Dear Editor Nguyen,

Thank you very much for helping with this manuscript. We also thank the reviewers for their precious input about improving the paper. Below is a detailed changes we have made to address the critiques.

From Editor:

**Critique 1.** Formatting: Acknowledgements – Who is Dr. XXX? We assume this is a placeholder.

**Reply:** Names of two colleagues helping with the manuscript were added.

**Critique 2.**Grammar:

-Line 44 – “Tubes will be regularly” – wrong verb tense

**Reply:** Changed to “were tapped regularly”

1.3 – “several pair” -

**Reply: Changed to “several pairs”.**

1.4 – “Dissolved in”

**Reply:** The sentence is changed to “add 50 ml RPMI 1640 media to dissolve the glucose.”

**Critique 3.**Additional detail is required: 6.1 note – Please describe the kind of methods used, at minimum.

**Reply:** A brief description of the method is added (6.2-6.10).

**Critique 4.**Branding: Charles River should be removed from the ethics statement and used in the materials table instead.

**Reply:** The information was moved to materials as suggested.

**Reviewer #1:** *Manuscript Summary:* Accept.

**Reply:** Thanks for the positive opinion.

**Reviewer #2:** *Manuscript Summary:* The authors describe an optimized protocol for islet isolation from neonatal mouse pancreata. This method should be useful for the developmental biology of pancreatic islets in mice.

**Reply:** Thanks for the positive opinion.

***Major Concerns****:* Possible effects of the genetic background should be clarified for the application of this method. As the authors describe that "In general, ICR and CBA/Bl6 pure-bred mice produce less islets than hybrids between ICR and CBA/Bl6 mice do", is it the size of their pancreata that results in different yields? Were these the only two strains of mice tested? What about C57BL/6 mice that are most commonly used?

**Reply:** Thanks for reading into these details. Now we have included more details to describe results of the regularly used CD1 and C57BL/6 lines and the pronuclear injection recipient line (B6CBAF1/J) of our studies.

To directly answer the reviewer’s question: the size of the pancreas is not directly related with islet number. We have tested more than three strains with different intercrosses. Unfortunately, due to the method-nature of the journal, providing all the details in islet size, number, and function in relation with genetic background, age, and pancreatic size is out of the scope.

***Minor Concerns:***

1. p.1. line 44: Change "will be" to "were".

**Changed as suggested.**

2. p.2. line 81: Change "laymen" to "researchers". Done

**Changed as suggested.**

3. p.4. line 139: Change "into" to "to". Done

**Changed as suggested.**

4. p.6. line 237 and 256: Change "jagged" to "rough". Done

**Changed as suggested.**

5. p.6. line 259: Change "+" to "±". Done

**Changed as suggested.**

6. p.7. line 314: Fill "Dr. XXX".  The names were now added.

**Changed as suggested.**