Dear Editor,

We thank for your and the reviewers’ valuable time and generous comments on our manuscript: Isolation and characterization of tumor-initiating cells from sarcoma patient derived xenografts (57011\_R0\_072117).

For the second time, we have revised and modified the text and figure according to the reviewers’ comments. These changes have improved the manuscript considerably. And we believe that the manuscript is now suitable for publication in JoVE.

Sincerely,

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Editorial comments:

Note that there have been some formatting changes, and some areas of concern have been highlighted in green (feel free to remove the latter after editing).

General:

1. Please proofread; there are still a few grammar and usage mistakes.

We proofread to correct the grammar mistakes.

Protocol:

1. “Matrigel” is commercial; please use a generic term that is subsequently defined in the Table of Materials.

We replaced it with basement membrane matrix and added the produce in the Table of Materials.

2. 1.1/1.2: Why are these in the third person? Will you be doing them yourselves? If not, they shouldn’t be marked for filming.

We obtained tumor samples from the clinical lab. Thus, we have removed this part from filming.

3. 1.1.1/3.2.4: What gauge needle for these injections? Please also include in the Table of Materials.

We used 25G needle and added the product into the Table of Materials.

4. 2.5: Please provide the isotope control antibodies in the Table of Materials.

We added the product into Table of Materials.

5. 3.1.1/3.3.6.2.3: How fine a filter, and what type? Please provide in the Table of Materials.

We used standard 0.2 μm cell culture filter. We added it into the Table of Materials.

6. 3.3.6.2.6: What sort of microscope is used for this?

We used standard light microscope. We clarified this in the text.

Results:

1. Can you explain somewhere here that “that HLA positive cells are fast dividing cells compared to HLA negative cells”, as you indicated in your response to reviewer 2?

This is a very important question that we should make it clear. Based on our results in cell population growth rate, cell cycle analysis, gene expression profiling. HLA-I(+) cells proliferate faster than their HLA-I(-) counterparts. Therefore, we argued that the difference in tumor formation assays truly showed the difference in tumorigenicity but not cell growth between HLA-I(-) and HLA-I(+) cells.

Figures:

1. Is it possible to split the current figure up into 2 or more figures (e.g., Isolation and Characterization figures)? There is no limit on the amount of figures, and this may make some things clearer.

This is a very good suggestion. We made the changes to split the results in two figures.

2. Please obtain explicit copyright permission to reuse Figure 1 (and any other figures you may make). Explicit permission can be expressed in the form of a letter from the editor or a link to the editorial policy that allows re-prints.

We obtained the permission for reuse in our figures.

3. 1D/G/H: Please explain the scale bars in the legend. Alternatively, if they all are the same length; please explicitly say so. Also, the white scale bar in H is hard to see.

We changed the color into black in that picture to make it easier to be seen (It is now in Figure 2 as we split the figure into two as suggested by the reviewer). We also added the scale bars in the legend as you suggested.

4. 1A: Per reviewer 3, can you also provide images from the HLA-1 (-) tumors created in step 3? This isn’t critical, though. Also (per reviewer 1), can you indicate the sarcoma subtype?

The tumor we presented in Figure 2B is from step 3. We clarified that in addition to the subtypes in the legend as suggested.

5. 1B: Can this be higher resolution?

We replaced it with a better picture as you suggested.

6. 1B: Are these just 3 different samples? Also, is this also showing isolation for HLA-I (+) cells? Is that the yellow box? Please explain this more in general in the legend.

This is three steps in flow cytometry to isolated purified HLA-I(-) cells from one sample. We made it clear in the legend.

7. 1C,E: Please explain the error bars (what they represent, what is n).

As you suggested, we added the information in the legend of Figure 2C.

8. 1C: Please explain this more in the legend .

We clarified this in the legend.

9. 1D: What are the insets showing? What is the black scale bar on the side for? What is the CD44 for?

To make our point clear without confusion, we remove this picture from the figure.