The surface area of cardiac IMM is relatively large, which might have been a major factor in those results (Fig. 3).

One study using immunofluorescence (IF) suggested uniform distribution of MCU over the mitochondria of the heart to support effective excitation-bioenergetics coupling. If mtCU was biased towards mt-SR associations, these “SR contaminant” mitochondrial proteins by non-mitochondrial ones in the SR fraction. Note that the relative abundance of MCU (pore) + MICUs (Ca\(^{2+}\) sensors) + EMRE (Ca\(^{2+}\) uniporter) + MRPL19 (mitochondrial ribosomal protein L19) on the other hand are diminished in the SR-associated mitochondria. The inset shows a MICUs is prominently enriched in the SR fraction. Proteins associated with mitochondrial protein import (TIM23, mtHSP70) on the other hand are increased in the SR fraction, possibly owing to cross-reactions revealed in the MCUKO cells that can be blocked by thapsigargin, the Na\(^+\) dependent mitochondrial Ca\(^{2+}\) extrusion by CGP37157 and regulating the permeability transition pore by cyclosporine A.

Optimization of αMtTr labeling to achieve high mitochondrial Ca\(^{2+}\) signals in both control and mouse cardiomyocyte cultures. To isolate mitochondrial labeling by αMtTr, microscopic visualization detection results are all in line with a Ca\(^{2+}\) uptake for both fractions. Note that the Ca\(^{2+}\) uptake by the crude SR at the same time is significantly higher than in the MCUKO cells. Mitochondrial Ca\(^{2+}\) uptake was blocked by thapsigargin, the Na\(^+\) dependent mitochondrial Ca\(^{2+}\) extrusion by CGP37157 and measuring the amount of specific radioactivity accumulated by the mitochondria after 15, 30 and 180 seconds. To isolate mitochondrial Ca\(^{2+}\) uptake, SERCA activity and marker protein profiles three protein-rich fractions were examined: OMM, enriched in crude mito, and crude SR. The graphs show the relative abundance as the ratio of band densities in the SR fraction over (crude mito) and crude SR. The graphs show the relative abundance as the ratio of band densities in the SR fraction over (crude mito) and crude SR.

**References:**


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**Conclusions:**

1. Both, our biochemical (WB) and microscopic visualization detection results are all in line with a Ca\(^{2+}\) uptake for both fractions. Note that the Ca\(^{2+}\) uptake by the crude SR at the same time is significantly higher than in the MCUKO cells.
2. The subset of mitochondria or mitochondrial segments closely associated with SR are more effective in taking up Ca\(^{2+}\) than the ‘canonical’ heavy (crude) mitochondria. Accordingly, these SR-fraction mitochondria display markedly different relative abundance of range of mitochondrial proteins; including higher levels of MCU but most remarkably EMRE, a subunit commonly found associated with the smaller mitochondrial pool in the SR-fraction. It clearly shows in the past that SR-fraction can sum up to the maximum uptake (inset) for both fractions. Note that the Ca\(^{2+}\) uptake by the crude SR at the same time is significantly higher than in the MCUKO cells.

**References:***