2B.

Authors apologize for the poor quality of Figure 2B. The contrast of each image was adjusted and normalized in order to increase the overall quality without tampering the results.

8. In Figure 3 the authors should show that the transfection of myoferlin siRNA is associated with the inhibition of myoferlin synthesis. Therefore, the authors should analyze myoferlin levels by western blotting analysis.

Author thank the reviewer for this suggestion. In Fig 3A, we have added the western-blot showing the siRNA transfection efficiency of the concordant migration assay.

9. In the subsection 2.5, the authors analyzed the gene expression of different metabolism-related proteins (Figure 5B). It could be interesting evaluate also PGC1-alpha expression levels, because it represents one of the main positive regulators of OXPHOS (PMID: 12588810).

Authors thank the reviewer for his/her sounding suggestion improving strongly our manuscript. PGC1-a is broadly described as a positive regulator of mitochondrial function and its expression level was checked by western blot in LM and HM clones. We observed a slight decrease of PGC1a in LM clones according to the injection round, and an increase of its abundance in HM clones following their migratory potential. This point confirms the metabolic switch occurring in migratory PDAC cells.

References