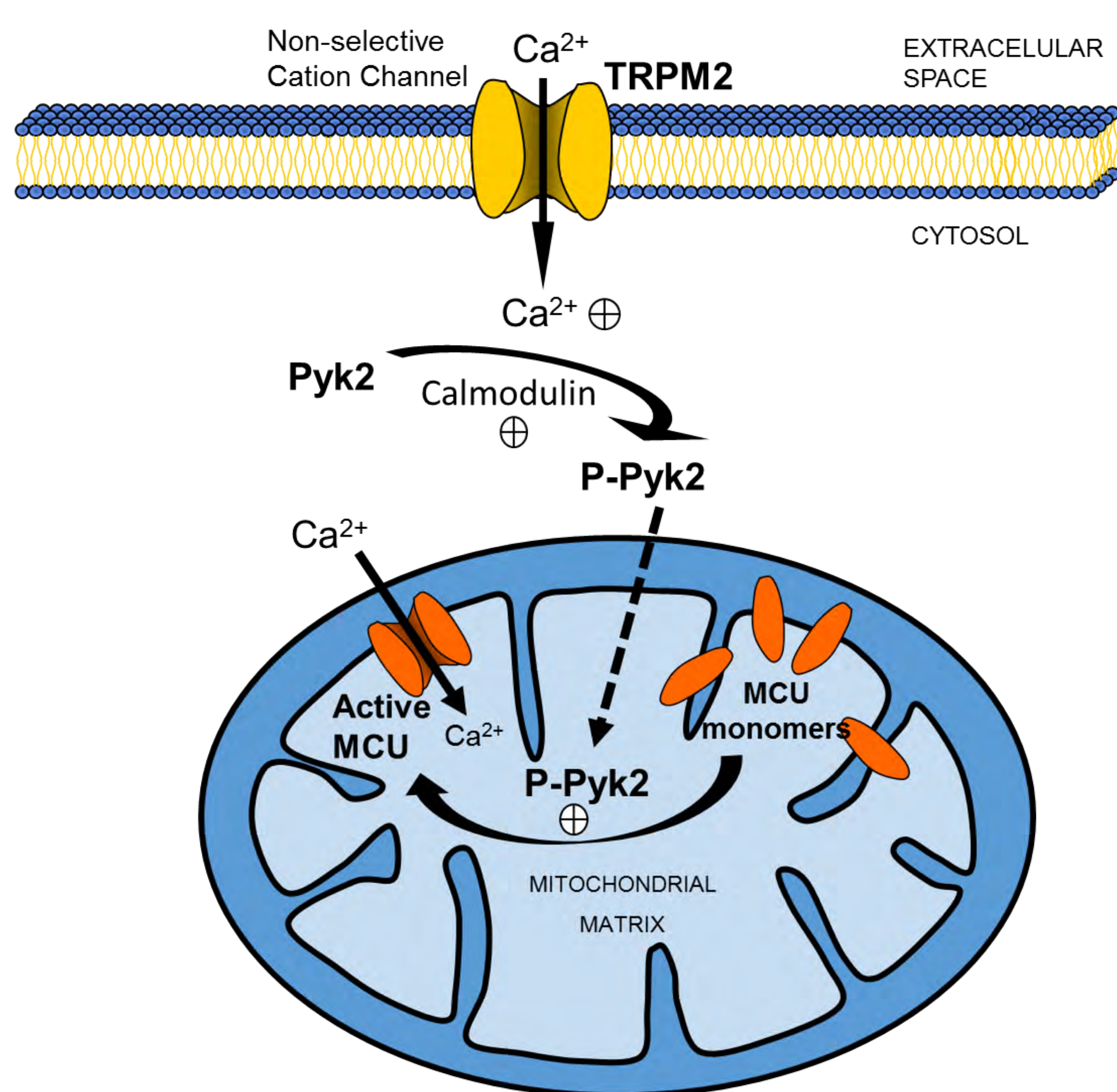


Introduction

TRPM2 is a non-selective cation channel located in the plasma membrane of the cell. Upon activation, the channel opens, allowing calcium to enter into the cytosol of the cell, leading ultimately to the phosphorylation and activation of the enzyme Pyk2 (proline-rich tyrosine kinase 2). Once phosphorylated, Pyk2 translocates from the cytosol to the mitochondria, where it regulates the formation of the pore component of the mitochondrial calcium uniporter (MCU) complex. Consequently, this interaction is a key factor in mitochondrial calcium uptake and therefore mitochondrial bioenergetics.

Hypothesis

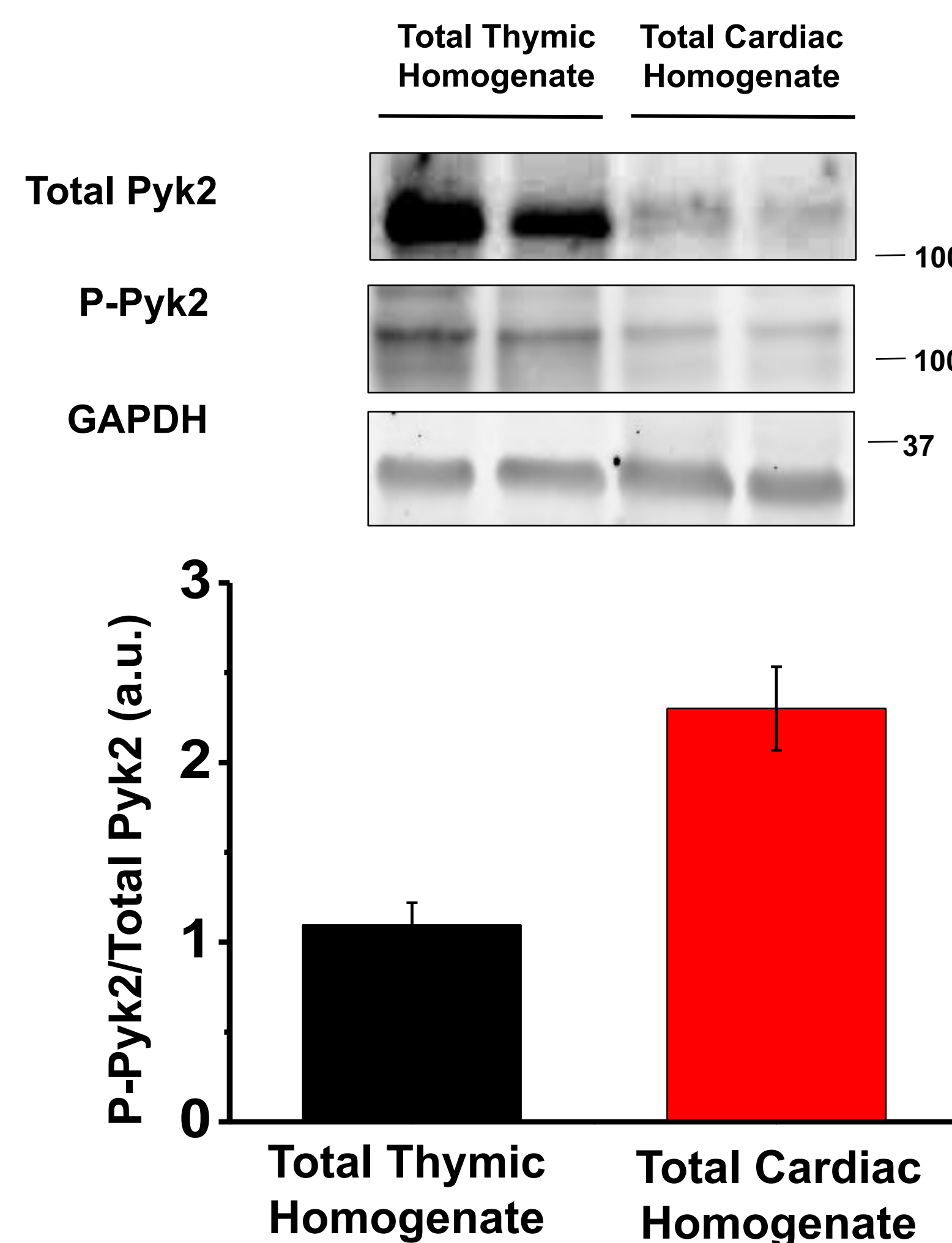
We hypothesize that tonic activation of TRPM2 facilitates mitochondrial Ca^{2+} uptake through the phosphorylation and translocation of Pyk2 and its interaction with the MCU complex. This pathway ensures the proper mitochondrial cardiac bioenergetics. Disturbance of this pathway leads to mitochondrial dysfunction due to the impairment in mitochondrial calcium dynamics.



Aim: To determine the role of Pyk2 in regulation of mitochondrial Ca^{2+} uptake via MCU complex phosphorylation and oligomerization

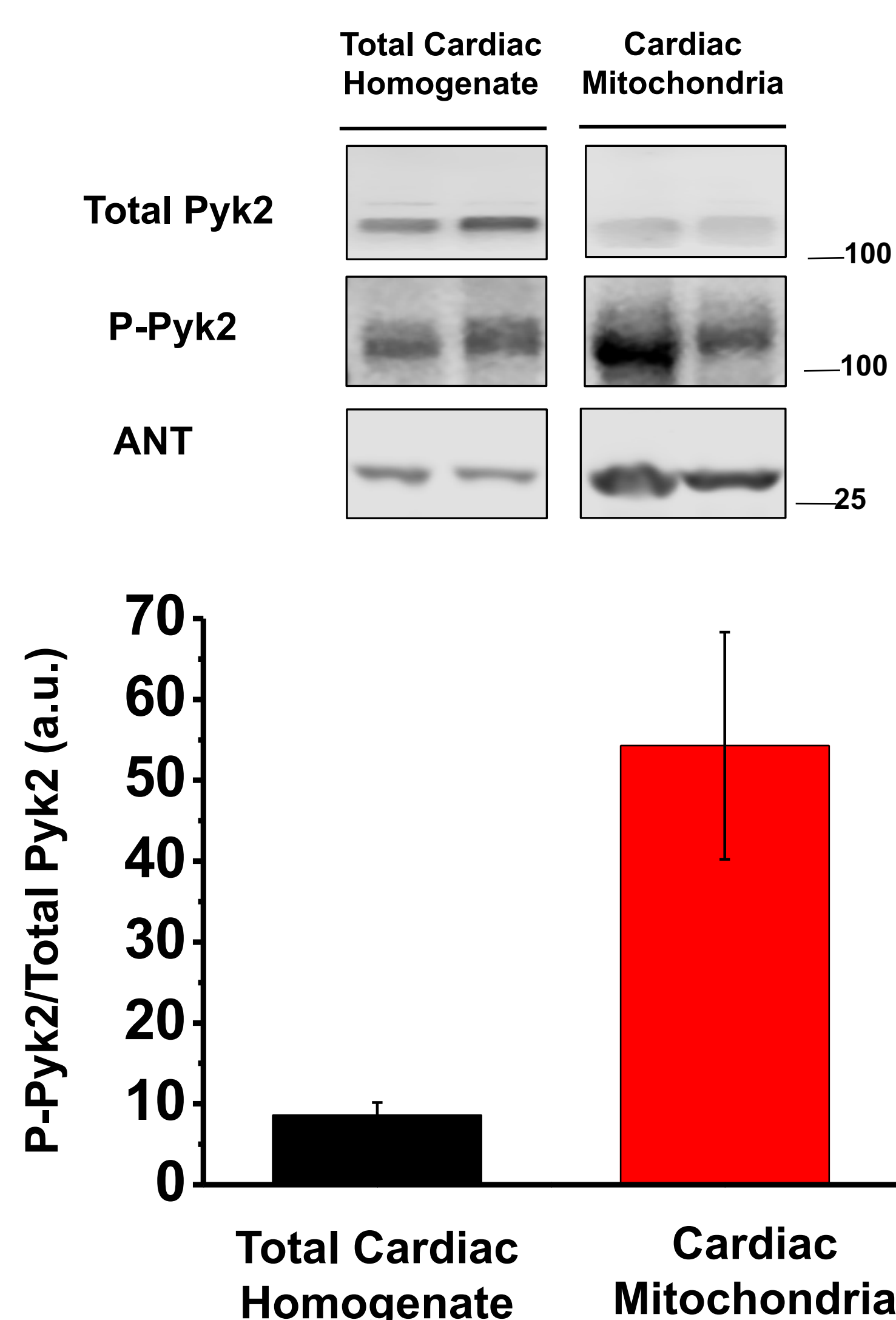
Results

1. High levels of activated Pyk2 are found in the cardiac tissue



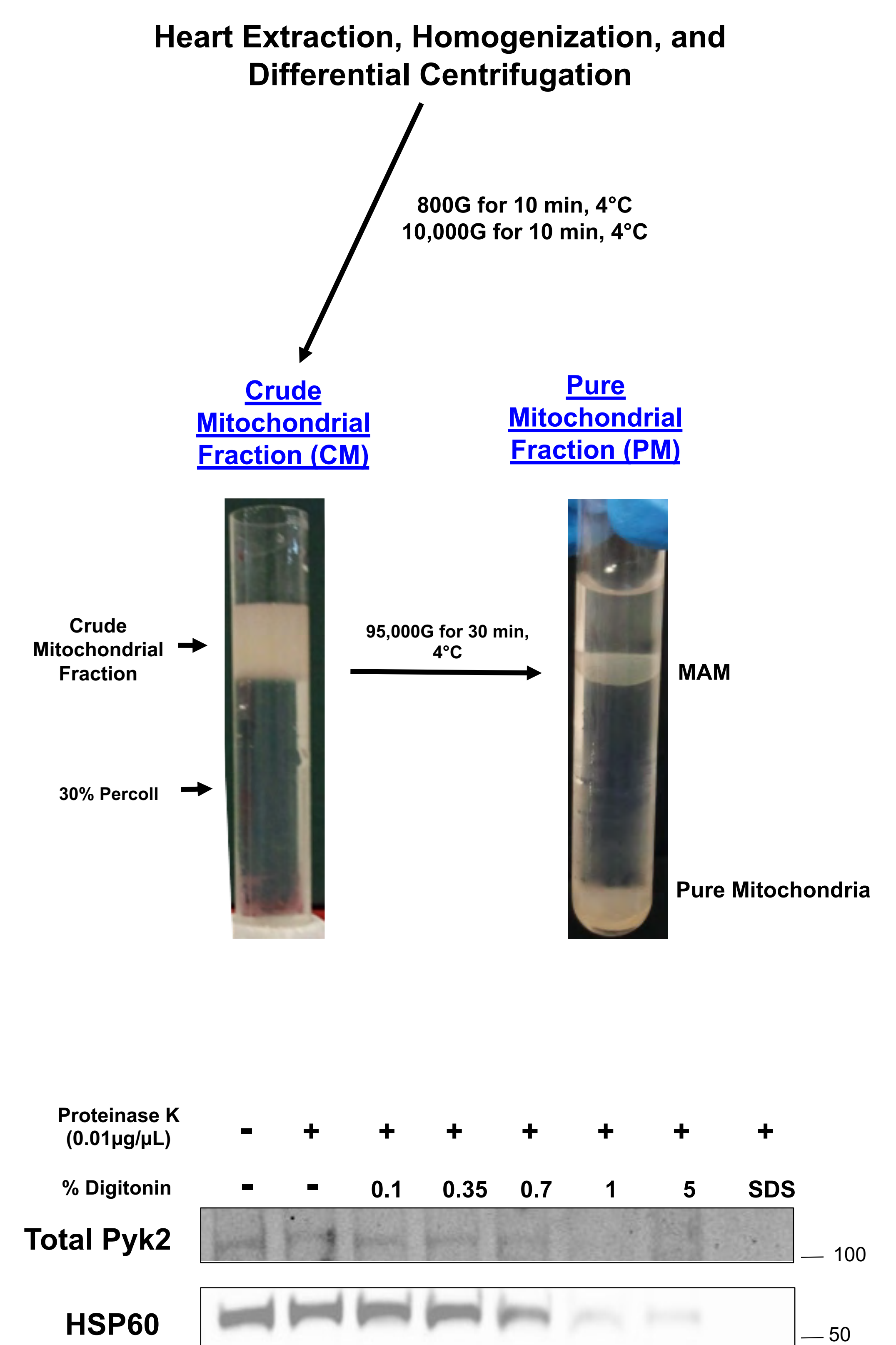
Levels of total Pyk2 and P-Pyk2 were compared in the total homogenate fraction of heart and thymus via Western Blot. Despite the low expression of Pyk2 in the heart, a high proportion of Pyk2 is phosphorylated and therefore activated.

2. In the heart, activated Pyk2 is preferentially located in the mitochondria



Mitochondria were isolated from cardiac tissue via differential centrifugation. Levels of total Pyk2 and phosphorylated (activated) Pyk2 were compared via Western Blot. Data shows a preferential localization of activated Pyk2 in the mitochondria of cardiac tissue.

3. Pyk2 localizes to the matrix of cardiac mitochondria



An accessibility assay was performed to determine the specific localization of Pyk2 within the mitochondria. Increasing amounts of digitonin were added to Percoll purified mitochondria along with a fixed amount of proteinase K.

The results were compared to known matrix proteins. The comparison between HSP60 and Pyk2 demonstrates that Pyk2 is located in the matrix of the isolated cardiac mitochondria.

Conclusion: In the heart, Pyk2 is localized to the mitochondrial matrix, where it can consequently regulate mitochondrial calcium uptake.