**Reviewers' comments:**

**Reviewer #1: Manuscript Summary:**

*The manuscript by Jourdain and coworkers describe two mouse models of cancer cachexia, the first already well established and characterized by several laboratories (including PMID: 27929469 in this same journal) and the second reported in a single paper (PMC5435723). The manuscript highlights methodological aspects relevant for providing reliable investigational tools. The subject is relevant and deserves attention, however some points should be clarified and/or improved.*

We would like to thank the reviewer for the constructive comments, which are highly appreciated. We have now modified the manuscript according to the reviewer’s suggestions.

*Major Concerns:*

*- Cancer cell lines: please provide a reference for the source (Cell bank, original paper where the line was established, commercial provider, etc.). Moreover, clarify whether CT26 cells are similar or the same cells that elsewhere are called C26.*

The mouse colon cancer cell line CT-26 was obtained from Dr. Chatenay-Rivauday at Novartis Pharma AG (Basel, Switzerland) and originally provided by Dr. Isaiah J Fidler’s lab at the MD Anderson Cancer Center in Houston TX. We now indicated the source of the cells and have also added a corresponding reference (Int J Cancer. 1992 Aug 19;52(1):98-104).

*- From the text it is unclear whether final body weight change in comparison to the initial body weight is takes in consideration the tumor mass. Please provide both data (with and without tumor mass).*

Tumor weight was not subtracted from final body weight when % of change from cell inoculation was calculated. This is now specified in the manuscript (legend Figure 1). In the case of CT-26, it was -8.4% with tumor weight and -11% without tumor (i.e. after subtracting tumor weight).

*- Please explain the reason for choosing the adopted temporal endpoint. One might argue that also A2058 cell induce severe cachexia if allowed to growth for longer.*

We fully agree with the reviewer and would like to thank for the comment. The endpoints have been selected based on cantonal regulations for animal experimentation. The experimental license BS2186 granted by the cantonal authorities of Basel requires animals to be euthanized as soon as certain predefined cut-off criteria are reached. In case of experiments involving CT-26 cells, mice reached the bodyweight loss cut-off (-20%) faster than the tumor volume cut-off (1500 mm3). In case of experiments involving A2058 cells, mice reached the tumor volume cut-off (1500 mm3) earlier and had to be sacrificed despite moderate bodyweight loss. This is now specified in the manuscript.

*- As for the food intake, few points are present in the curves in comparison to the body weight data. I suppose that each point represent the average amount of food intake consumed in the period starting from the previous measurement. Such approx. impedes to highlight a potential severe anorexia present in the last days before sacrifice.*

Food intake is shown as the average food consumption during the indicated days. We now more clearly state this in the manuscript.

*- Please comment on the value and feasibility of measuring evoked force as compared to voluntary strength (e.g. grasping test or others). Moreover, the force in the graph is expressed in grams, not the right measure unit (Newton), please convert.*

The disadvantage of other tests (e.g., grasping test) is that they depend on the motivation of the animals, which is an enormous confounding factor. The evoked force occurs under anesthesia and is triggered by increasing electrical stimulation, which allows assessments over the entire range of muscle contractions from the minimal to the maximal force. We have now added a corresponding explanation. The unit has been converted to Newton.

*- MRI for detecting calf volume reduction was performed only at 7 days of tumor growth. Such result is intriguing since highlights the precocious loss of muscle mass, however it would be useful to provide a time course or at least the final point before sacrifice in order to validate the data as compared to muscle mass measured at necropsy.*

We fully agree with the reviewer. We wanted to assess, whether at the 7, which is prior to body weight loss, there is already a muscle weight loss which could be used as an early, non-invasive marker for muscle wasting. We now highlight our rationale in the manuscript.

*Minor Concerns:*

*- Please clarify better the area where cell injection is performed.*

Cells are injected subcutaneous on the left or right upper flank side, in order to allow proper grabbing for tumor measurements or other manipulation without touching the tumors. This is now described in the manuscript.

**Reviewer #2: Manuscript Summary:**

*The authours address an important question in muscle wasting field: can skeletal muscle wasting precede body weight loss in cancer bearing-mice? Two unrelated cancer cachexia models are used the C26-bearing mouse and the human melanoma bearing-ones. The first is in an immunocopetent backgroud and the second in immunodeficient mice.*

*The comparison between the two models helped to answer the main question of the paper*

*Major Concerns:*

*Some important dataset are missing:*

*In fig 1 survival plot shall be added*

We are not allowed to run survival experiments under our experimental licenses. However, we have recently published surrogate survival plots based on our predefined cut-off criteria (body weight loss or tumor size), which are now specified in the method and have now added the corresponding reference.

*In Fig 2 is food intake measured for each animal that has been kept alone in each cage? Or is the food intake the mean of the food eaten from multiple animals for the same cage and divided for the n? That should be clarified*

The experimental license BS2186 granted by Basel-City cantonal authorities does not allow us to keep animals alone unless there is a major incompatibility. Therefore the food intake data is calculated by dividing the total amount of food eaten per cage by the number of mice present in this cage. This is now specified in the manuscript.

*In fig 3 soleus decrease in size shall be shown alone as example of oxidative muscle, please show that out. At which day from tumor injection have these muscles been weighed? If different for each mouse (data come from a survival experiment), they cannot plot altogether and the experiment has to be repeated choosing a predefined timepoint. The same holds for the fig 3B*

In these experiments, soleus muscle weight is not available because we measured the weight of gastrocnemius-plantaris-soleus muscle complex and did not separate each muscle. With regard to the timing to weigh the muscles, it was performed one to two days after the last measurements of body weight and tumor volume, not different for each mouse.

*in fig 4 what are the calf measurements by MRI in A2058-carrying mice? Please add them, if they do not show muscle wasting ,discuss why that in the appropriate section*

MRI measurement was not performed with A2058.

*Minor Concerns:*

*CT-26 shall be replaced with C26*

*why just 3X105 cells of C26 are injected if most researchers do 1 million or half millions of cells?*

The starting cell number has been determined in pilot experiments. The amount indicated was appropriate to induce substantial tumor growth with sufficient window for later treatment in the model. This is now more clearly stated in the manuscript.

*cubic mm is unappropriate, please correct it as well as many mispellings and gramatcal mistakes throughout all the text*

Cubic mm has been replaced with mm3, and typos have been corrected.

*Discussion shall be enriched with references and points coming out from the data provided*

We thank for the suggestion. Discussion is enriched with references and key points from the data.

**Editorial comments:**

*Changes to be made by the Author(s):*

The changes requested by the editor have been applied and the essential steps of the protocol are highlighted in yellow.

*1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. The JoVE editor will not copy-edit your manuscript and any errors in the submitted revision may be present in the published version.*

*2. For in-text formatting, corresponding reference numbers should appear as numbered superscripts after the appropriate statement(s).*

*3. Please upload each Figure individually to your Editorial Manager account as a .png or a .tiff file. Please combine all panels of one figure into a single image file. Please also provide a one liner title for the figures in the legend.*

*4. Please remove the titles and Figure Legends from the uploaded figures. Please provide the Figure and Table Legends after the Representative Results.*

*5. Please upload each table as .xls/.xlsx file separately into your editorial manager account.*

*6. Please revise the table of the essential supplies, reagents, and equipment. The table should include the name, company, and catalog number of all relevant materials in separate columns in an xls/xlsx file.*

*7. Unfortunately, there are a few sections of the manuscript that show significant overlap with previously published work. Though there may be a limited number of ways to describe a technique, please use original language throughout the manuscript. Please see lines: 47-50, 166-167, 209-211, etc.*

*8. JoVE cannot publish manuscripts containing commercial language. This includes trademark symbols (™), registered symbols (®), and company names before an instrument or reagent. Please remove all commercial language from your manuscript and use generic terms instead. All commercial products should be sufficiently referenced in the Table of Materials and Reagents.*

*For example: Betadiene, Bruker Avance 7 T / 16 cm, Bruker BioSpin Inc., Billerica, MA, USA, SA Instruments, Inc, Stony Brook, NY, USA, Matlab (Mathworks, Natick), MA, USA, PowerLab, etc.*

*9. Please revise the Introduction to include all of the following:*

*a) A clear statement of the overall goal of this method*

*b) The rationale behind the development and/or use of this technique*

*c) The advantages over alternative techniques with applicable references to previous studies*

*d) A description of the context of the technique in the wider body of literature*

*e) Information to help readers to determine whether the method is appropriate for their application*

*10. Please use S.I. abbreviation throughout the protocol.*

*11. Please add more details to your protocol steps. Please ensure you answer the “how” question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action.*

*12. 1.1.1-1.2.1: Please explain where is the cell line from? How do you put the frozen cell in culture? Do you thaw the cells or just transfer the cells from the cryovial to the tube and then add medium to it? How do you ensure the cell number at this stage?*

*13. 1.1.2.- 1.2.2: How do you passage the cells? Please add a note stating why 2-3 or 3-4 passages of the cells. What is the cell confluency while trypsinisation? What other criteria do you look for before ensuring cells are ready for further processing- cell count, etc?*

*14. 1.3.1 – How much cells are injected? Cell number/kg body weight? Do you check anything before and after cell injection? What is the age and gender of the mice being inoculated? Anything specific to keep in mind.*

*15. 2.1- This should come before the animal injection occurs. This will be the order of filming.*

*16. 3.2: Please provide GUI if this step needs to be filmed. The video will show how to do the things.*

*17. 4.2: Please explain do you use sterile instrument? How do you identify the Achilles tendon?*

*18. 4.4: Please explain when was the sciatic nerve exposed?*

*19. Line 178-181: Please mark as a note.*

*20. Please ensure that a single line space is left between each step and sub-step of the protocol. The font used us Calibri and font size is 12 with one inch margin on all sides.*

*21. Please highlight 2.75 pages or less (hard cut limit) of the Protocol (including headings and spacing) that identifies the essential steps of the protocol for the video, i.e., the steps that should be visualized to tell the most cohesive story of the Protocol. The highlighted steps should form a cohesive narrative with a logical flow from one highlighted step to the next. Remember that non-highlighted Protocol steps will remain in the manuscript, and therefore will still be available to the reader.*

*22. Please also ensure that the steps involving anesthesia, steps involving calculations and computational steps without GUI cannot be filmed.*

*23. Line 190: Please explain what is non- tumor group. What was injected in the non-tumor group? What is being measured here?*

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*25. As we are a methods journal, please revise the Discussion to explicitly cover the following in detail in 3-6 paragraphs with citations:*

*a) Critical steps within the protocol*

*b) Any modifications and troubleshooting of the technique*

*c) Any limitations of the technique*

*d) The significance with respect to existing methods*

*e) Any future applications of the technique*