**אוניברסיטת בן-גוריון בנגב** **BEN-GURION UNIVERSITY OF THE NEGEV**

**ת.ד. 653 באר-שבע 84105 P.O.B. 653 84105 BEER-SHEVA, ISRAEL**

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| **Regenerative Medicine & Stem Cell Research Center and the Cardiac Research Laboratory** | **המרכז לרפואה מחדשת ומחקר בתאי גזע**  **והמעבדה לחקר הלב** |

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| **Sharon Etzion, PhD**  **Senior Staff Scientist** | Email: [shar@bgu.ac.il](mailto:shar@bgu.ac.il),  [sharetz@gmail.com](mailto:sharetz@gmail.com)  Tel: 0747795225 | **דר' שרון עציון**  **עמית מחקר א'** |

November 2023

Prof. Dr. Manuel J Santos,

Editor-in-Chief, *Biological Research*

Dear Prof. Dr. Manuel J Santos:

Attached please find our manuscript entitled “Plekhm2 acts as an autophagy modulator in murine heart and cardiofibroblasts but is not vital for myocardial function under stress”, which we would like to submit for publication in the *Biological Research* Journal.

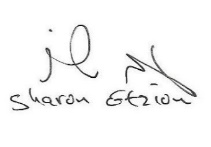
We previously associated a recessive inherited mutation in *PLEKHM2* with a familial form of dilated cardiomyopathy (DCM) and left ventricular non-compaction (1). Plekhm2 interacts with kinesin-1, and the lysosomal GTPase Arl8 affecting endosomal trafficking and lysosome distribution. In line with that, human primary fibroblasts from the inflicted patients exhibited abnormal lysosomes distribution and impairment in autophagy. Recently we also found impaired autophagy, contraction, calcium handling and sarcomeres orientation in human iPSC-derived cardiomyocytes from 2 patients as well as MRI evidence for massive and progressive myocardial tissue loss in one of the patients (2). Since autophagy have been shown to play a critical role in maintaining normal cardiac function limiting damage to the cardiac cells during stress, we hypothesized that dysfunction of Plekhm2 impairs cardiac function and leads to dilated cardiomyopathy mainly by affecting the autophagy process.

In the present study, we investigated the role of Plekhm2 in autophagy and cardiac function in normal conditions and under stress in mice homozygous for global knockout of the *Plekhm2* gene (PLK2-KO), as well as in culture neonatal cardiomyocytes and cardiofibroblasts lacking the *plekhm2* gene. Our findings indicate that Plekhm2 is indeed important for maintaining normal autophagy in the murine heart as well as in the cultured cardiofibroblasts but not in the cardiomyocytes. In addition, we found that the PLK2-KO heart was less sensitive to neurohormonal stress compared to WT. Our findings suggest that in contrast to the human heart, the global absence of Plekhm2 in the murine heart may stimulate compensatory mechanisms that enable the cardiomyocytes to maintain normal autophagy and cope with neurohormonal stress.

This manuscript is original, no part of it has been published before, nor is any part of it under consideration for publication elsewhere. We have no conflict of interest to disclose. All authors approved the written content and figures in the submitted manuscript.

Thank you for your consideration of this manuscript.

Sincerely,

Sharon Etzion

**References**

1. Muhammad E, Levitas A, Singh SR, Braiman A, Ofir R, Etzion S, et al. PLEKHM2 mutation leads to abnormal localization of lysosomes, impaired autophagy flux and associates with recessive dilated cardiomyopathy and left ventricular noncompaction. *Human molecular genetics*. 2015;24(25):7227-40.

2. Korover N, Etzion S, Cherniak A, Rabinski T, Levitas A, Etzion Y, et al. Functional defects in hiPSCs-derived cardiomyocytes from patients with a PLEKHM2-mutation associated with dilated cardiomyopathy and left ventricular non-compaction. *Biological Research*. 2023;56(1):34.