

## ROUND 1

Reviewer #1:

**Comment 1.** The study by Jaing is to delineate the role of TRPC6 in the Diabetic cardiomyopathy. In addition, to study the involvement of TRC6 and P- CaMKII in cardiomyopathy. The study is interesting; however, the mechanism of TRC6 and CaMKII is not studied in-depth. The study by Xie et al. 2012 have shown that the TRC6 downregulation showed prevention of stress induced cardiac modelling. Further Anderson et al. 2011 also showed that increased expression of CaMKII showed cardiac hypertrophy.

**Reply:** Thank you for your insightful and objective comments. In the future study, we will made much deeper study on this topic. If you still have any problem, please do not hesitate to contact us. We will try our best to solve them. Have a nice day. Best regards.

**Comment 2.** Figure 3A, the author showed a significant difference in the G1 cells; however, it is very clear on the increased G1 in DCM model.

**Reply:** Thank you for your insightful comment. The Figure 3A were mainly used to confirm the successful establishment of DCM model. In the future study, we will made much deeper study on this topic. If you still have any problem, please do not hesitate to contact us. We will try our best to solve them. Have a nice day. Best regards.

**Comment 3.** Figure 3B, whether the level of PCNA and Cyclin D normalized with GAPDH?

**Reply:** Thank you for your insightful comment. In our present study, the level of PCNA and Cyclin D was normalized with GAPDH. If you still have any problem, please do not hesitate to contact us. We will try our best to solve them. Have a nice day. Best regards.

**Comment 4.** Figure 4B, TRC6 Fluorescence is not clear, although the plot showed significant difference in DCM.

**Reply:** Thank you for your insightful comment. Based on your comment, we have revised figure 4B. If you still have any problem, please do not hesitate to contact us. We will try our best to solve them. Have a nice day. Best regards.

**Comment 5.** Figure 8A, The level of p-CaMKII in western of all groups is not clear and acceptable, This should be repeated.

**Reply:** Thank you for your insightful comment. Based on your comment, we have revised figure 8a. If you still have any problem, please do not hesitate to contact us. We will try our best to solve them. Have a nice day. Best regards.

**Comment 6.** Table 1, glucose level in 4 weeks showed a decreased trend in STZ? Not clear. The mechanism of TRC6 and CaMKII is not studied that would have provided more information.

**Reply:** Thank you for your insightful comment. I am sorry for the mistakes I made in the table 4. There was no statistical significance between 1 week and 4 weeks. I have revised the table 1. If you still have any problem, please do not hesitate to contact us. We will try our best to solve them. Have a nice day. Best regards.

**Comment 7.** Moreover, there is TRC6 and PI3K involvement is there.

**Reply:** Thank you for your insightful comment. In our present study, we did not study PI3K. In the future study, we will do further exploration about PI3K. If you still have any problem, please do not hesitate to contact us. We will try our best to solve them. Have a nice day. Best regards.

**Reviewer #2:**

**Comment 1.** This paper does not fit in WJSC. The role of WJSC is to provide research trend various fields of stem cells. However, this paper is not stem cell research. Let your choice of different journal for your great results.

**Reply:** Thank you for your objective comment. Our paper has been transferred to World Journal of Diabetes. If you still have any problem, please do not hesitate to contact us. We will try our best to solve them. Have a nice day. Best regards.

**Reviewer #3:** Comments on the manuscript "The roles of TRPC6 in glucose-induced cardiomyocyte injury" Diabetic cardiomyopathy is a complication of diabetes characterized by heart enlargement and failure. The aim of the work presented here was to explore whether TRPC6 which is a calcium channel linked to various cardiomyopathies affects cardiomyocyte apoptosis and proliferation inhibition in diabetic cardiomyopathy. This work brings interesting and useful results. However, it is necessary that the manuscript be improved before considering its publication. Here are a few remarks.

**Reply:** Thank you for your insightful and objective comments. Our paper has been transferred to World Journal of Diabetes. If you still have any problem, please do not hesitate to contact us. We will try our best to solve them. Have a nice day. Best regards.

**Comment 1.** Experimental animals and protocol: "Modeling method: STZ solution with a concentration of 6.5 mg/ml was prepared with citric acid buffer": specify the pH of the buffer.

**Reply:** Thank you for your insightful comment. the pH of the buffer was 4.5. Based on your comment, I have revised it in the text. If you still have any problem, please do not hesitate to contact us. We will try our best to solve them.

**Comment 2.** cell culture: this paragraph is not clear. "...control group: a certain amount of mannitol was added to 5.5 mmol/L low-glucose medium to make the osmotic pressure same as that of the high-glucose group": it is not clear, specify whether the osmolarity to be obtained is 33 mMol / L and what was the quantity of mannitol used or specify whether mannitol was added until the correct osmolarity was obtained. How was osmolarity measured?

**Reply:** Thank you for your insightful comment. control group: 275ul mannitol solution of 500 mmol/L was added to 5ml low-glucose medium of 5.5 mmol/L to obtain the osmotic pressure of 33 mmol / L measured by OsmoPRO from Advanced Instruments Inc. Based on your comment, I have revised it in the text. If you still have any problem, please do not hesitate to contact us. We will try our best to solve them.

**Comment 3.** "High-glucose group (HG): a certain amount of glucose powder was added to the low-glucose medium to form a high-glucose medium with a concentration of 33 mmol/L;": this part is not clear: what does "a certain amount of glucose" mean? See also the note above.

**Reply:** Thank you for your insightful comment. High-glucose group (HG): 275ul of glucose solution of 500 mmol/L was added to 5ml low-glucose medium of 5.5 mmol/L to obtain the osmotic pressure of 33 mmol / L measured by OsmoPRO from Advanced Instruments Inc. Based on your comment, I have revised it in the text. If you still have any problem, please do not hesitate to contact us. We will try our best to solve them. Based on your comment, I have revised it in the text. If you still have any problem, please do not hesitate to contact us. We will try our best to solve them.

**Comment 4.** immunofluorescence: specify the characteristics of PBS (pH, osmolarity) Write "Then, the samples were washed with PBS solution three times" instead of "Then, the samples were washed with PBS solution three times"

**Reply:** Thank you for your insightful comment. Based on your comment, I have revised it in the text, as following "Then, the samples were washed with phosphate buffered saline (PBS, pH 7.4, 0.1mol/L) solution three times for 8 min each time". If you still have any problem, please do not hesitate to contact us. We will try our best to solve them. If you still have any problem, please do not hesitate to contact us. We will try our best to solve them.

**Comment 5.** "Anti-fluorescence quenching sealing agent was added": what was this agent?

**Reply:** Thank you for your insightful comment. Based on your comment, I have revised it in the text, as following "Anti-fluorescence quenching sealing agent (ProLong® Gold Antifade Reagent, CST) was added to the samples and pictures were took with confocal microscope". If you still have any problem, please do not hesitate to contact us. We will try our best to solve them. If you still have any problem, please do not hesitate to contact us. We will try our best to solve them.

**Comment 6.** CCK-8 test: quickly describe the test.

**Reply:** Thank you for your insightful comment. Based on your comment, I have revised it in the text, as following "Cells were seeded at 1000 cells per well density in 96-well plates. Culture plates were pre-cultured in a 37C, 5% CO2 incubator, 10ml CCK-8 was added to each well. 96-well plates were placed in the incubator for 1-4h, then absorbance was determined at 450nm.". If you still have any problem, please do not hesitate to contact us. We will try our best to solve them. If you still have any problem, please do not hesitate to contact us. We will try our best to solve them.

**Comment 7.** "After the present time, the supernatant was aspirated and put into a centrifuge to centrifuge for about 5minutes": give the centrifugation speed in number of g.

**Reply:** Thank you for your insightful comment. Based on your comment, I have revised it in the text, as following "After the preset time, the supernatant was aspirated and put into a centrifuge to centrifuge for about 5minutes at the speed of 300g". If you still have any problem, please do not hesitate to contact us. We will try our best to solve them. If you still have any problem, please do not hesitate to contact us. We will try our best to solve them.

**Comment 8.** ROS detection: write ", and it can be freely penetrated the cell membrane" or ", and it can freely penetrate the cell membrane" instead of ", and it can be freely penetrate the

cell Membrane”

**Reply:** Thank you for your insightful comment. Based on your comment, I have revised it in the text, as following “, and it can freely penetrate the cell membrane, ”. If you still have any problem, please do not hesitate to contact us. We will try our best to solve them. If you still have any problem, please do not hesitate to contact us. We will try our best to solve them.

**Comment 9.** Detection of apoptosis by flow cytometry: “The cells of each group were collected, resuspended in the culture medium, and centrifuged at 4°C for 5 min”: specify the centrifuge speed.

**Reply:** Thank you for your insightful comment. Based on your comment, I have revised it in the text, as following “The cells of each group were collected, resuspended in the culture medium, and centrifuged at 4°C for 5 min at the speed of 300g”. If you still have any problem, please do not hesitate to contact us. We will try our best to solve them. If you still have any problem, please do not hesitate to contact us. We will try our best to solve them.

**Comment 10.** histological analysis: “Masson dye is one of the main methods to show the dyeing of fibers in tissue. Masson stains muscle fibers in red and collagen fiber in blue, which is mainly used to distinguish collagen fiber from muscle fiber”: in Masson's trichrome (not Masson's dye), fuchsin stains muscle fibers and certain other components red; aniline blue stains collagen blue. The nuclei are stained by hemalum. Specify the staining.

**Reply:** Thank you for your insightful comment. Based on your comment, I have revised it in the text, as following “The cells of each group were collected, resuspended in the culture medium, and centrifuged at 4°C for 5 min at the speed of 300g”. If you still have any problem, please do not hesitate to contact us. We will try our best to solve them. If you still have any problem, please do not hesitate to contact us. We will try our best to solve them.

**Comment 11.** “In histology, periodic acid schiff (PAS) staining is mainly used to detect glycogen or other polysaccharide substances, which makes glycogen and the neutral mucilage material red and the nucleus blue.”: nuclei are blue stained when the dye is hematoxylin. Specify.

**Reply:** Thank you for your insightful comment. Based on your comment, I have revised it in the text, as following “The cells of each group were collected, resuspended in the culture medium, and centrifuged at 4°C for 5 min at the speed of 300g”. If you still have any problem, please do not hesitate to contact us. We will try our best to solve them. If you still have any problem, please do not hesitate to contact us. We will try our best to solve them.

**Comment 12.** Echocardiographic detection: write “Mice” instead of “mice”. “We removed the hair around the heart”: I guess it's in the heart region on the body? Specify.

**Reply:** Thank you for your insightful comment. Based on your comment, I have revised it in the text, as following “Mice were injected intraperitoneally with 3% sodium pentobarbital at 40 Mg/kg. After reaching the state of mild anesthesia, they were fixed on the animal fixation plate in the supine position, and we touched them with fingers for apical pulsation. We removed the hair in the heart region on the body”. If you still have any problem, please do not hesitate to contact us. We will try our best to solve them. If you still have any problem, please do not

hesitate to contact us. We will try our best to solve them.

**Comment 13.** write "western" instead of "watern"

write "Sonnevedl" instead of "Sonnevedl".

**Reply:** Thank you for your insightful comment. Based on your comment, I have revised it in the text. If you still have any problem, please do not hesitate to contact us. We will try our best to solve them.

**Comment 14.** In the text, it is the reference 43, but in the list it is 44 "Gao et al found that AngII..." in the text it is the reference 44, but I didn't find it in the list.

**Reply:** Thank you for your insightful comment. Based on your comment, I have revised it in the text. If you still have any problem, please do not hesitate to contact us. We will try our best to solve them.

**Comment 15.** conclusions: write "suggested" instead of "sugggested".

**Reply:** Thank you for your insightful comment. Based on your comment, I have revised it in the text, as following " In conclusion, the results of flow cytometry, immunofluorescence and western blot experiments suggested that high glucose environment might lead to cardiomyocyte apoptosis and inhibition of cardiomyocyte ". If you still have any problem, please do not hesitate to contact us. We will try our best to solve them.

**Comment 16.** References: references should be carefully checked by checking both the number given in the text and the reference in the list.

**Reply:** Thank you for your insightful comment. Based on your comment, I have carefully checked both the number given in the text and the reference in the list. If you still have any problem, please do not hesitate to contact us. We will try our best to solve them.

**Comment 16.** Figures: in a general manner, I have had some difficulties to understand the figures. The figures need some explanations, the different pictures are presented but not explained and finally the figures are difficult to be understood. Exemple: Figure 2A: what are the differences between the two pictures? What does the graph on the right mean?

**Reply:** Thank you for your insightful comment. Based on your comment, I have carefully revised the figure legends and . If you still have any problem, please do not hesitate to contact us. We will try our best to solve them.

**Comment 17.** The author needs to supplement the flow chart of animals and experiments for readers to better understand.

**Reply:** Thank you for your insightful comment. Based on your comment, I have added the flow chart in the text. If you still have any problem, please do not hesitate to contact us. We will try our best to solve them.



## ROUND 2

Reviewer #1:

**Comment 1.** I went through the rebuttal letter of the revised manuscript by Jiang et al., and it looks OK. Language editing should be done.

**Reply:** Thank you for your positive and insightful comments. Based on your comments, our paper was edited and proofread by the English native speaker again. If you still have any problem, please do not hesitate to contact us. We will try our best to solve them. Have a nice day. Best regards.