**Answers to the reviewers’ specific comments**

**Reviewer: 2**

**General comments:** The manuscript “Myoferlin Contributes to the Metastatic Phenotype of Pancreatic Cancer Cells by Enhancing Their Migratory Capacity Through the Control of Oxidative Phosphorylation” by Rademaker et al. The authors found that myoferlin expression is positively associated with the cell migration in pancreatic cancer cells, and that knock-down of myoferlin or treatment with mitochondrial respiratory chain uncoupler FCCP or mitochondrial respiratory chain complex 3 inhibitor antimycin A could suppress the cell migration. The authors collected tissues from primary tumor sites and metastatic sites in mice bearing injected tumor cells and compared the expression levels of Myoferlin, E-Cadherin and Vimentin, and found increased expression of Myoferlin, Vimentin, Snail, and decreased expression of E-Cadherin in selected metastatic cells. The authors claimed that myoferlin increases OXPHOS activity in metastatic tissues, and that knock-down of myoferlin in both primary and metastatic tissue clones would suppress the cells’ response to oligomycin, FCCP or antimycin A. The authors conclude that Myoferlin would promote oxidative phosphorylation to facilitate cell migration.

**Specific comments**
1. It is unclear to me that for the 2nd (and 3rd round) of injections, are the LM#1 and HM#1 cells mixed and injected together?

   We apologize for the lack of details in the Materials & Methods section. LM and HM cells were injected separately. The Materials & Methods section was amended to explain clearly this point.

2. Based on the fact that the cells are from the same original cell line, and there is no mutagenesis performed, it would be unlikely that the cells would acquire a lot of mutations during the limited cell cycles. It would be helpful for the authors to provide molecular mechanisms associated with those clones, and check what is contributing to the increased expression of myoferlin and OXPHOS activity.

   The reviewer raises a pertinent point regarding the establishment of the model used in our study. The in vivo selection of liver-tropic cells by successive rounds of injection in immunocompromised mice is a broadly accepted metastasis model [1-4]. This model relies on the observation of tumor or cell line heterogeneity, where highly metastatic cells are present as a subpopulation in primary tumor [5] or cell line, including Panc-1 [6]. The progressive selection of these highly metastatic cells is probably the main driver for the establishment of the described model. This explanation was added to the Discussion section.

3. Also, if Myoferlin is knocked down in HM3 clone, is the cell migration decreased correspondingly? That could serve as a direct evidence of whether Myoferlin and OXPHOS are required for metastasis, as in LM3 the Myoferlin level is relatively high too (almost equivalent to HM3, Fig5C), while their metastatic potential are expected to be quite different.
Authors thank the reviewer for his/her relevant suggestion. The silencing of myoferlin in HM3 clone was performed and 2D migration was assayed. Myoferlin silencing reduced significantly (p=0.002) the migration ability of HM3 clone by >30%. The results were included in the result section and as Fig. 5D. The initial Fig. 5C concerning LM3 was moved to supplemental figure 3B.

We apologize for the quality of our initial Fig. 5C where myoferlin abundance in LM3 clone appeared as high as in HM3 clone. However, western-blots were performed neither on the same membrane nor with the same ECL exposure. As consequence, it is very tricky to compare them. In order to make the myoferlin difference between LM and HM clones more convincing, we add, in Fig. 4C, a low exposure western blot and performed quantifications.

4. Also, the Western blotting data and mRNA expression data of Myoferlin are discordant in low and high metastatic clones (Fig.4C&D).

Authors agree that myoferlin mRNA expression data were apparently discordant with the western blot data. This was due to a voluntary ECL over-exposition in order to show the appearance of the 180 kDa myoferlin isoform. To solve this issue, we have added a lower exposition of the same western blot. The quantification of this western blot is in accordance with the mRNA expression data.

Minor revision
1. Move the full name of FCCP to the first place it appears

The full name of FCCP was moved to the first place it appears.

2. In Fig3D, the color indication of each line is missing. Is the color labeling same as that in 3A?

Reviewer is right, the color labeling of Fig. 3D was the same than in Fig. 3A. A color indication was added in Fig. 3D.

3. Please add Western blotting data of Scale to Fig4C and mRNA data of E-cad to Fig.4D.

Authors are grateful to the reviewer for his/her suggestion. Western blots in Fig. 4C were quantified. We performed RT-qPCR for E-cadherin in LM and HM Panc-1 clones. Gene expression data are concordant with the western blot data and correlate negatively with the myoferlin gene expression.

References

