POINT-BY-POINT RESPONSE TO REVIEWERS

We would like to thank the reviewer for the points raised, which we hope we have solved in the revised version of the manuscript.

1) Many comparisons are made between attached culture and spheres, however it is not appropriate to compare spheres to adherent cultures and conclude on CSC functions, gene expression and treatment results. 2D and 3D cultures are different by nature in many aspects, not only about CSCs, and therefore differences detected are not necessarily attribute to or even related to CSC. A better comparison would be sorted CSC population versus non-CSC population from the same culture by flow cytometry.
Thank you for this fair comment. In fact, although the ideal methodology to compare CSC vs non-CSCs frequently involves the isolation of such populations from the same cell culture, FACS sorting can also affect redox metabolism during the inherent manipulation and cellular stress induced by the process. In order to minimize unspecific background introduced by comparing two culture types, we: 1) always incubate cells cultured in either adherent or sphere conditions in the same media; 2) confirm the most relevant results using CD133 staining by flow cytometry when possible. In the present manuscript, our main conclusions involving GSH content (Figure 3) and differential sensitivity to BSO / BSO+ Gem treatments (Figure 4C and 6B-C) in CSC *vs* non-CSC were confirmed in both settings (adherent *vs* sphere cultures and $CD133^-$ *vs* $CD133^+$ cells).

2) The "CSC-enriched condition" is not confirmed, only assumed by suspension culturing. Surface markers such as CD133 used in this manuscript (or other means) could be used to confirm that the suspension culture is actually CSC-enriched. This is critical because many conclusions in the manuscript is based on the comparison between "CSC-enriched" culture versus attached culture.
The enrichment in CSCs by culture in low attachment conditions for the PDX models included here has been previously validated and published by the group (see for example: Sancho et al, Cell Metabolism 2015, 22(4):590-605). For clarification, we have now included in Figure1A a representative measurement of the enrichment of $CD133^+$ cells in sphere vs adherent cultures.

3) The "Results" subsection 1 paragraph 2 is confusing about how the analysis was done. Did the authors use the 5 PDX samples for analysis of correlation between the up-regulated genes and "stemness"? Or did they use TCGA and GTEx data on normal vs pancreatic cancer tissues?
The correlation studies were performed in TCGA and GTEx data on normal vs PDAC samples. We have now slightly rephrased the paragraph to facilitate comprehension.

4) Materials and Methods: Please make it clear whether "the PDXs-derived tumor tissue fragments" were primary tumor tissues from patients, or PDX tumors passaged in mice/rodents? And what's the number of passages if passaged? PDX stands for "patient derived xenograft".
The PDX tumors were previously passaged in mice (passages 1 to 13). We have now slightly modified that section including this information.

5) Fig1B, what are the dotted lines?

As indicated in the figure legend, the dotted lines denote the 95% confidence intervals for the Mantel-Cox test.

6) Figure 2 has no label on y-axis, I assume its fold changes vs attached culture?
We apologize for this omission. Y-axis labels have been now included in the figure.