MCU Up-regulation contributes to myocardial ischemia- reperfusion Injury through calpain/OPA-1–mediated mitochondrial fusion/mitophagy Inhibition

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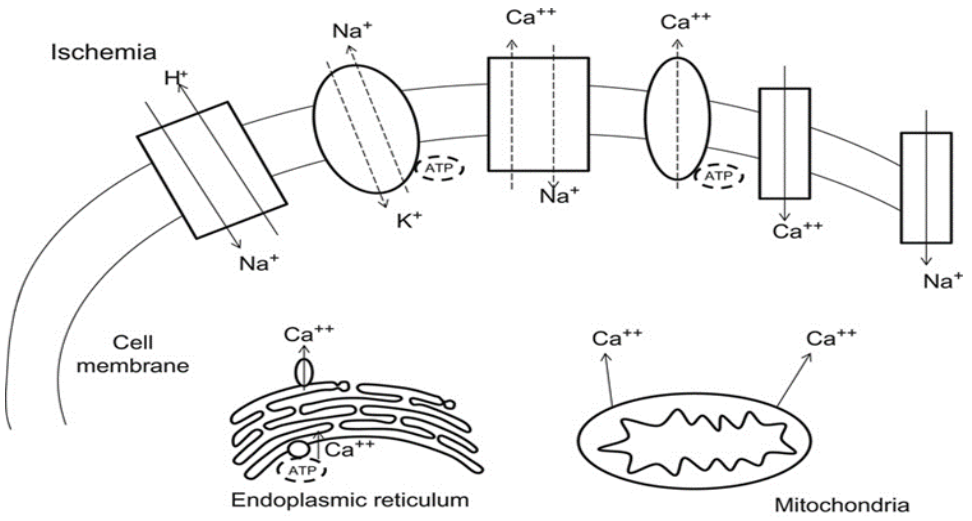
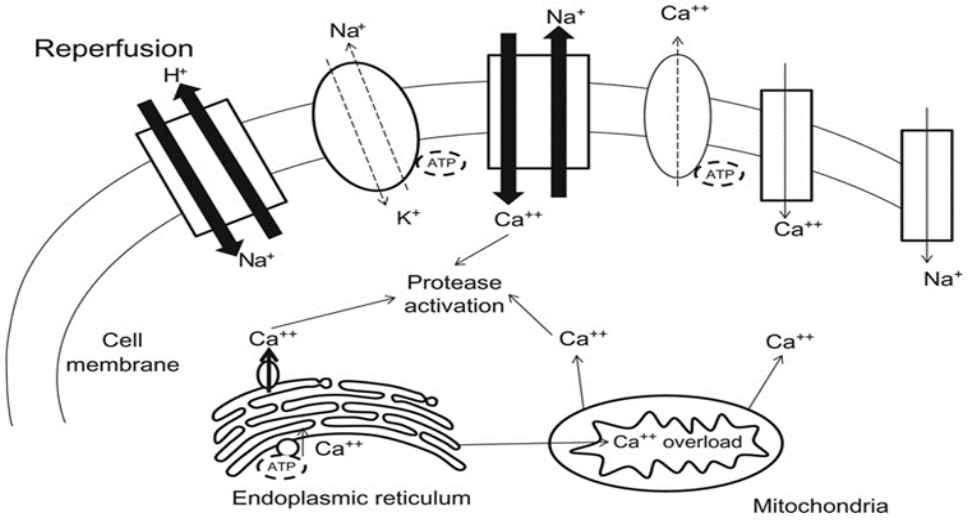
Ischemia/reperfusion (IR) injury in the cell

- During ischemia:
  - Ion imbalance (↑ $Ca^{2+}_{Cytosolic}$)
  - pH decreases
  - ATP production ceases



- Reperfusion:
  - pH is reestablished
  - Ca^{2+} overload
  - Oxidative damage



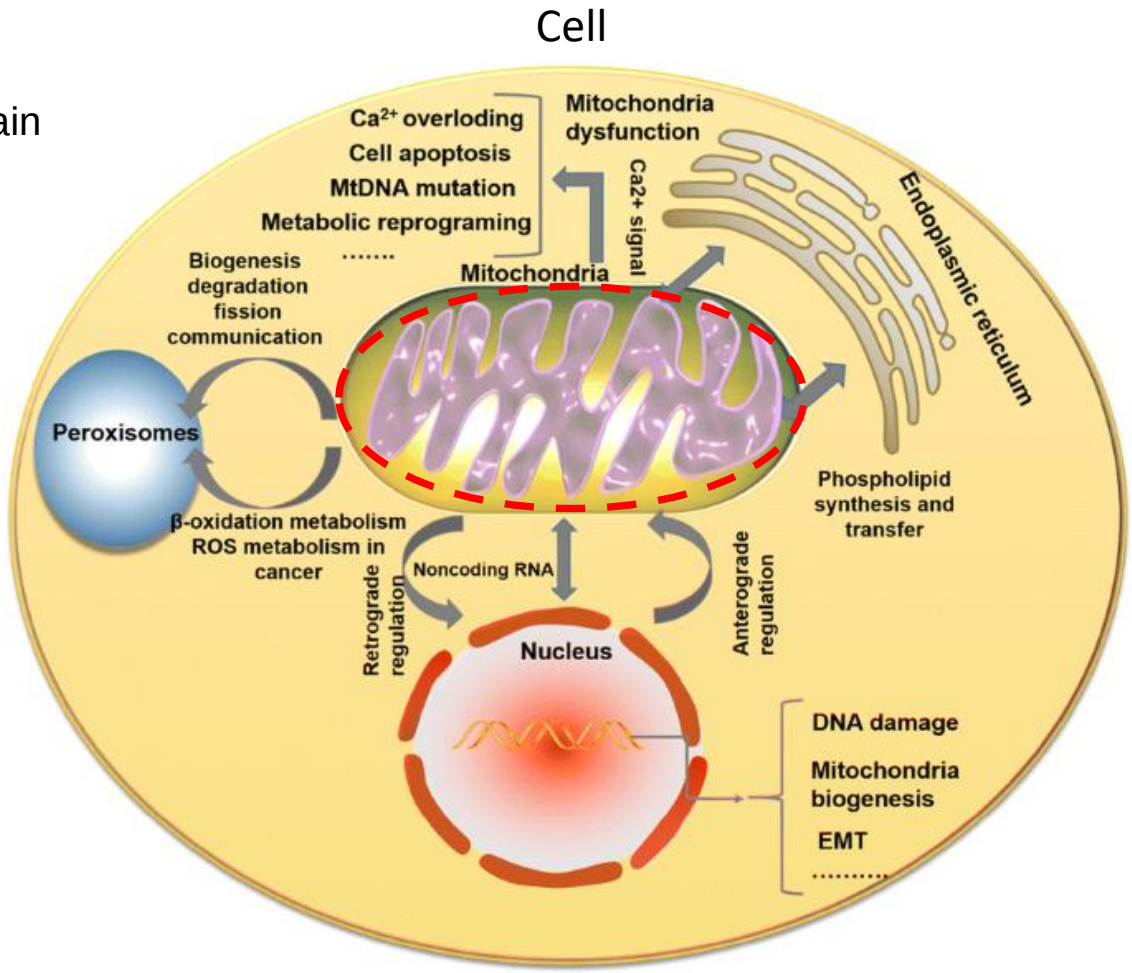
Mitochondria

Characteristics

- Double membrane organelle
- Human cardiac mitochondria contain over 1500 different proteins
- Origin: bacterial invasion into eukaryotic cell endosymbiosis.
- Diameter: 0.5 to 3 μm.

Functions:

- Primary source of energy
- Metabolism
- Biosynthesis of hormones
- ROS production
- Cell death regulation
- Ion homeostasis (regulating Ca^{2+})



Role of Calcium in the mitochondria

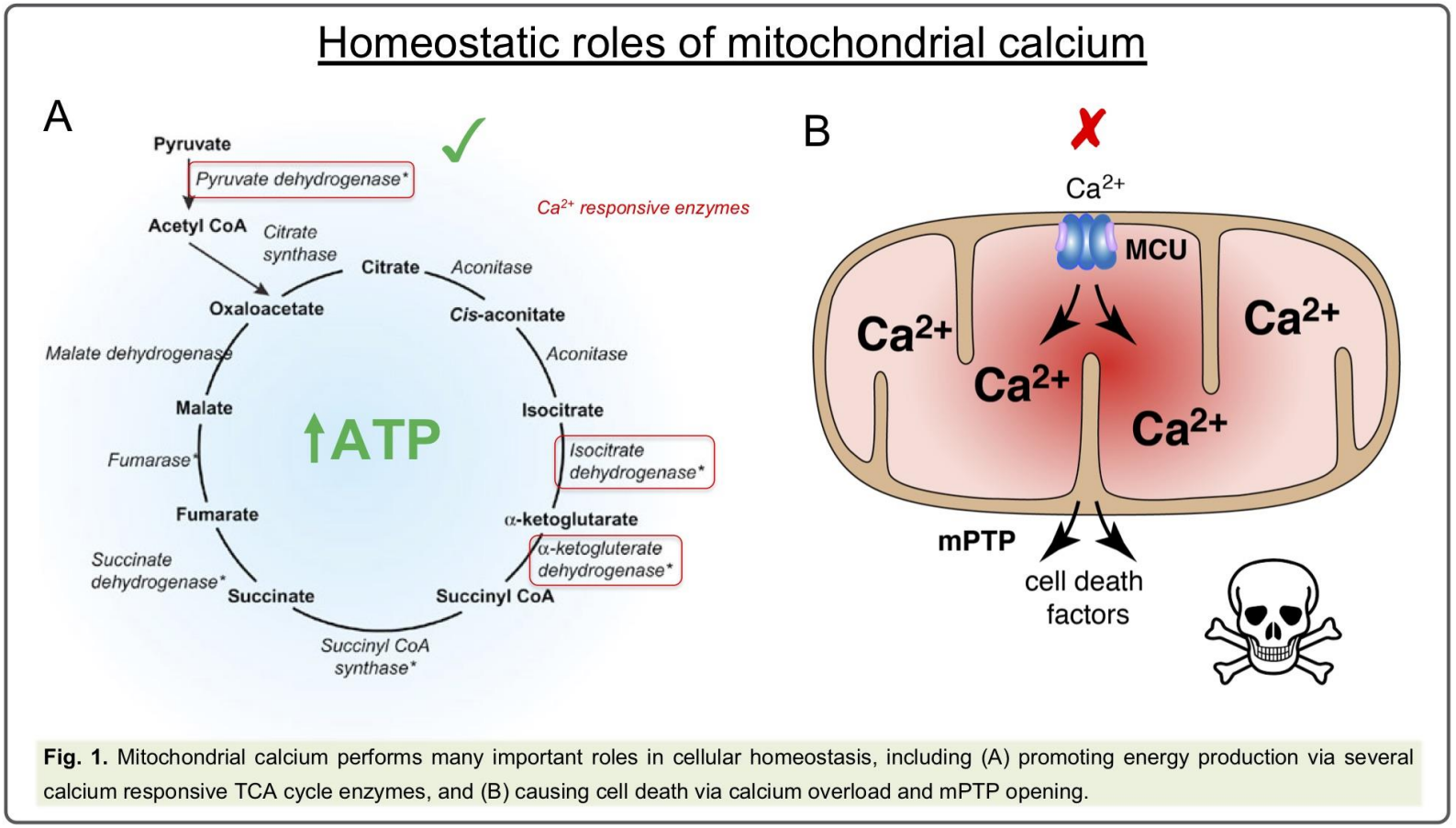
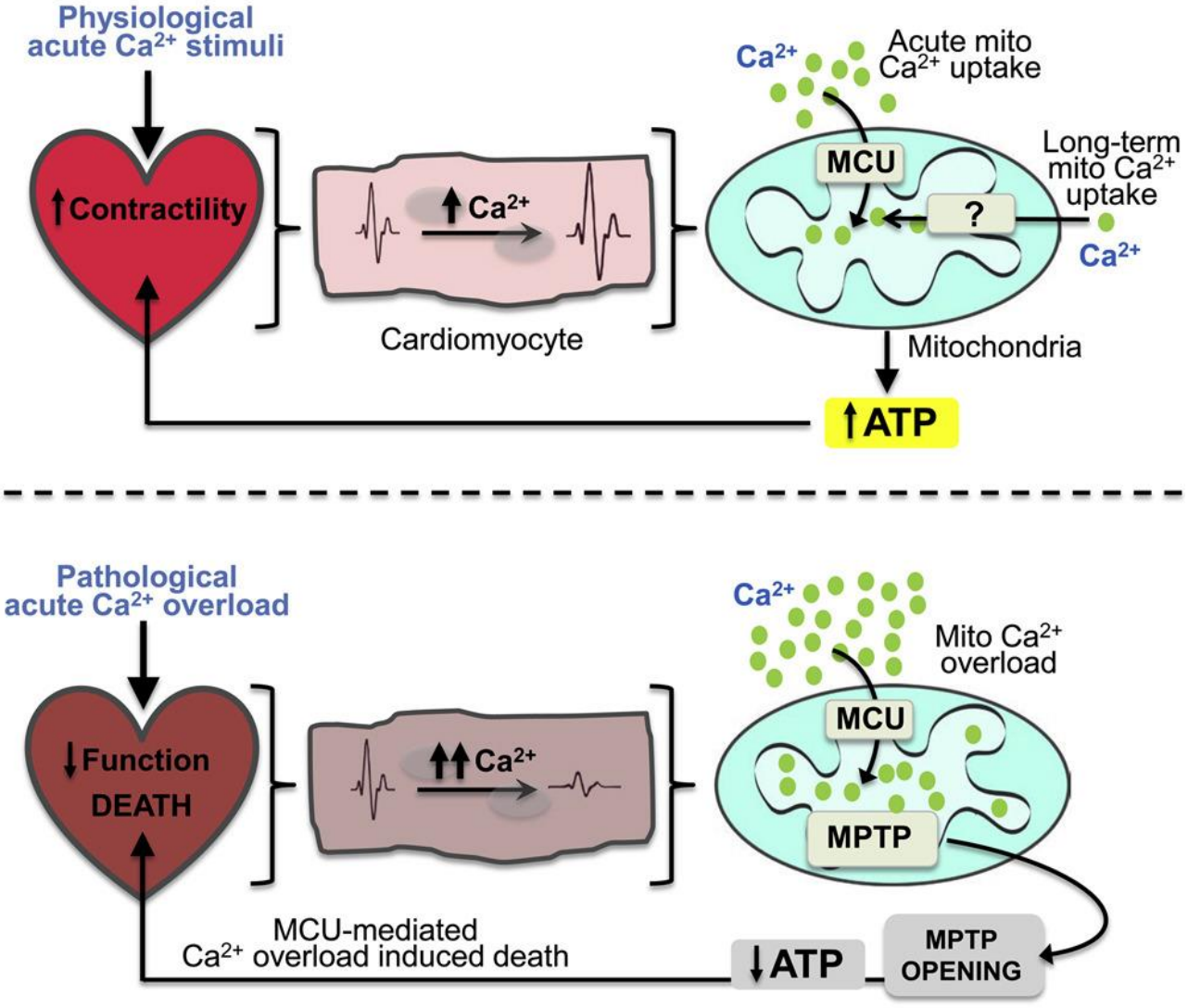


Fig. 1. Mitochondrial calcium performs many important roles in cellular homeostasis, including (A) promoting energy production via several calcium responsive TCA cycle enzymes, and (B) causing cell death via calcium overload and mPTP opening.

Low Ca^{2+} levels

High Ca^{2+} levels

The relationship between Ca^{2+}, mitochondria, and heart



Mitochondrial dynamic: fusion, fission, and death pathways

Fusion proteins:

- OPA-1
- MFN-1/2

Fission proteins:

- DRP1

Mitochondrial degradation proteins:

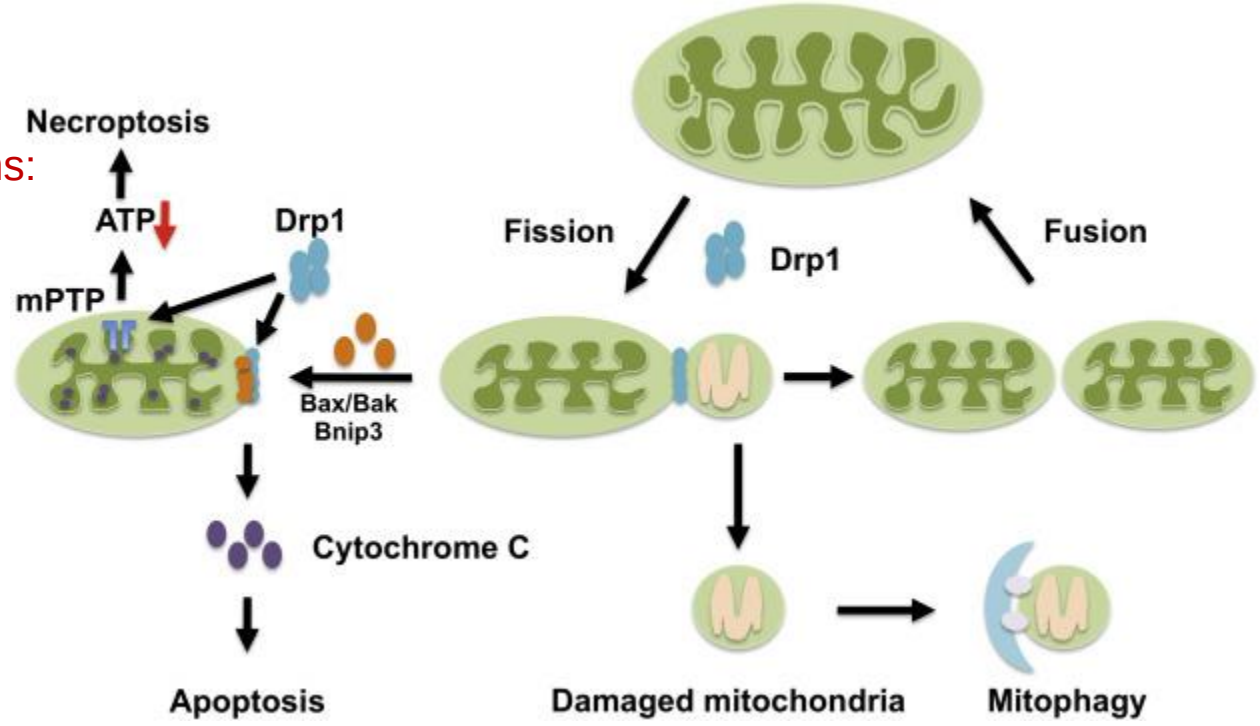
- Pink1
- Parkin
- LC3 I/II

Death pathway proteins:

- Bax/Bak
- Cytochrome C
- Caspase 3

Anti-death pathway proteins:

- Bcl-2

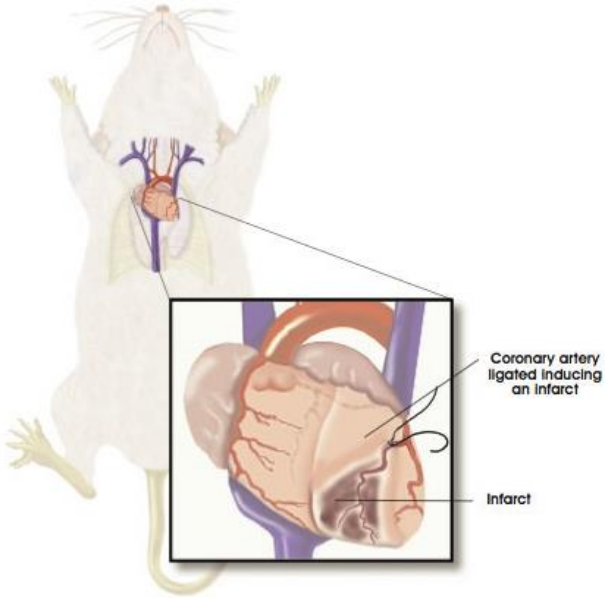


Objective

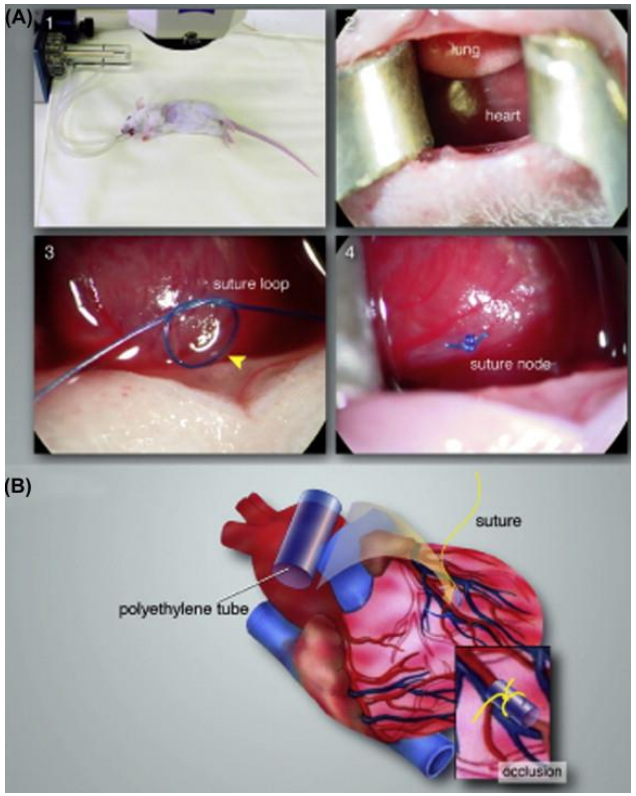
- Investigate the role of MCU expression in IR and its impact on mitochondrial fusion, fission, and mitophagy via calpain/OPA1 expression.

Ischemia/reperfusion model

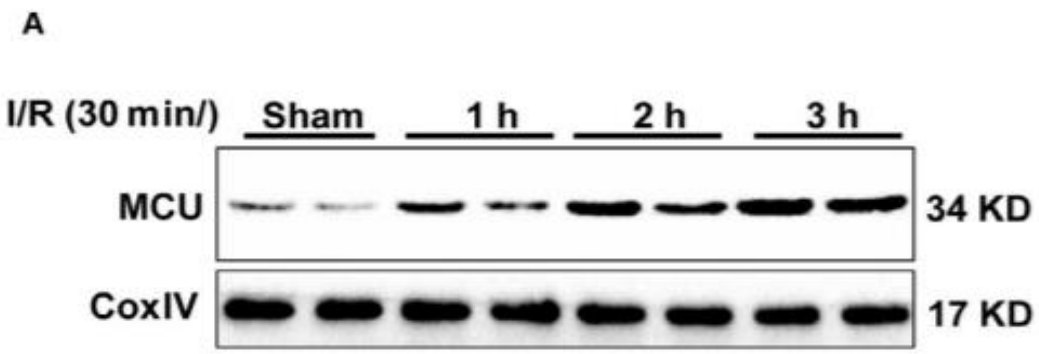
- Ru360 was dissolved in 0.9% saline solution and administered via intraperitoneal injection 30 minutes prior to the surgery.
- Surgery consisted of the ligation of the left coronary artery for 30 minutes. (Ischemia)
- Release of the knot and blood flow for 120 minutes. (Reperfusion)
- In sham groups the chest was opened but no LCA ligation was performed.

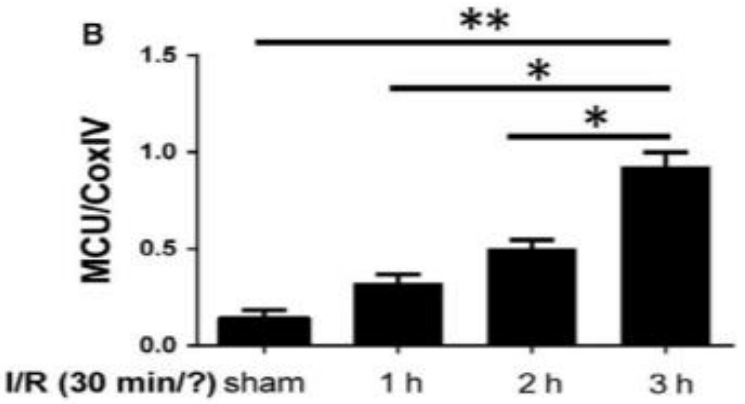


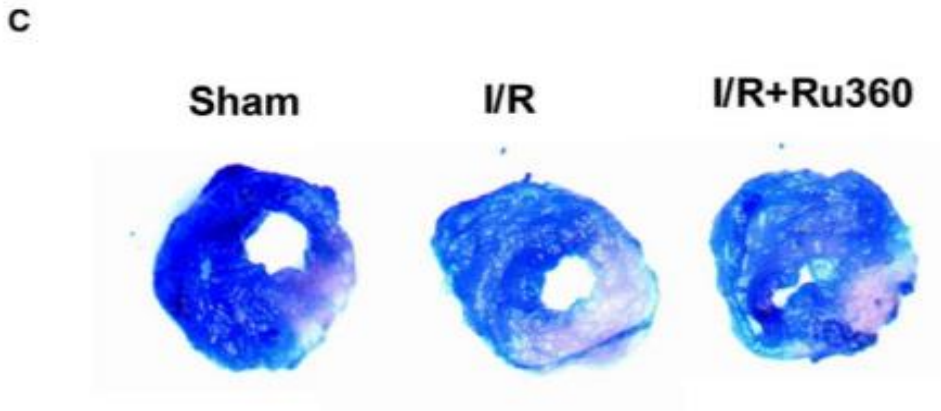
In-Vivo Coronary Artery Ligation

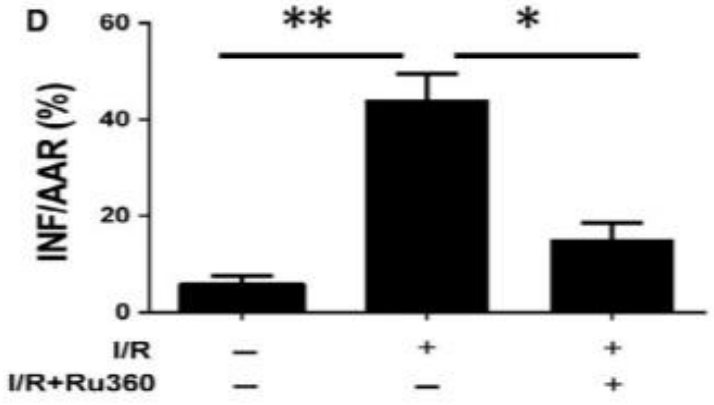


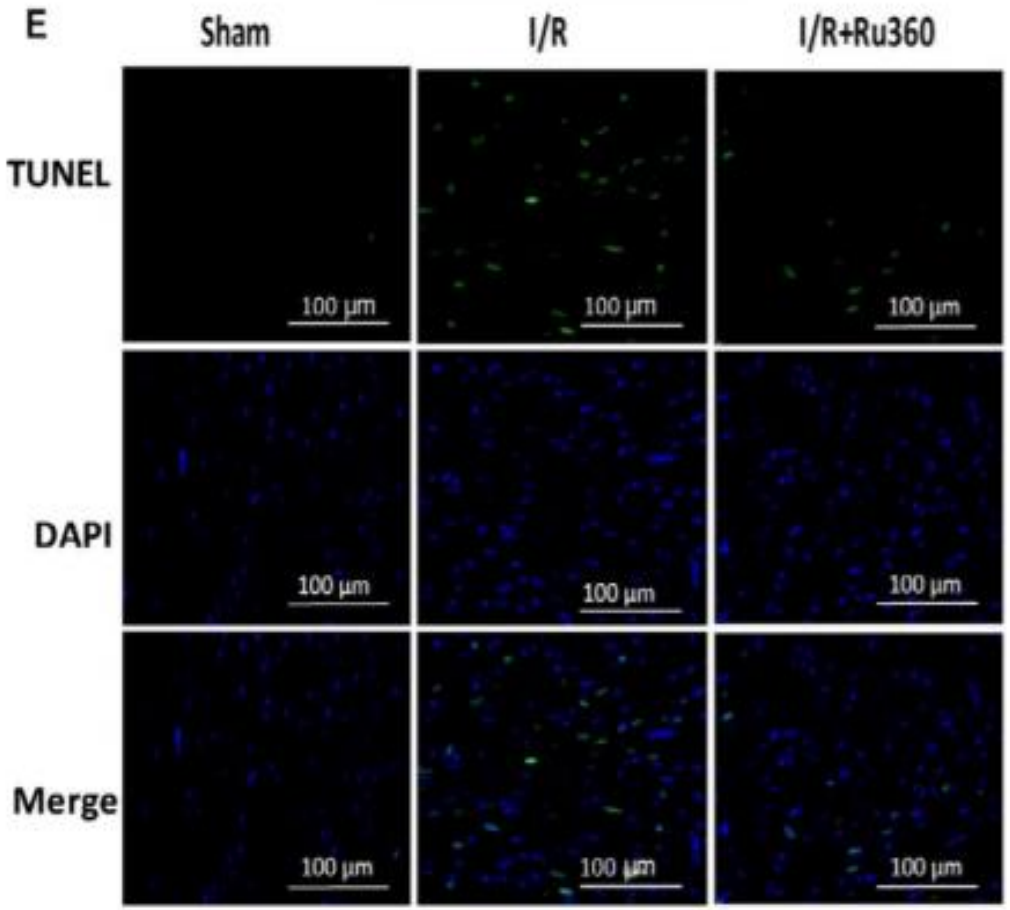
MCU was up-regulated under I/R injury and MCU inhibition reduced infarction size and apoptosis in vivo

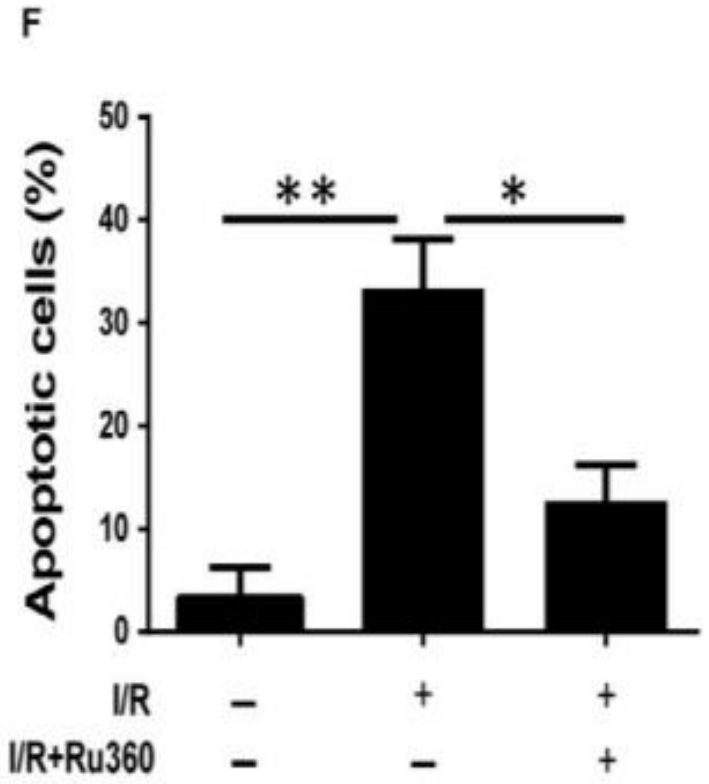






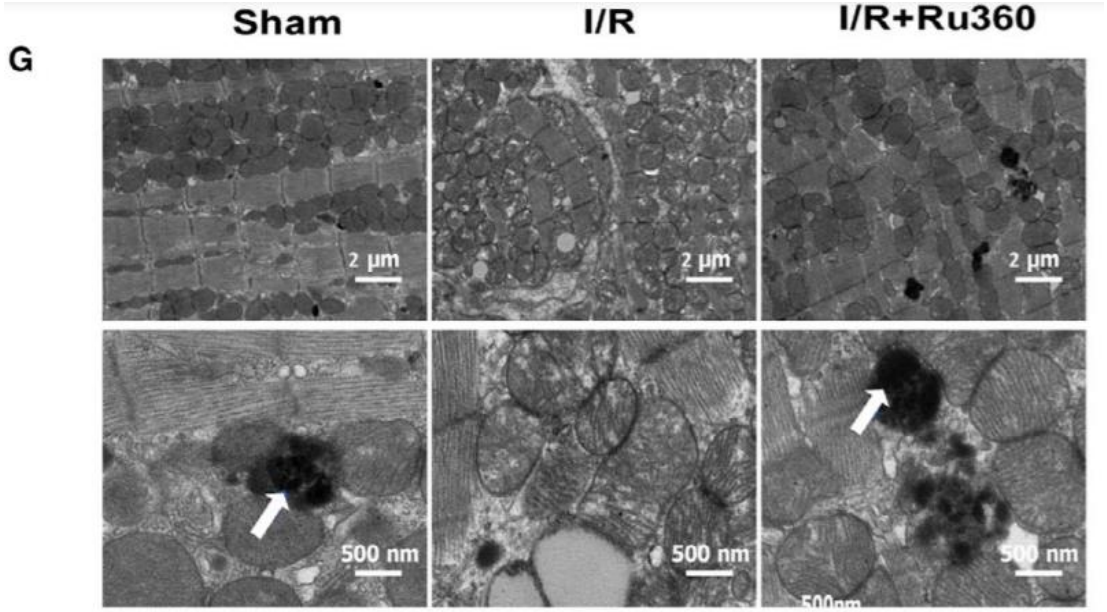




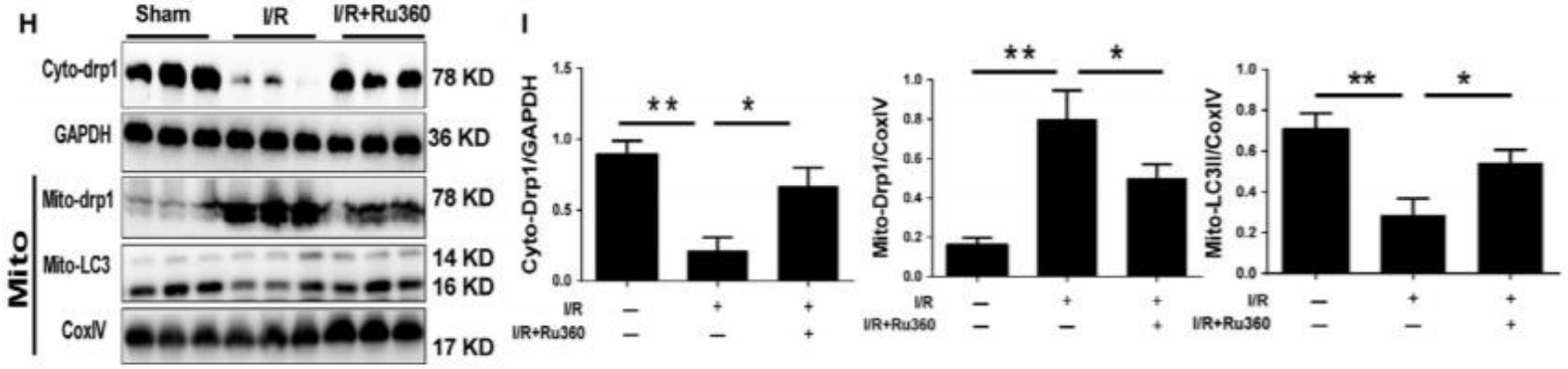


I/R injury up regulates the MCU and increases the infarct area and the percentage of apoptotic cells. Inhibition of the MCU via Ru360 lessens these effects.

MCU inhibition maintained mitochondrial morphology and homeostasis during I/R in vivo



I/R resulted in damage of mitochondrial structure, increase in fission and decrement in mitophagy. MCU inhibition might benefit the balance of fission/fusion and mitophagy and protect mitochondrial integrity.

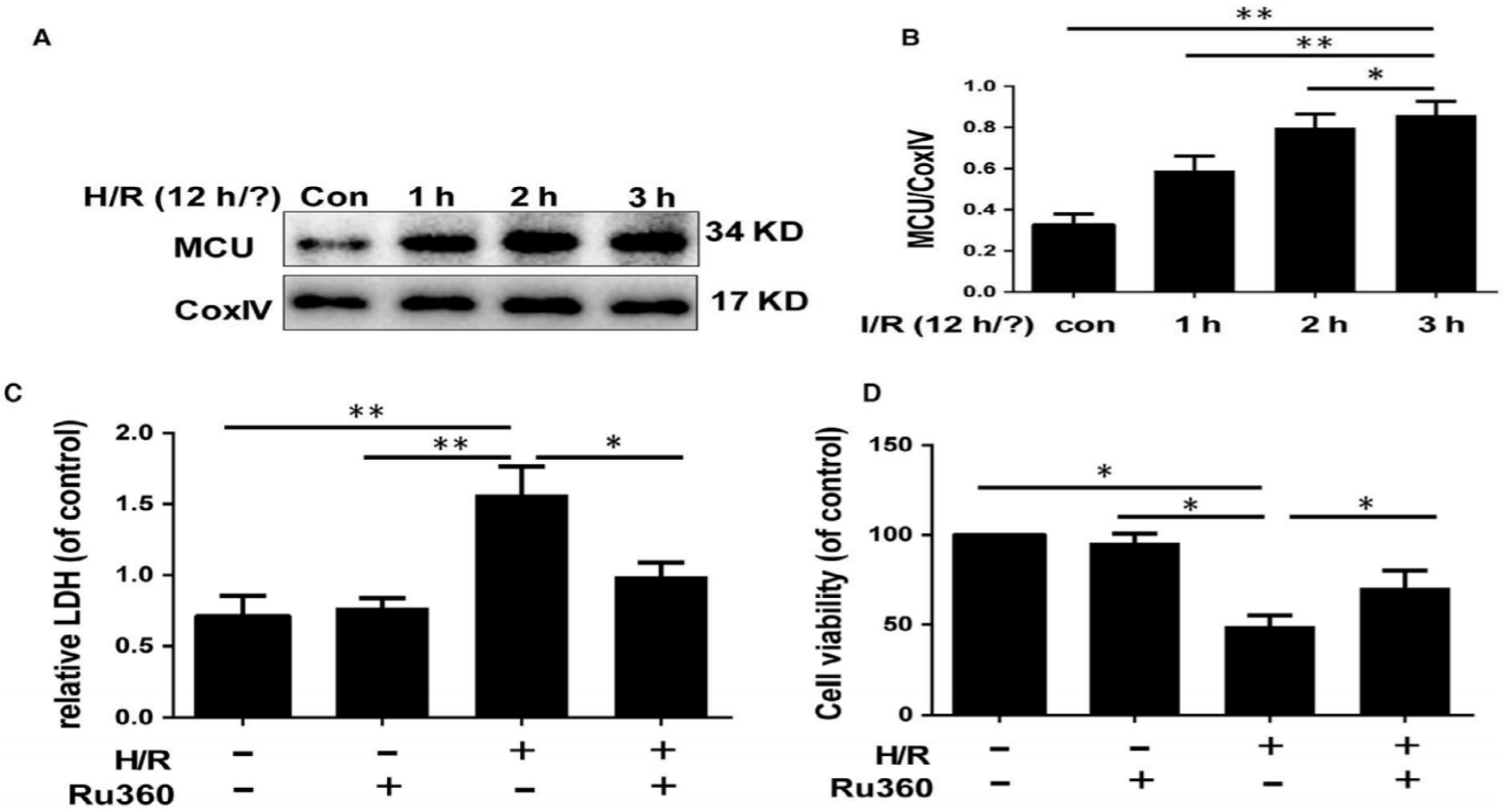


Induction of Hypoxia/reperfusion in cardiomyocytes

- Isolated myocardial cells were submitted to conditions of 1% oxygen, 94% nitrogen, and 5% carbon dioxide in a hypoxia incubator for 12 hours.

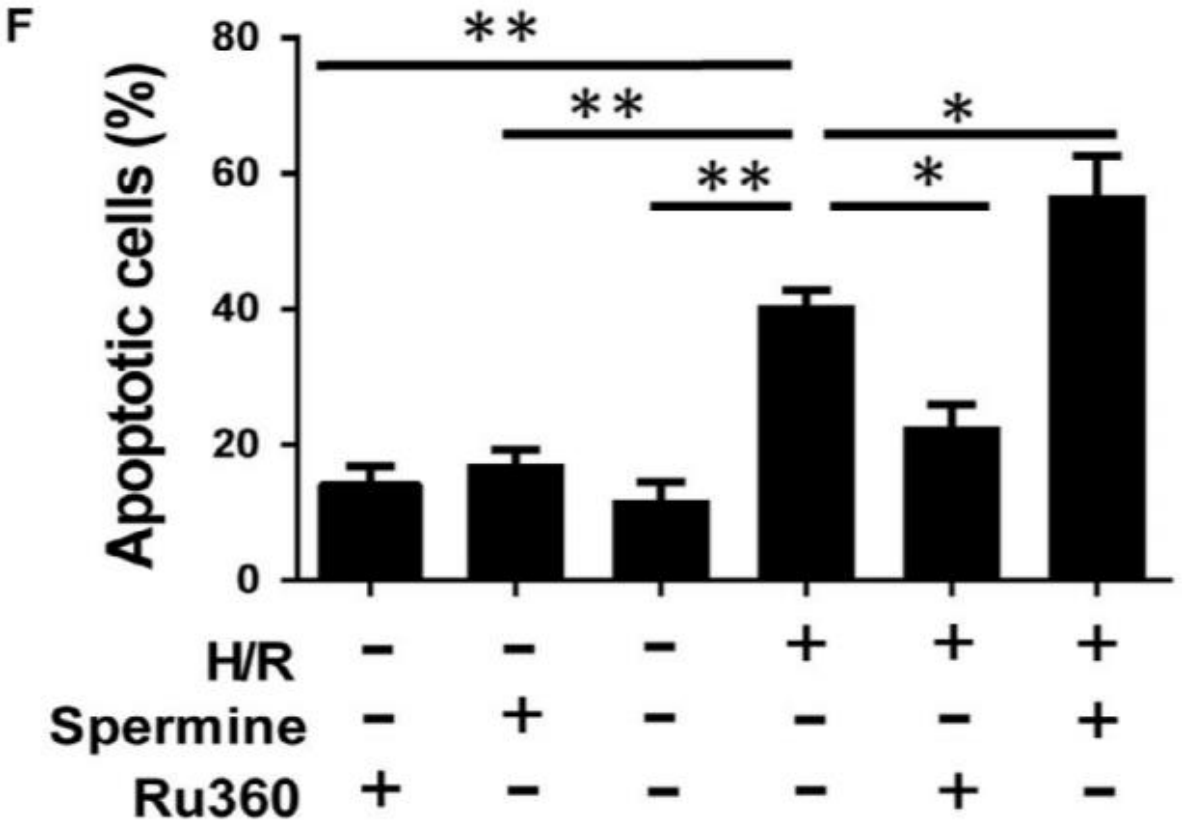
- Followed by reoxygenation in normal conditions (20% oxygen) for 3 hours.

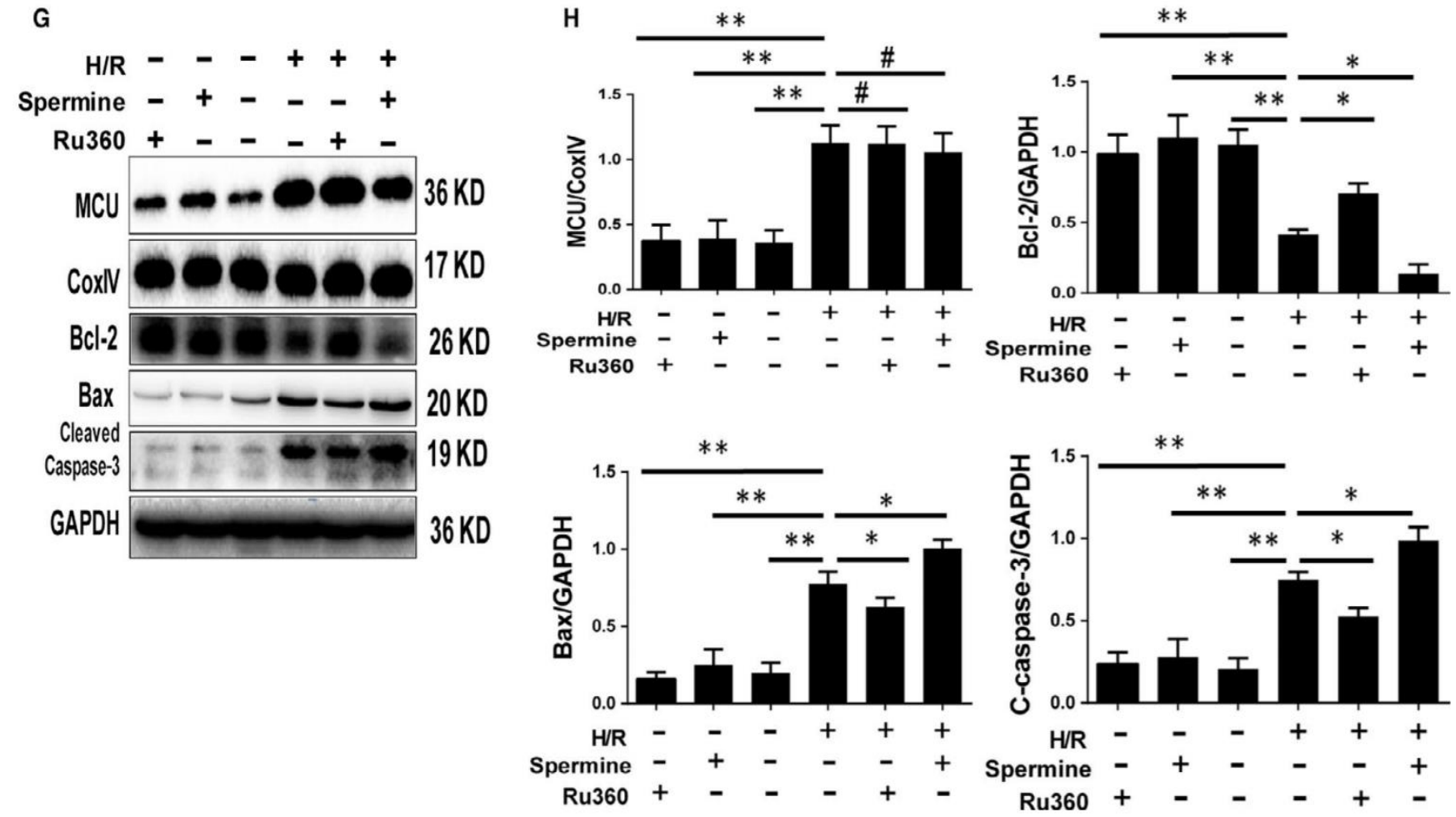
MCU up-regulation played a role in H/R induced myocardial injury in vitro



H/R resulted in loss of viability and elevation of LDH release. These injuries could be attenuated by Ru360 treatment or worsened by spermine.

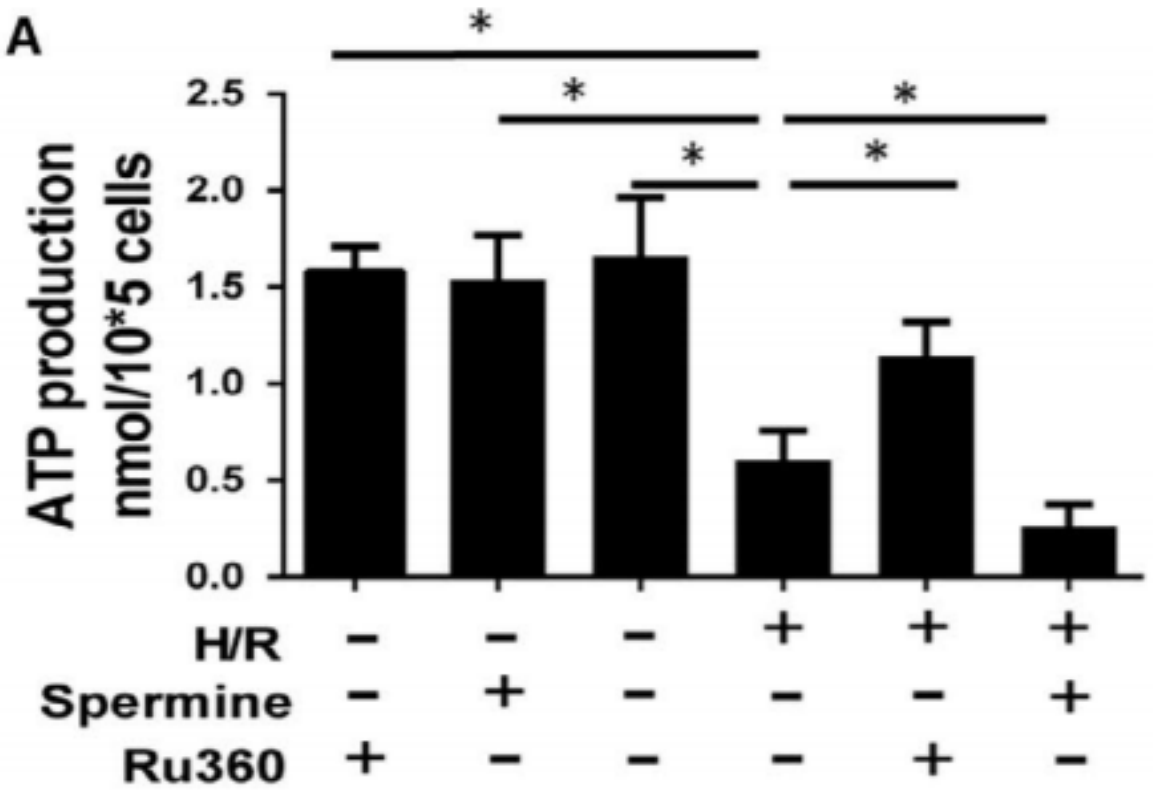
Apoptotic cell death in H/R induced myocardial injury in vitro



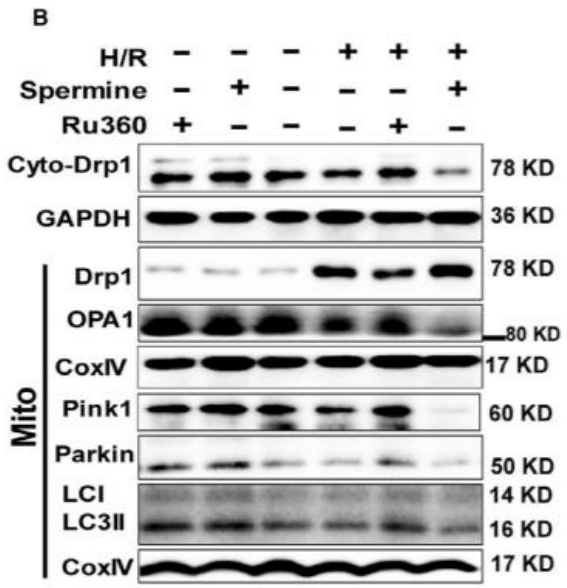


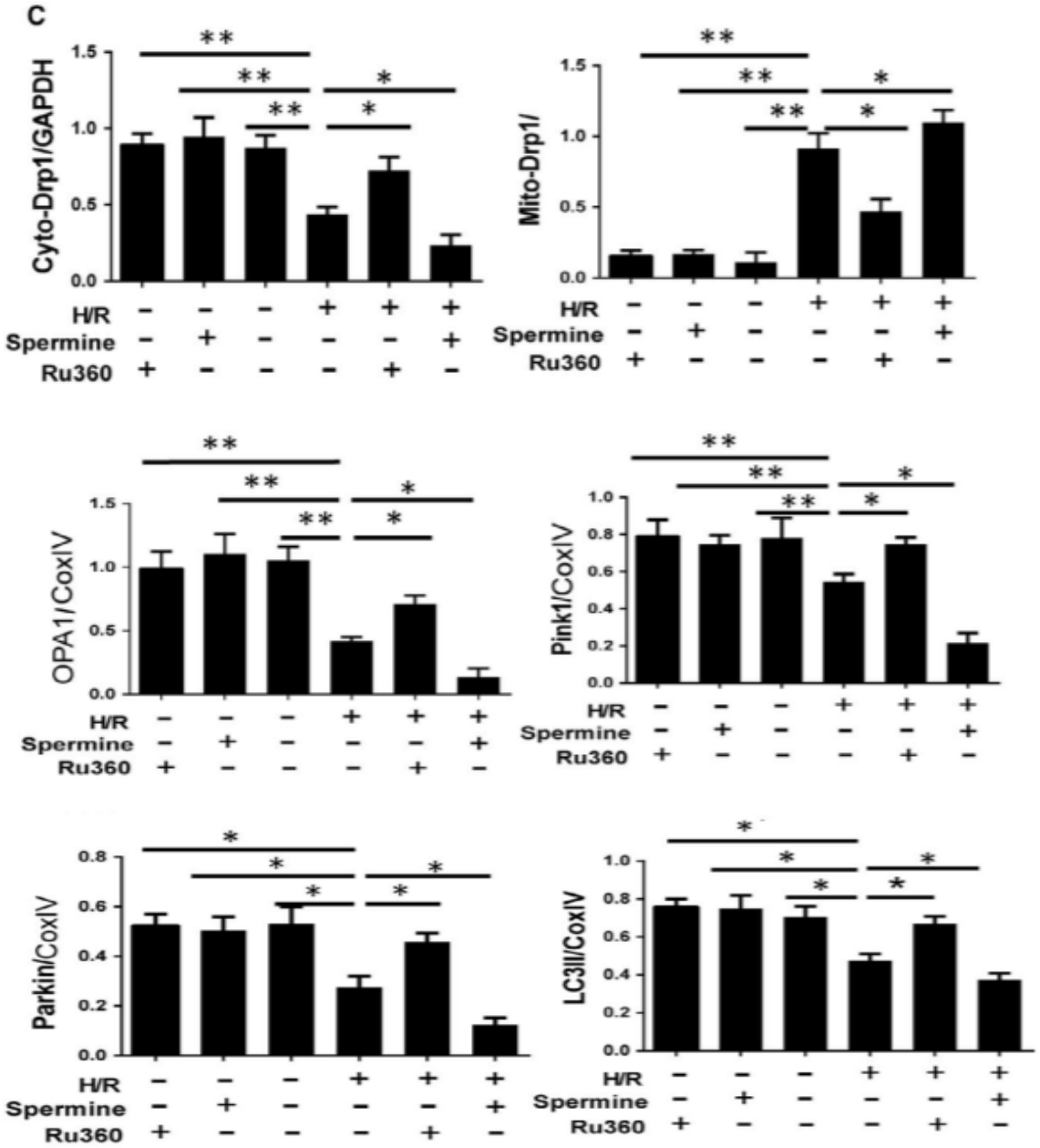
H/R induced the increment of Bax and cleaved caspase-3, and the decrement of Bcl-2. These findings confirm that MCU was up-regulated during I/R and contributed to I/R injury.

MCU activity modulated H/R-induced mitochondrial fission/fusion, mitophagy and ATP production in vitro

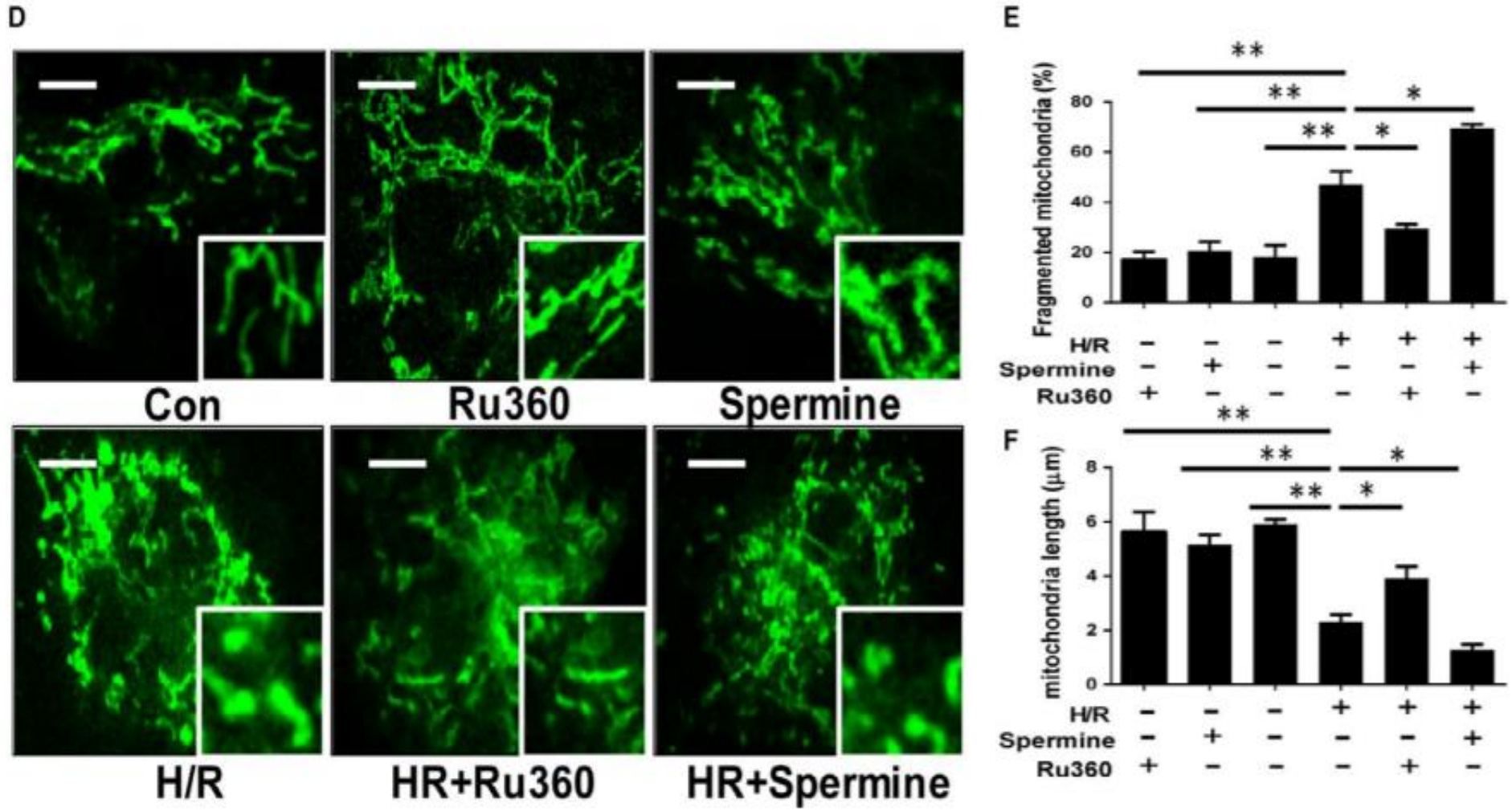


ATP production dropped after H/R, while recovered with Ru360 , and showed additional decrement with spermine treatment.



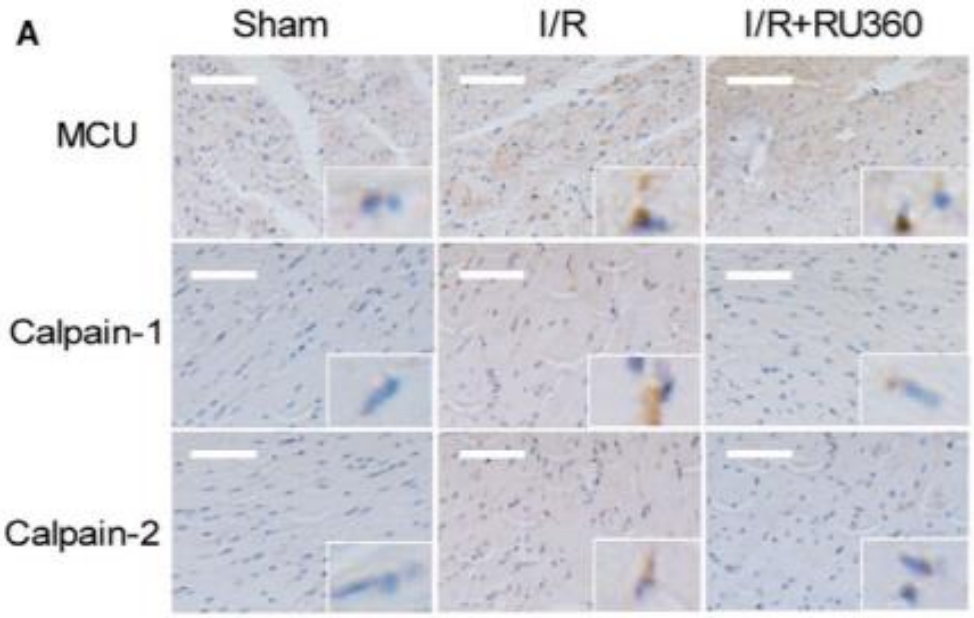


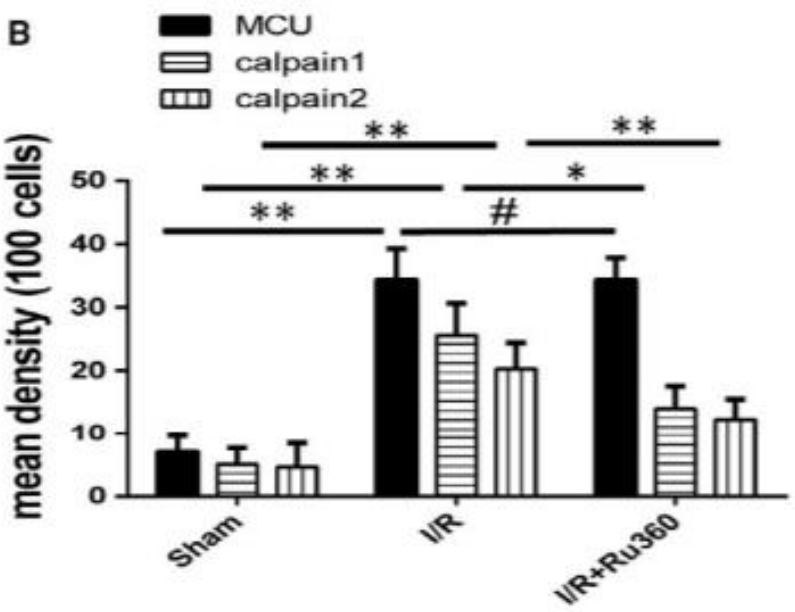
Drp1 transferred from cytoplasm to mitochondria after H/R, as well as OPA1, Parkin and LC3II of mitochondria down regulated, indicating fission enhancement and fusion and mitophagy inhibition.



The percentage of mitochondrial fragment rose in H/R group, while the average length of mitochondria dropped. This data clarifies that MCU up-regulation damaged the mitochondrial function, inhibited fusion and down-regulated mitophagy, and thus produced excessive fragments.

MCU modulated calpains expression and activity under I/R stress in vivo and in vitro





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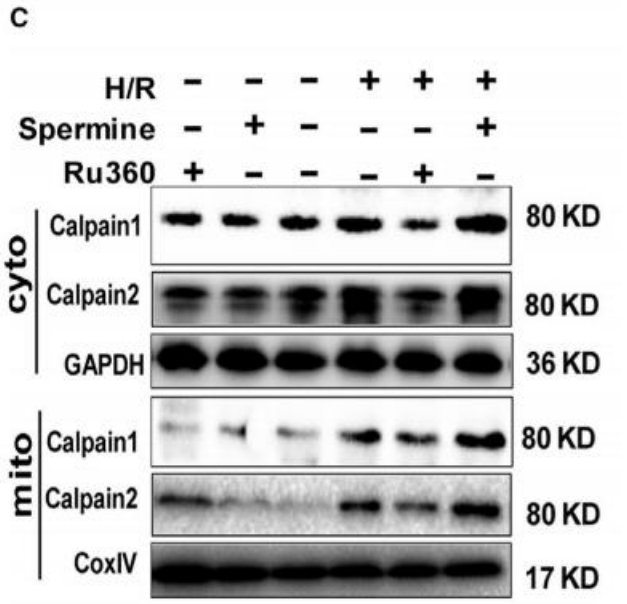
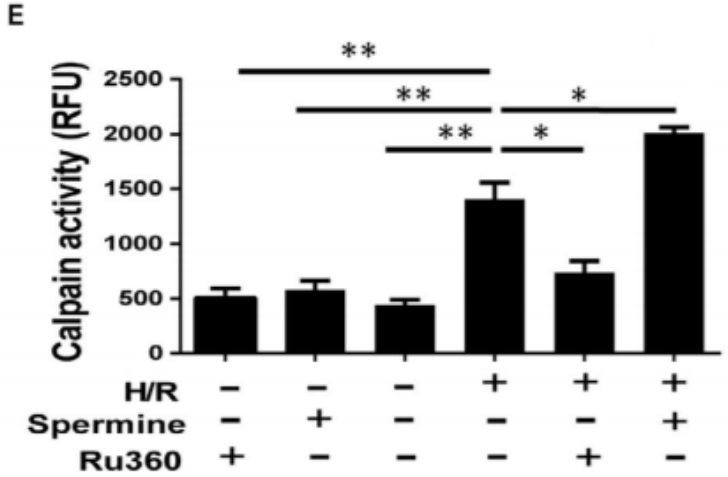
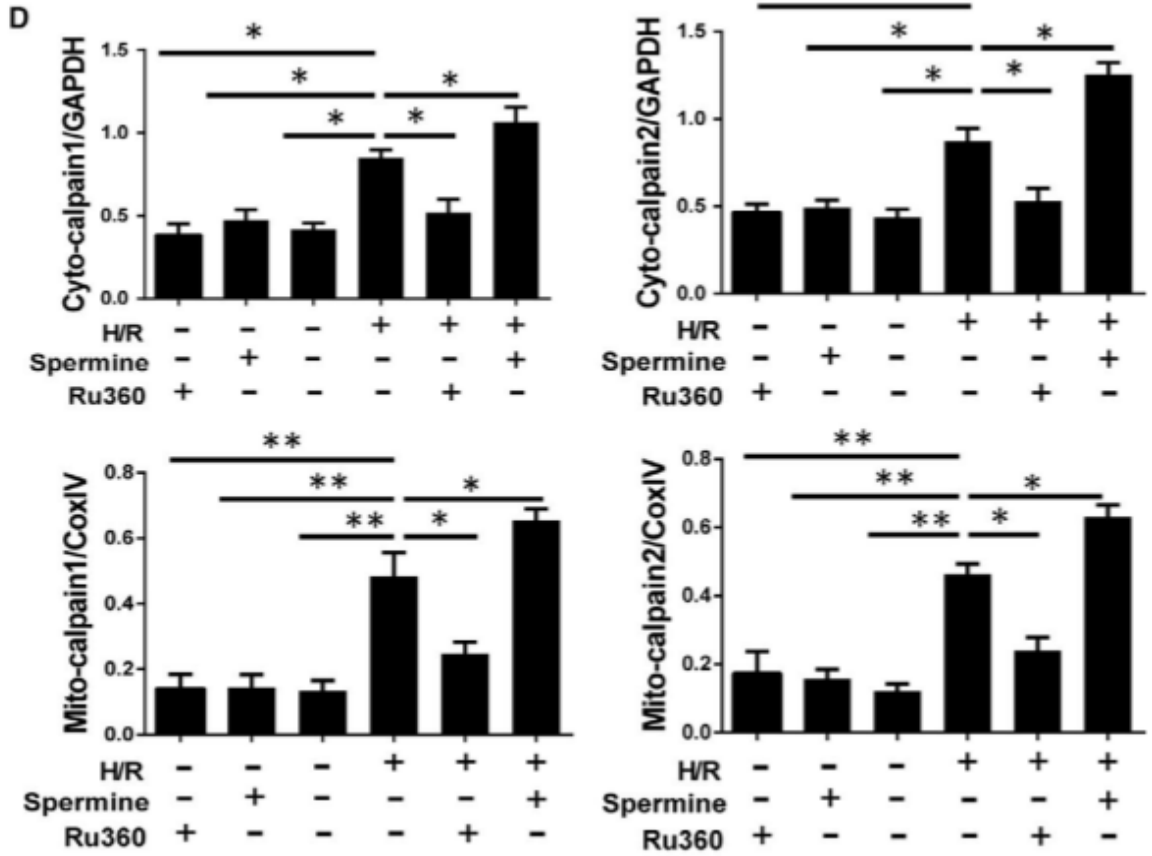
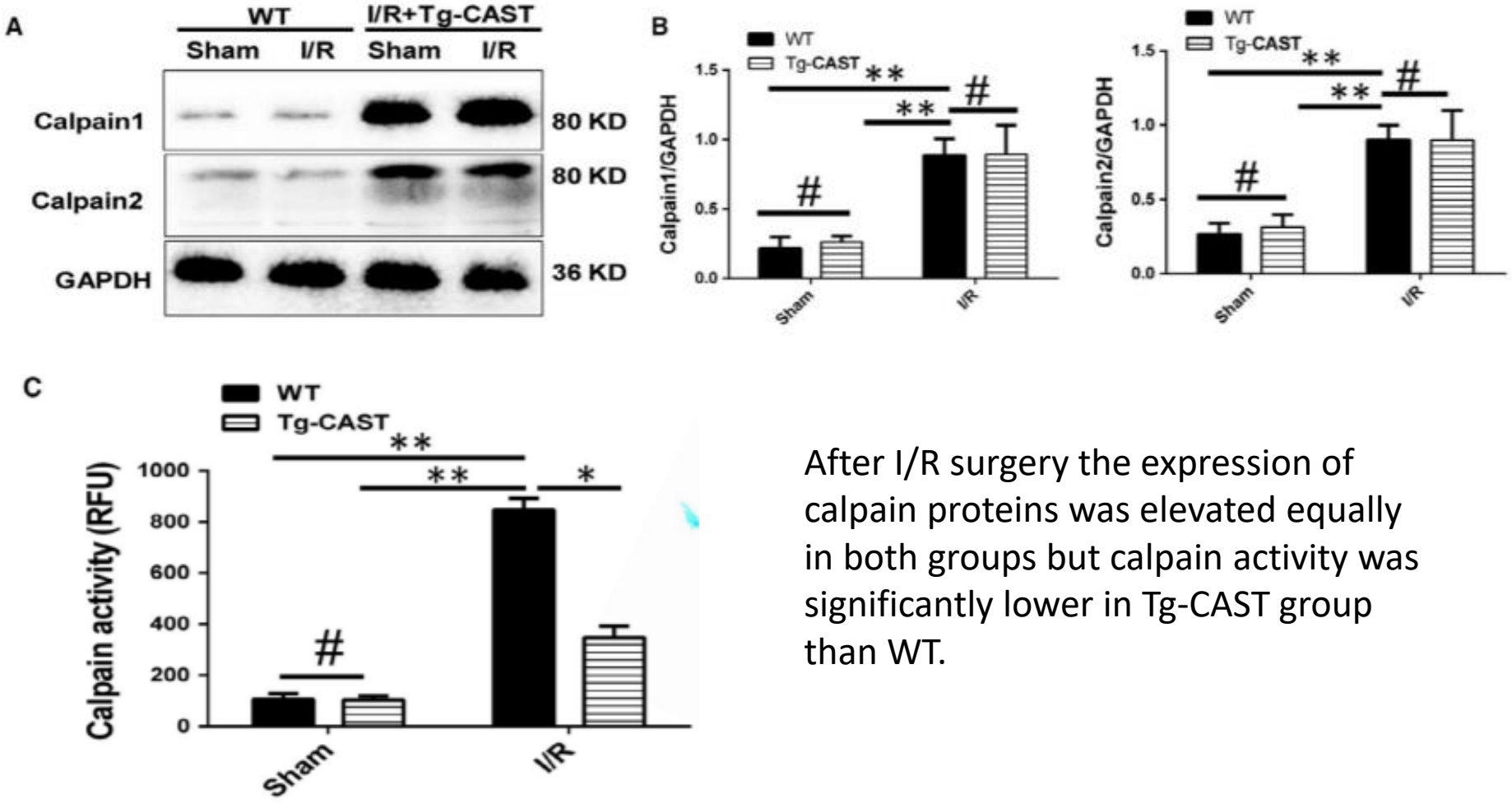






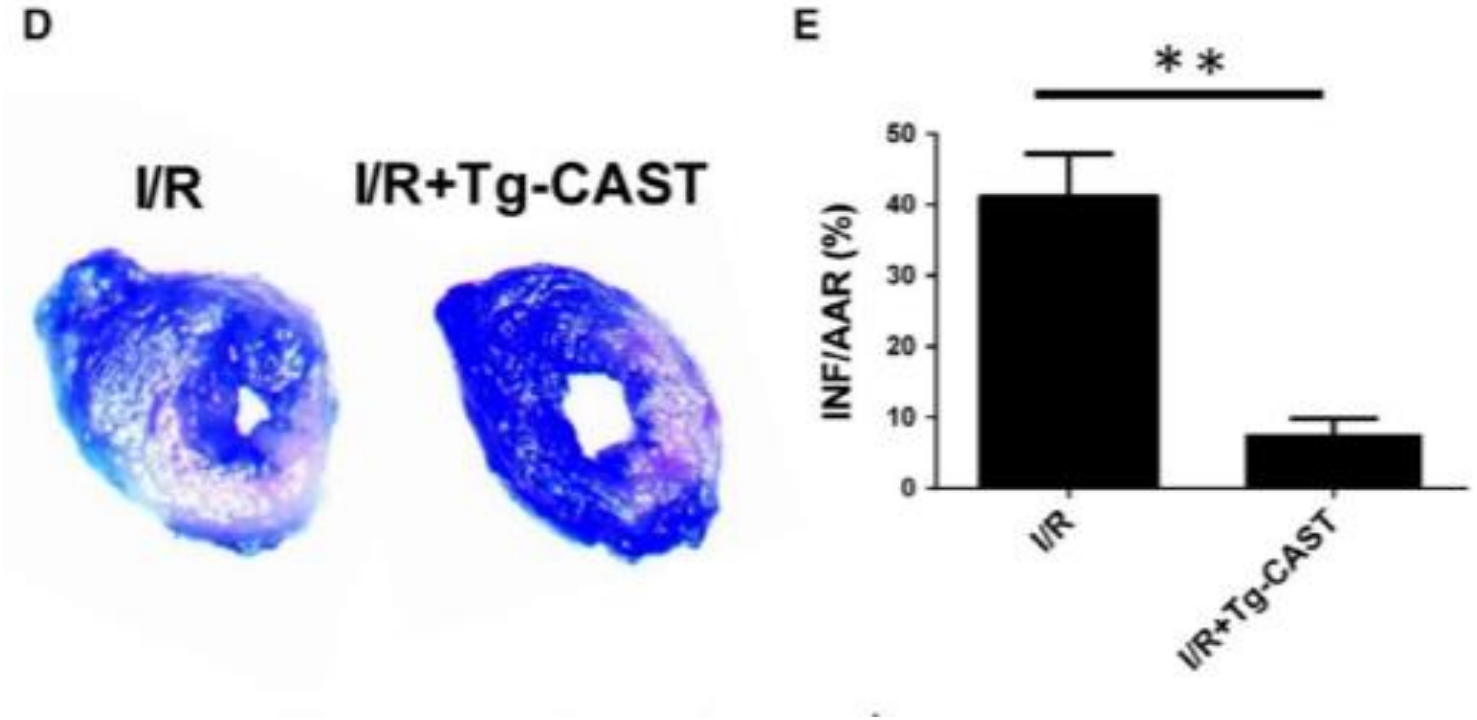
MCU modulates calpain expression via cellular calcium homeostasis.

Tg-CAST mice showed less myocardial injury, reduced mitochondrial fission and improved mitochondrial fusion and mitophagy during I/R by calpain inhibition

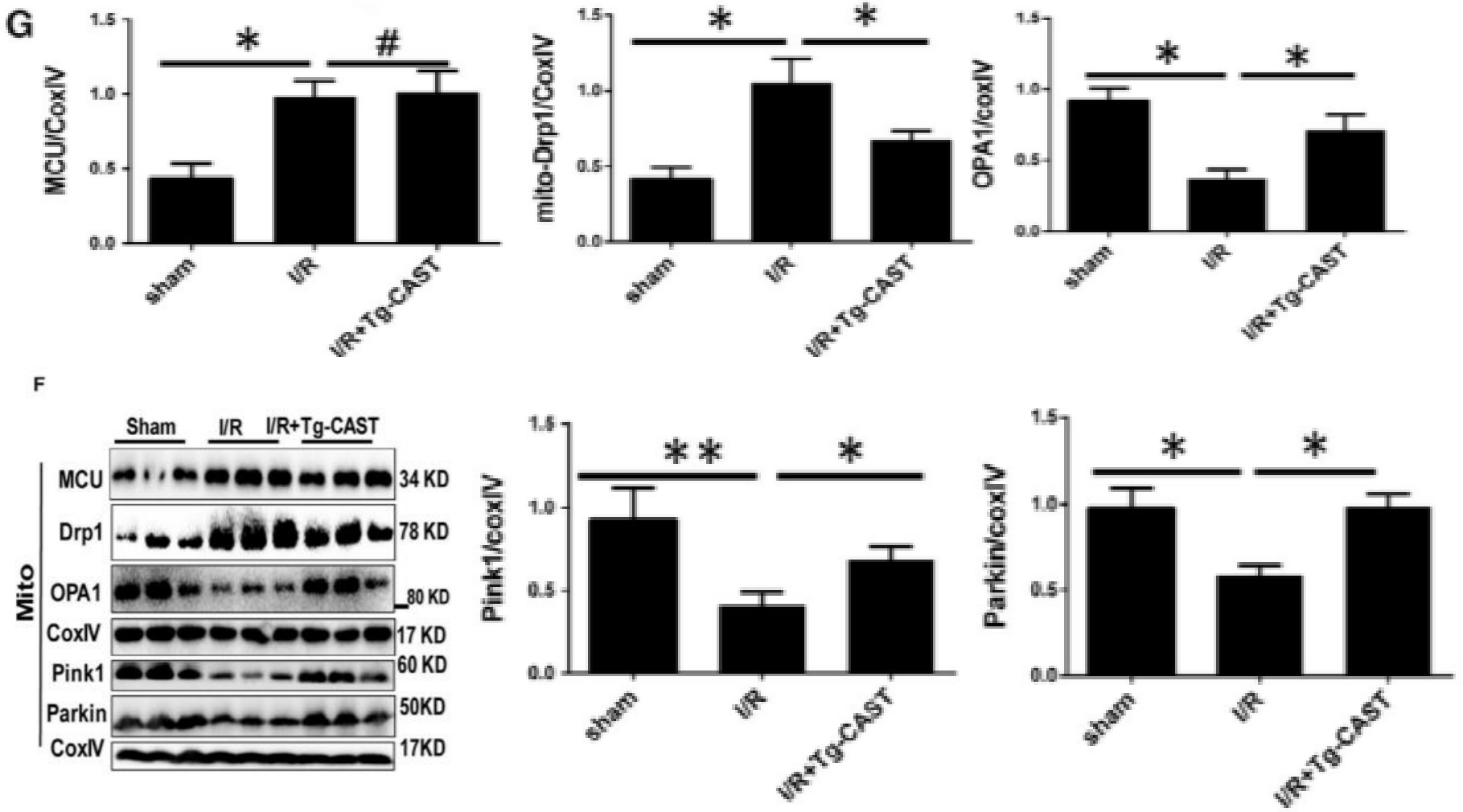


After I/R surgery the expression of calpain proteins was elevated equally in both groups but calpain activity was significantly lower in Tg-CAST group than WT.

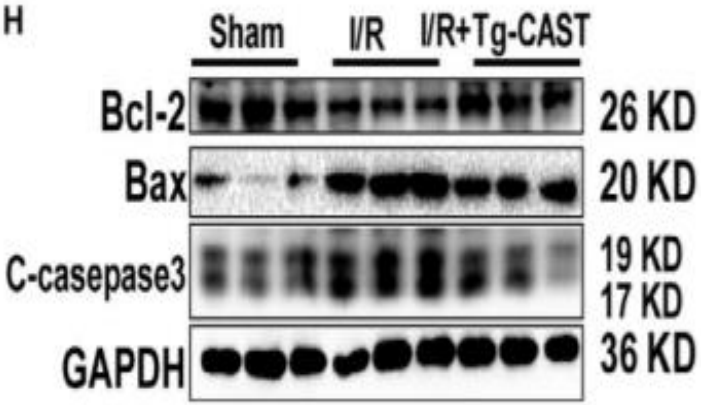
Tg-CAST mice showed less myocardial injury, reduced mitochondrial fission and improved mitochondrial fusion and mitophagy during I/R by calpain inhibition

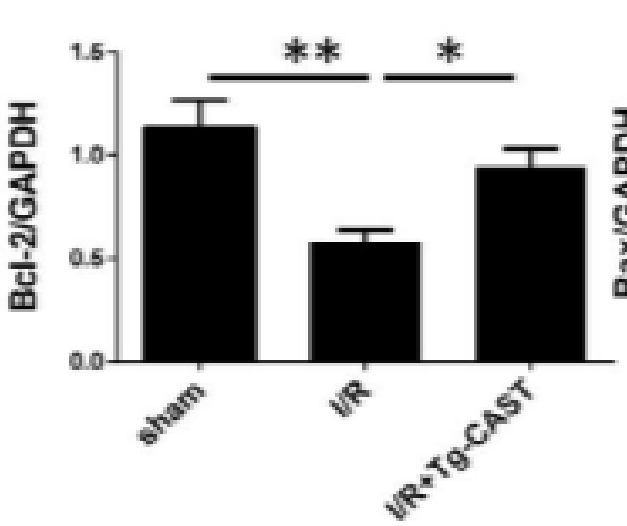


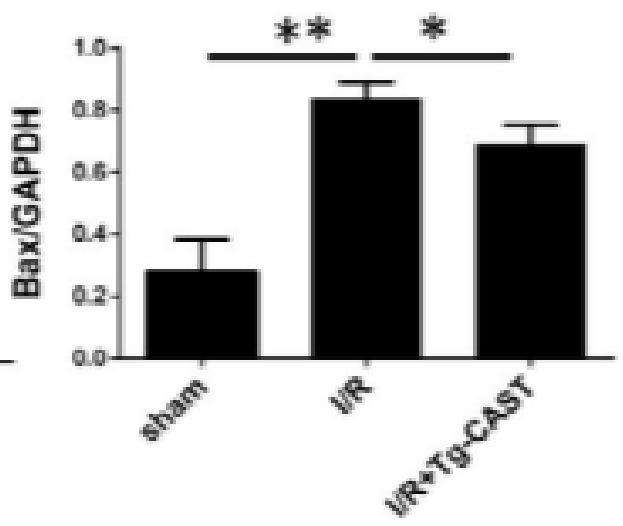
Myocardial infarction size of Tg-CAST mice was reduced after I/R surgery.

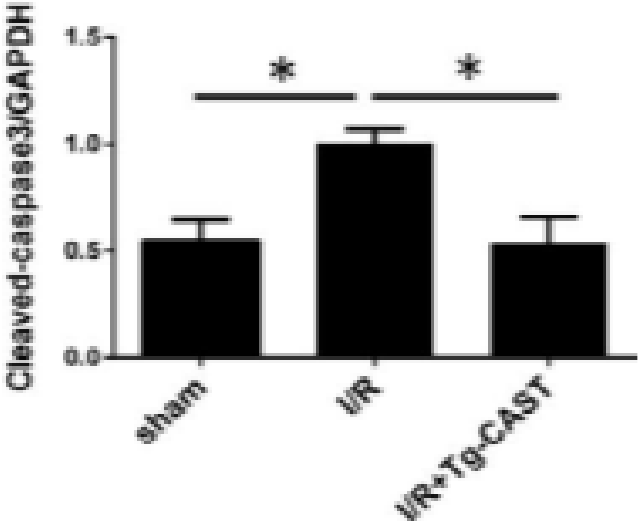


I/R induced up-regulation of Drp1 expression and down-regulation of OPA1, PINK1 and Parkin expression, while calpain inhibition neutralized these effects.





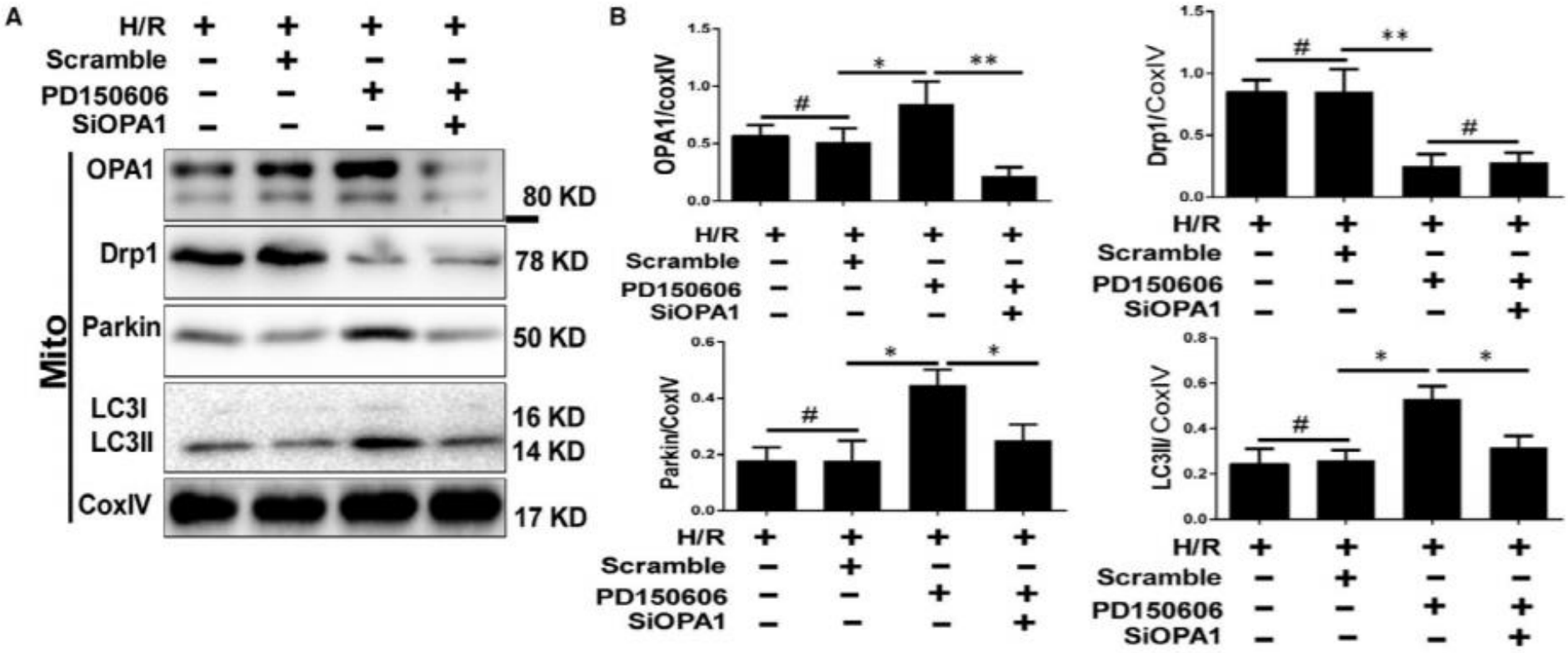




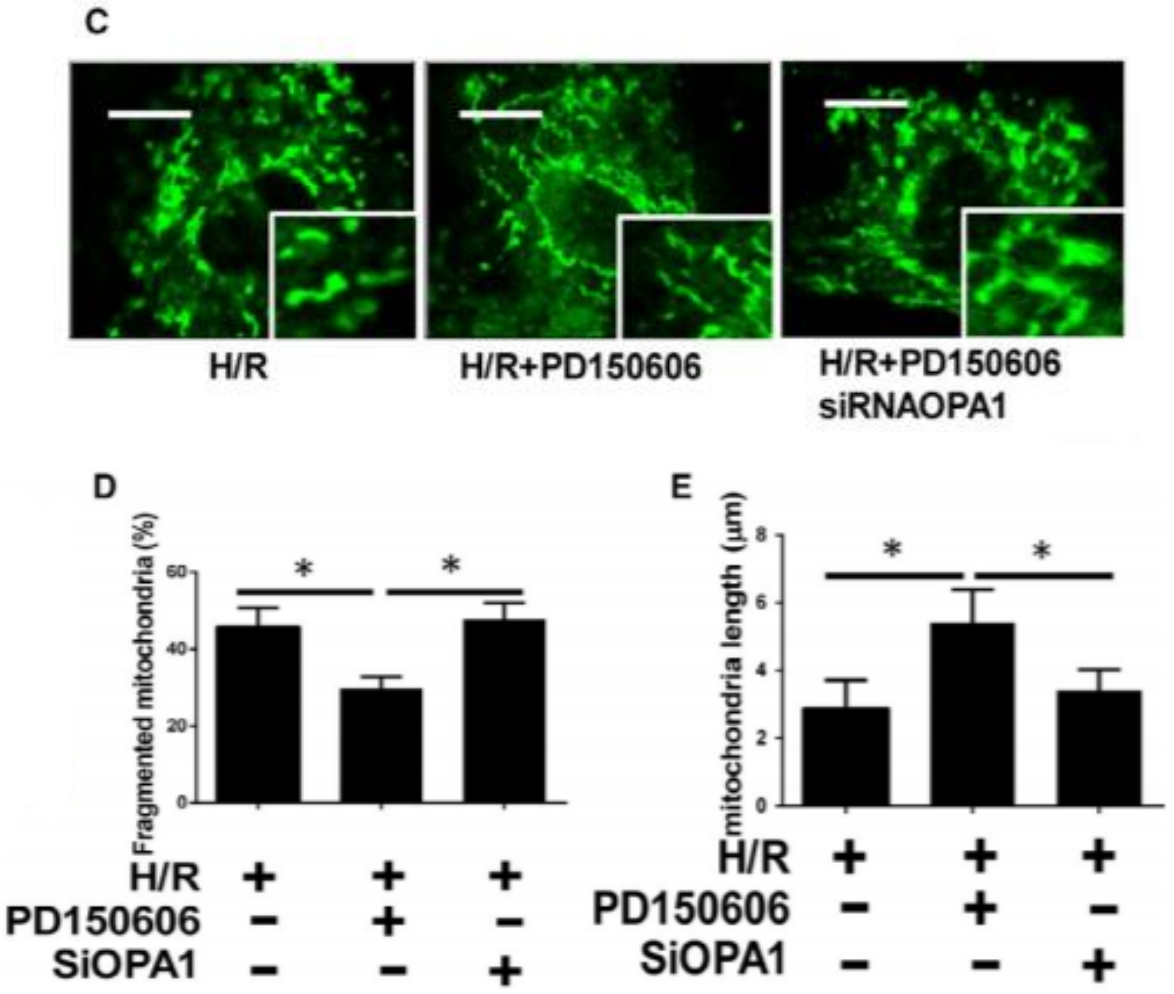
I/R increased the expression of cleaved capase-3 and Bax, and the decrement of Bcl-2. Calpain inhibition recovered the balance between Bax and Bcl-2, and reduced caspase-3.

Calpain up-regulation and activation plays a role in I/R injury and MCU induced mitochondrial fission and suppressed fusion/mitophagy.

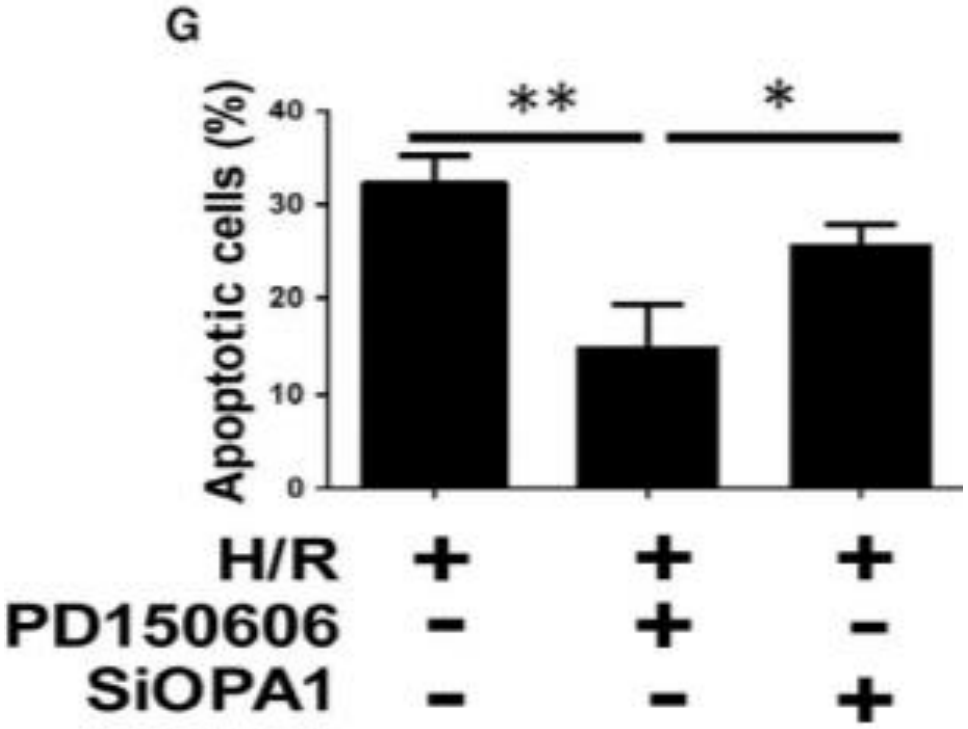
The protective effect of calpain inhibition on mitochondrial fission/fusion and mitophagy could be abolished by OPA1 knockout



OPA1 knockout nullified the protective effect of PD150606 on OPA1, LC3II, and Parkin, except Drp1.



Calpain inhibition normalized the balance between fission and fusion, and OPA1 knockout increased the fragmentation and decreased the length.



PD150606 treatment protected myocytes from apoptosis while OPA1 deletion rose the percentage of apoptotic cells.

This data suggests that the role of OPA1 in the MCU-calpain mediated mitochondrial fusion/mitophagy inhibition , which results in mitochondrial apoptotic fission.

Conclusions

- MCU is up-regulated during myocardial I/R, which increases the expression of cytoplasm and calpains and activates them.
- Activated calpains down-regulate OPA1-mediated mitochondrial fusion and mitophagy, promote mitochondrial fission, damage mitochondrial homeostasis and mitochondrial function, and promote apoptosis.

Limitations

- It is not clear whether calpain has a direct or indirect effect on OPA1.
- Direct relationship between MCU and OPA1 was not elucidated, however the relationship between the MCU expression and calpain was explored in the study.
- In addition to calpains, OPA1 cleaving proteins such as OMA1 or YMEL1 were not discussed in this paper.