



Distinction between mitophagy and ferroptosis in cardiomyocytes

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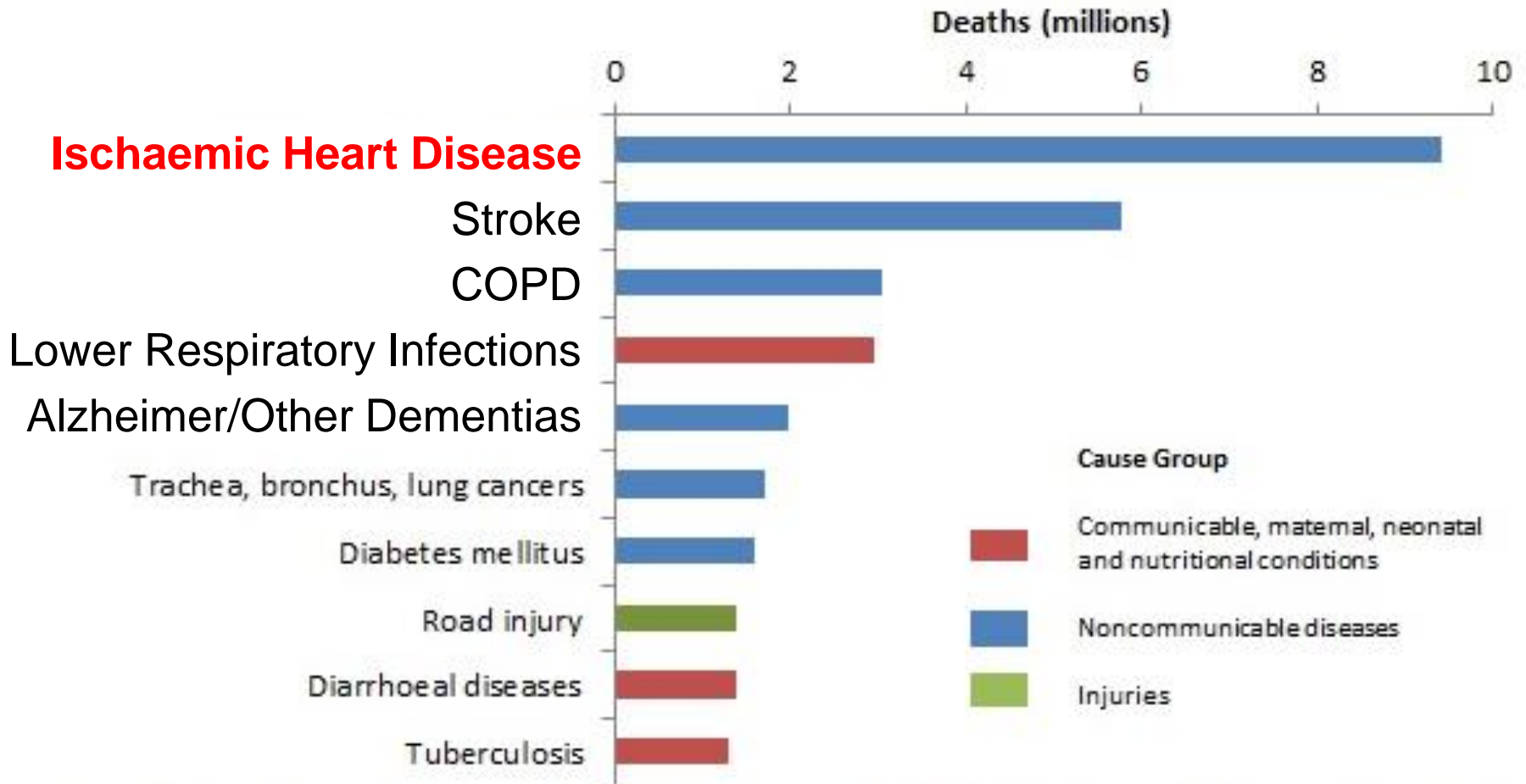
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Center for Cardiovascular Research

John A. Burns School of Medicine

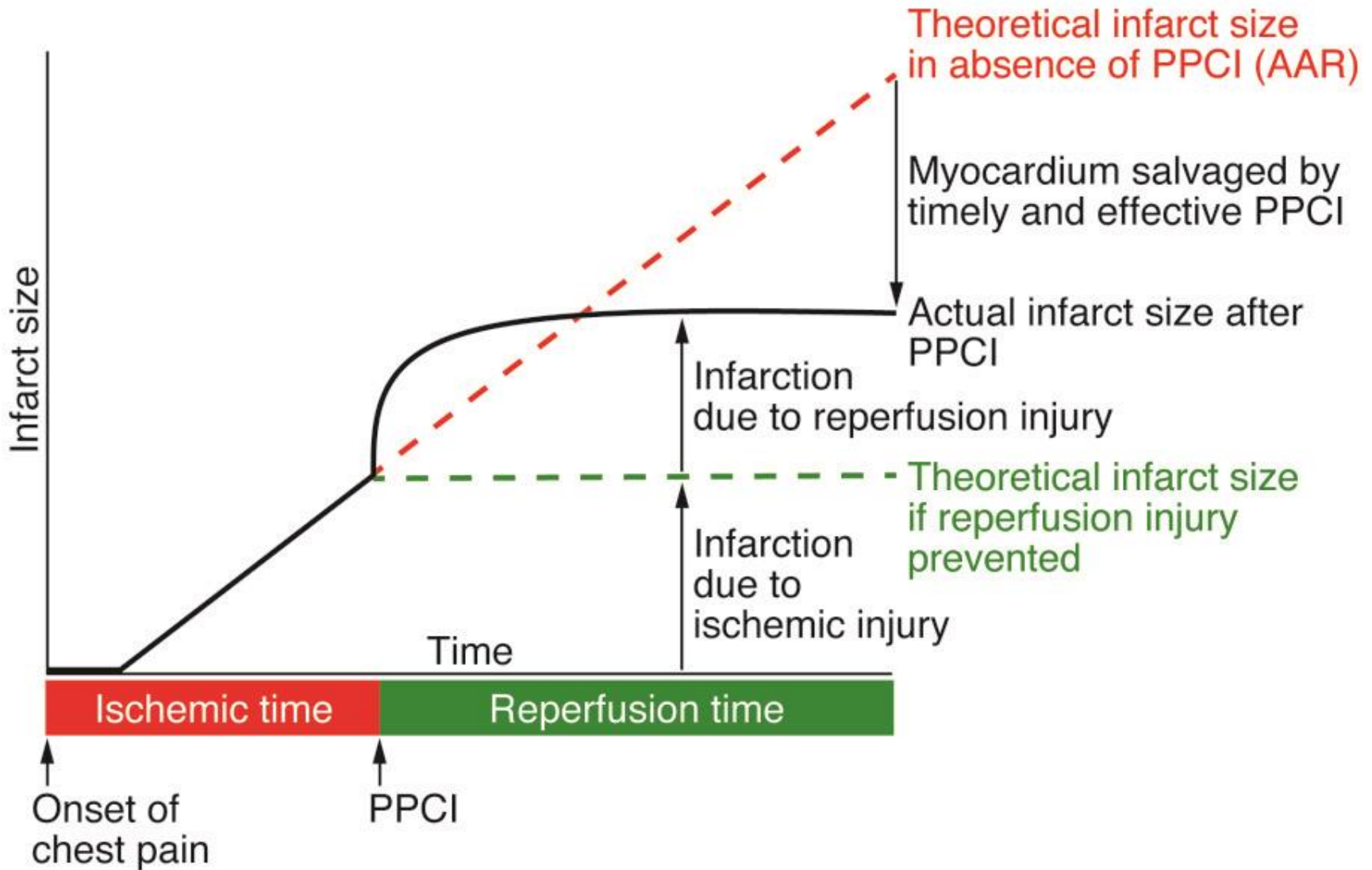
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Top 10 Global Causes of Death, 2016



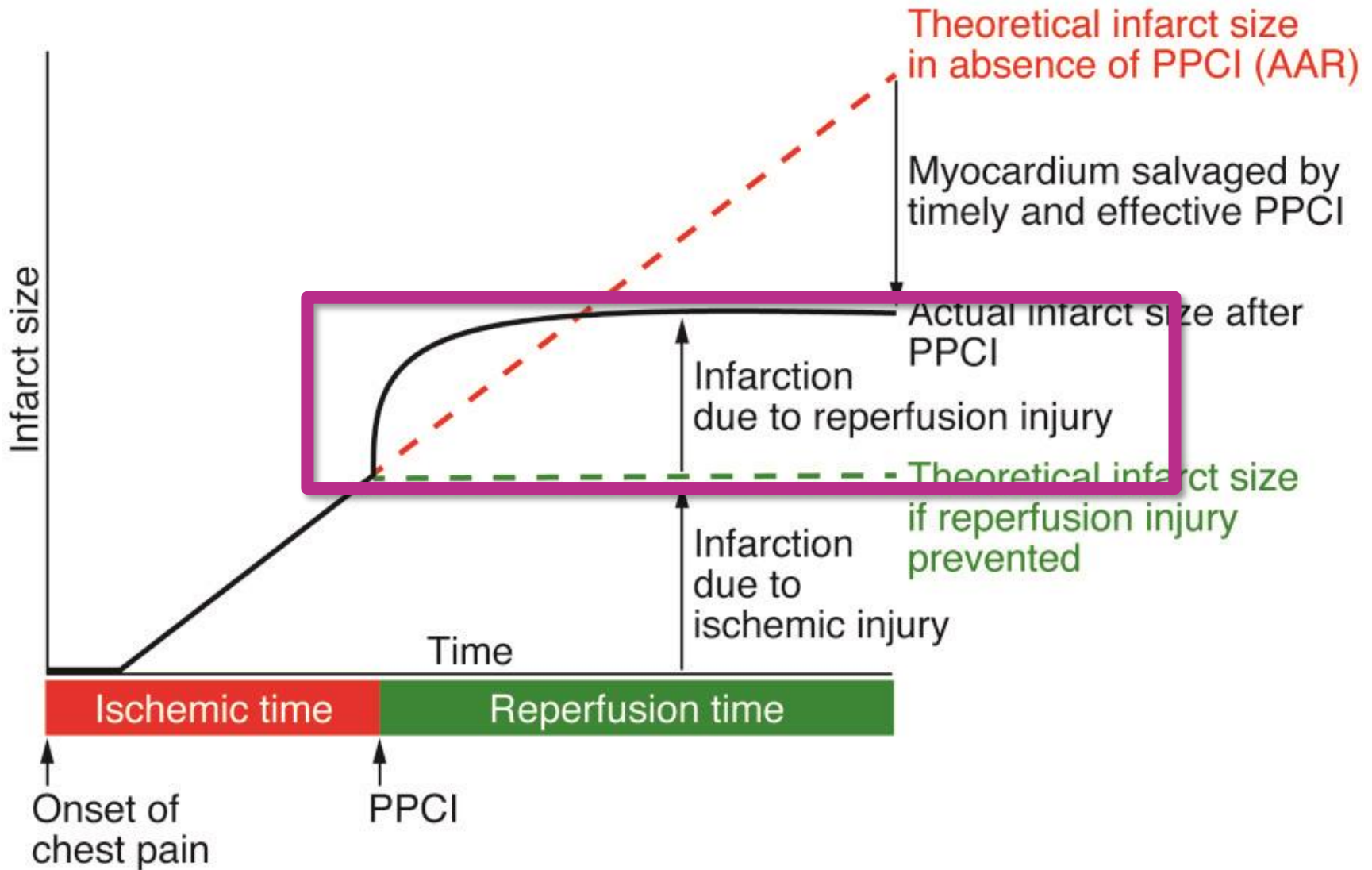
Source: Global Health Estimates 2016: Deaths by Cause, Age, Sex, by Country and by Region, 2000-2016. Geneva, World Health Organization; 2018.

Ischemia-Reperfusion Injury



(Hausenloy, D. J. and Yellon, D. M. 2013 J Clin Invest.)

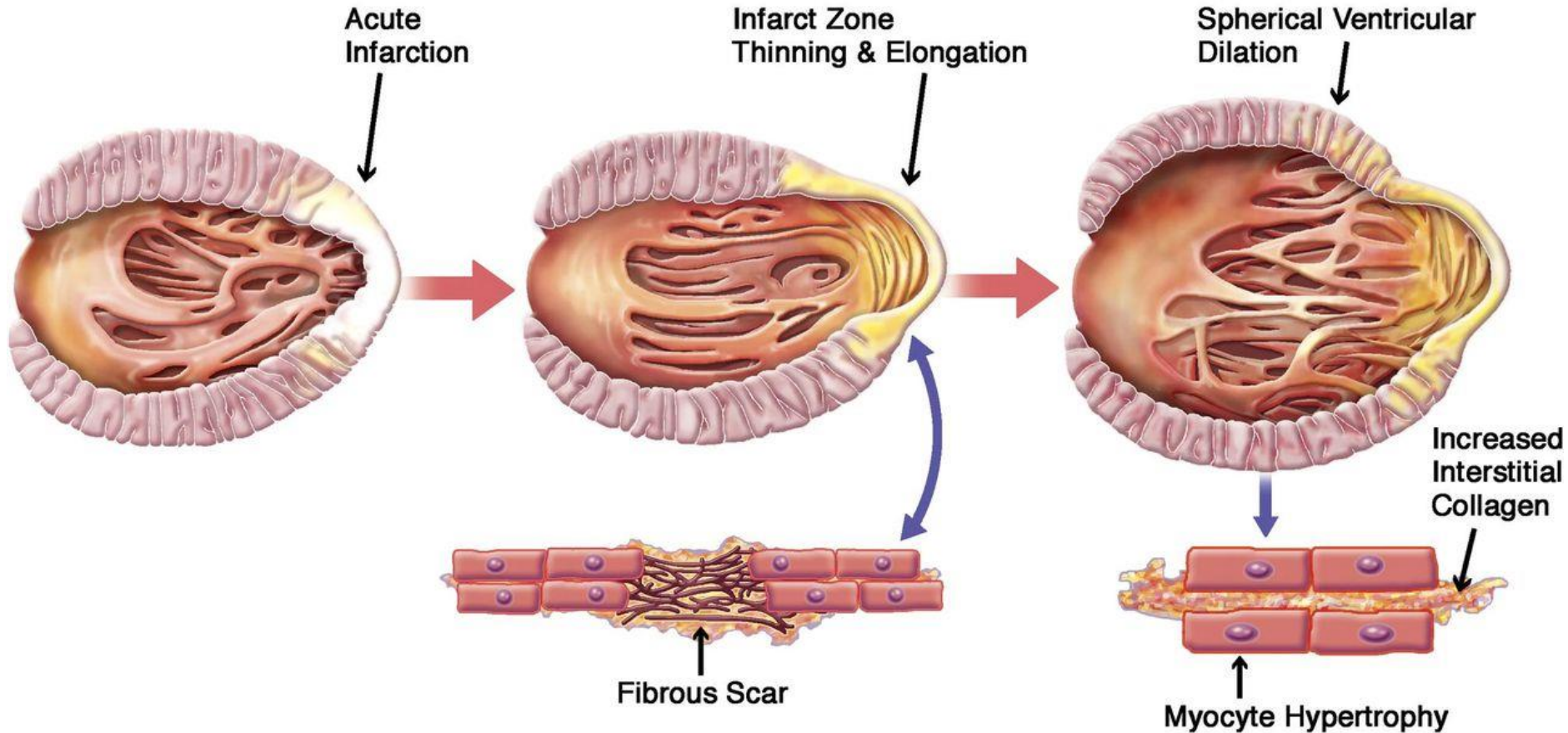
Ischemia-Reperfusion Injury



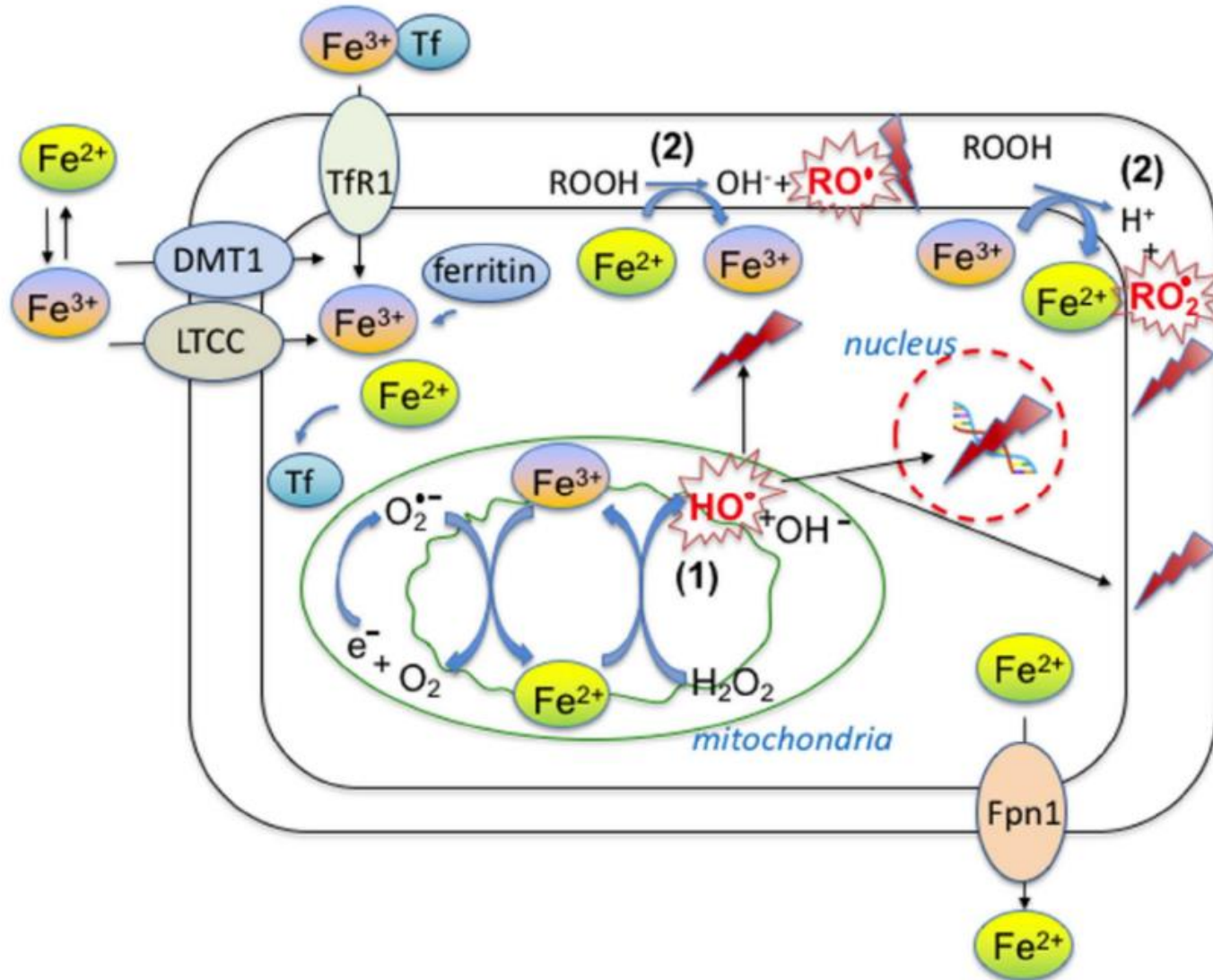
(Hausenloy, D. J. and Yellon, D. M. 2013 J Clin Invest.)

Left Ventricular Remodeling

POST-MI REMODELING

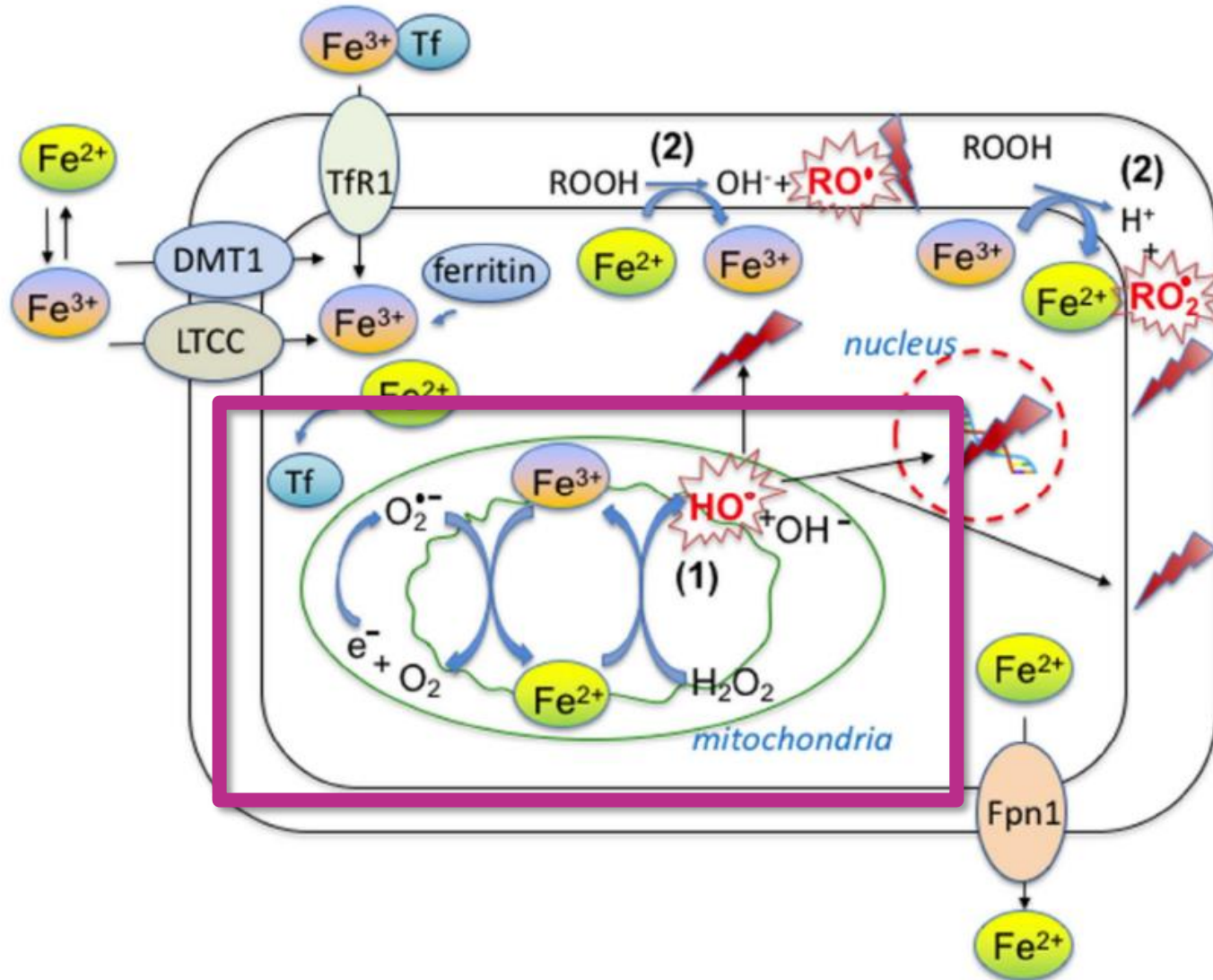


Role of Excess Iron



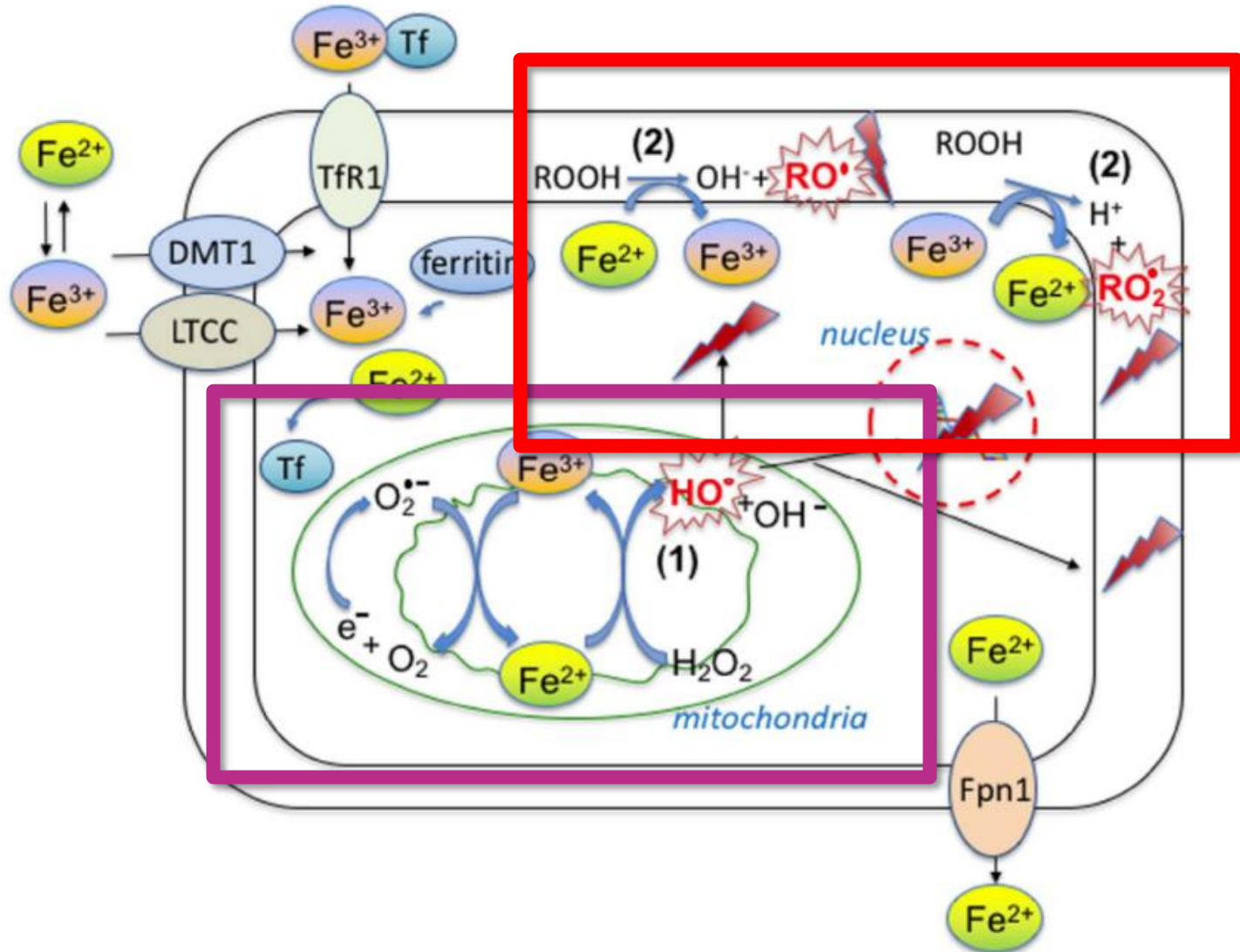
(Kobayashi, M., et al. 2018 Curr Drug Targets)

Role of Excess Iron



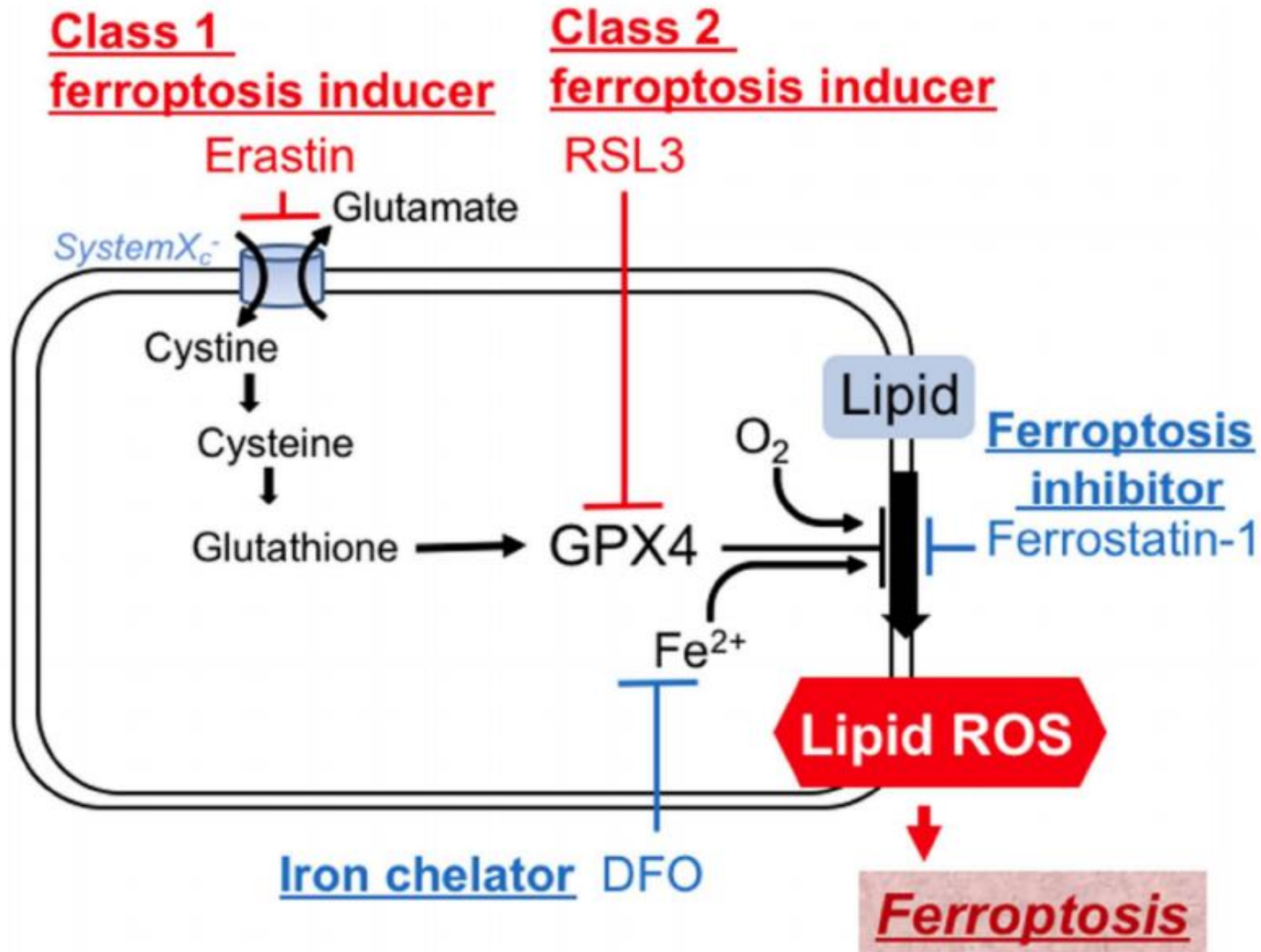
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Role of Excess Iron

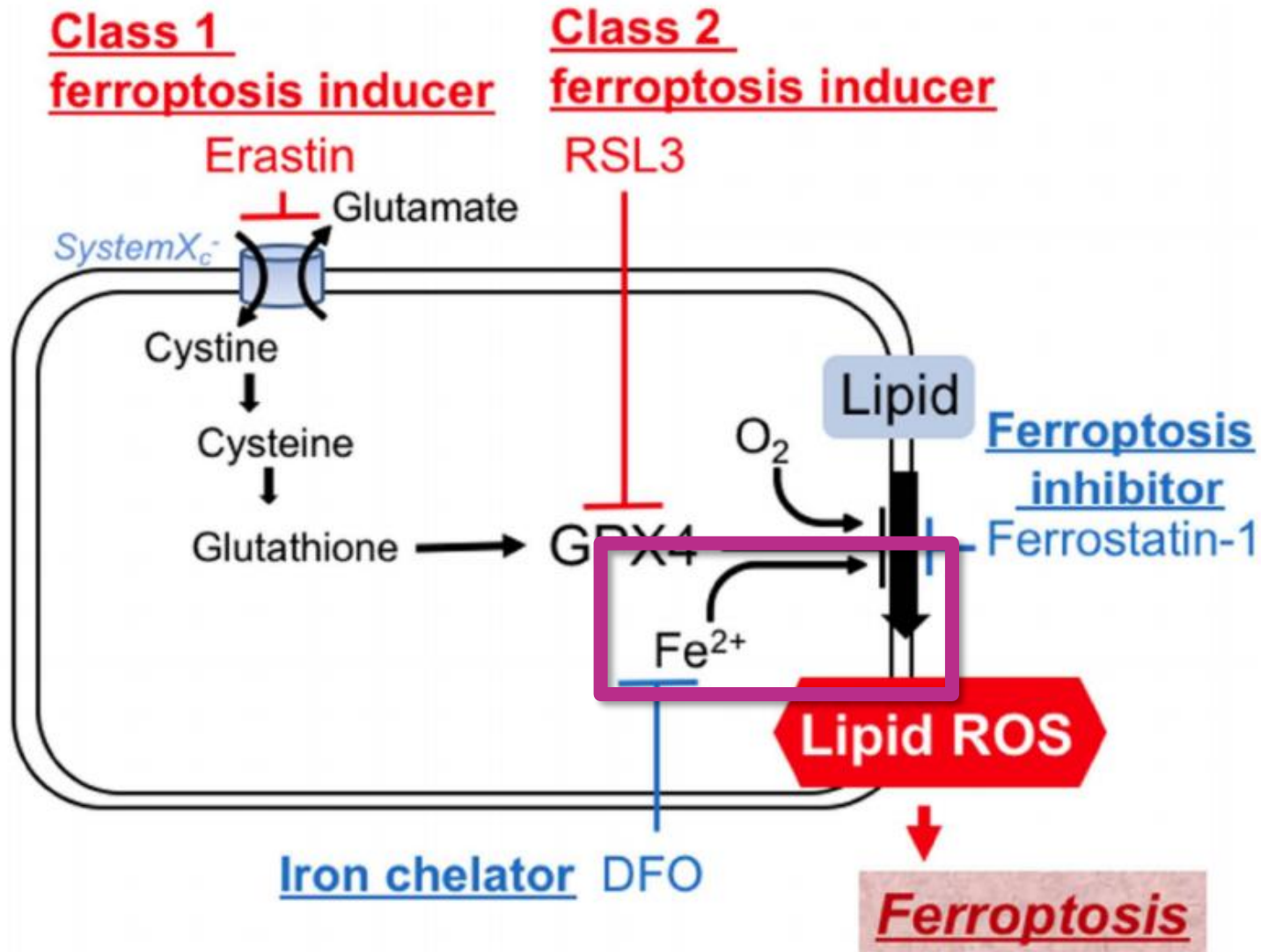


(Kobayashi, M., et al. 2018 Curr Drug Targets)

Ferroptosis

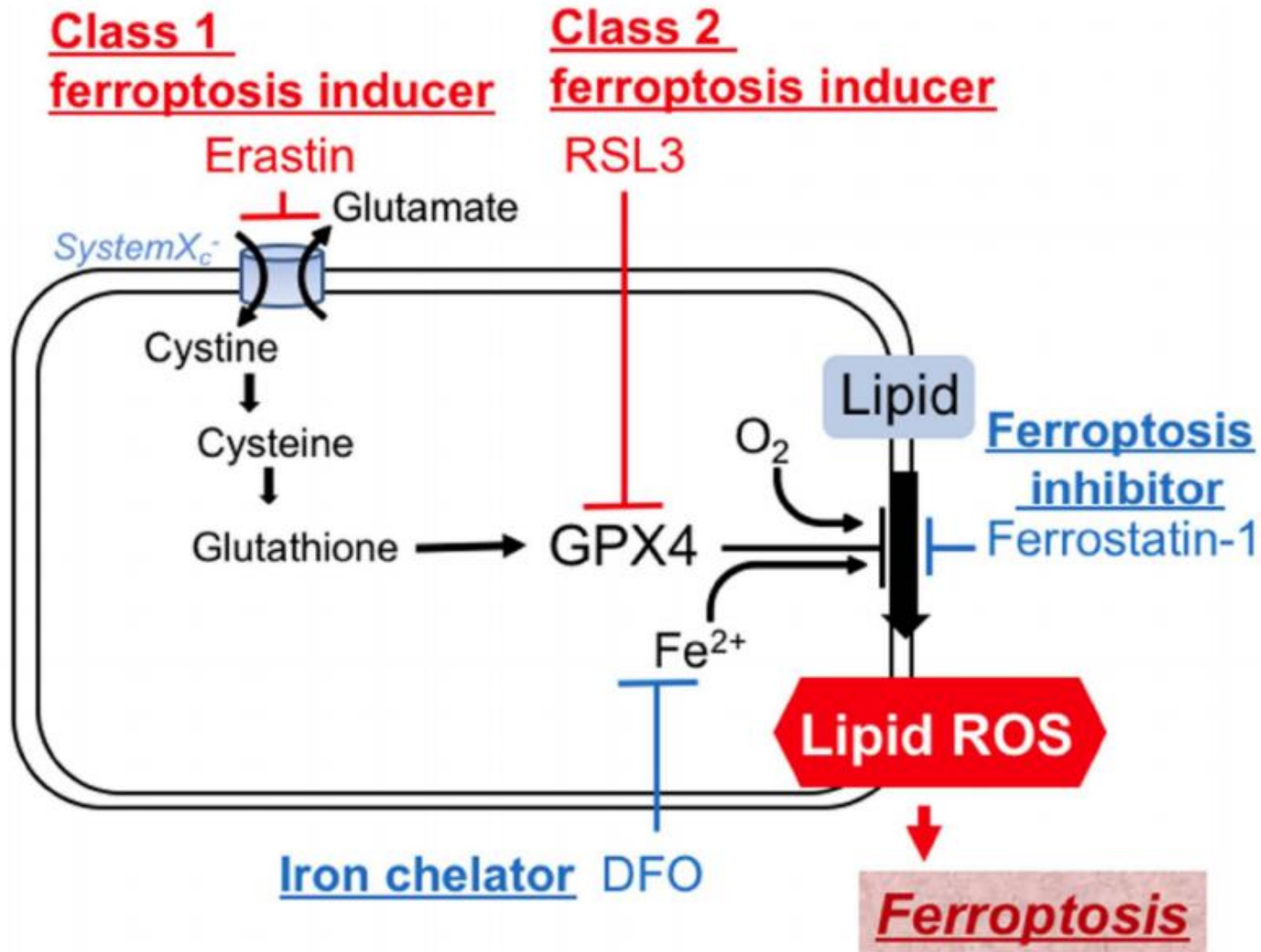


Ferroptosis

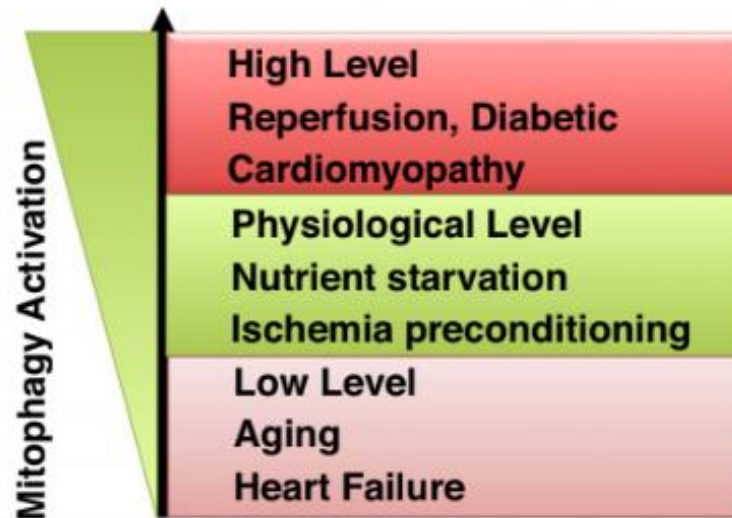
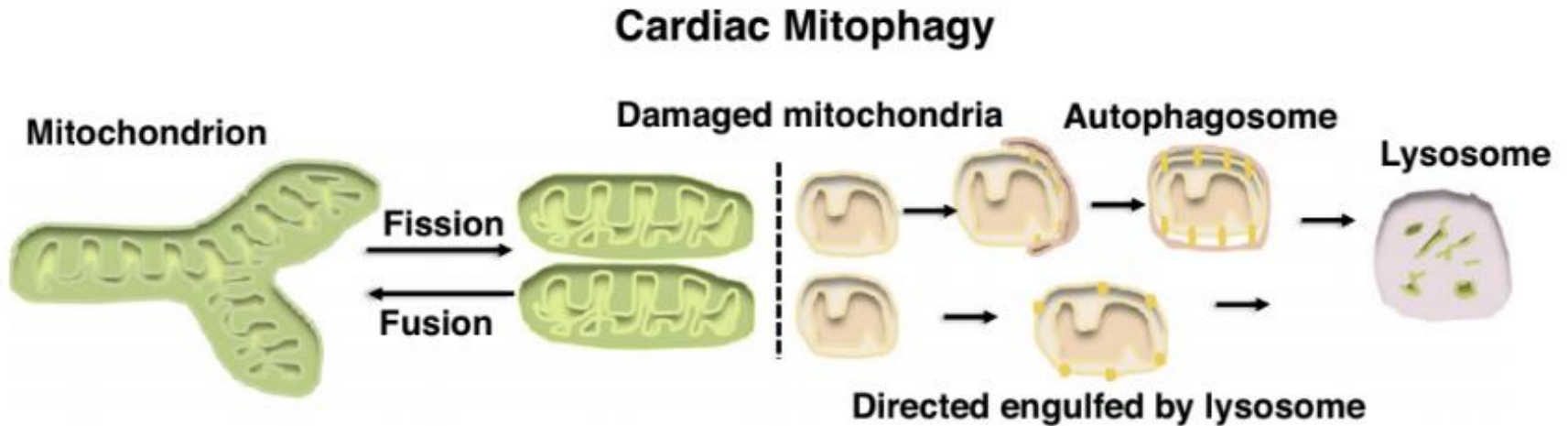


(Kobayashi, M., et al. 2018 Curr Drug Targets)

Ferroptosis



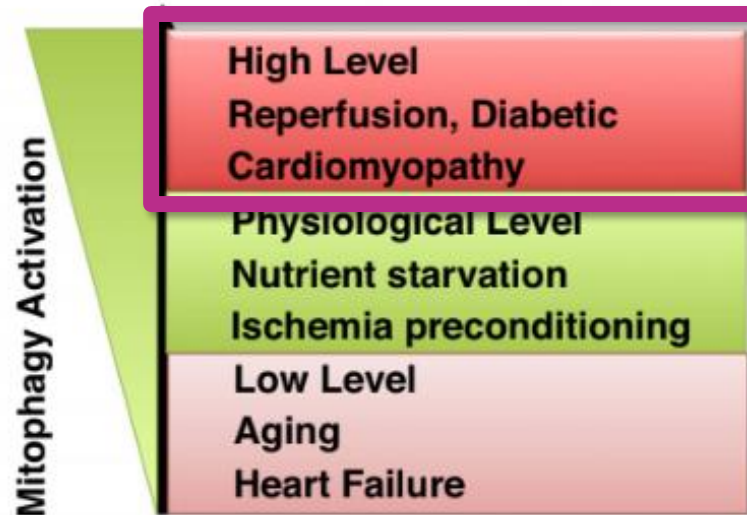
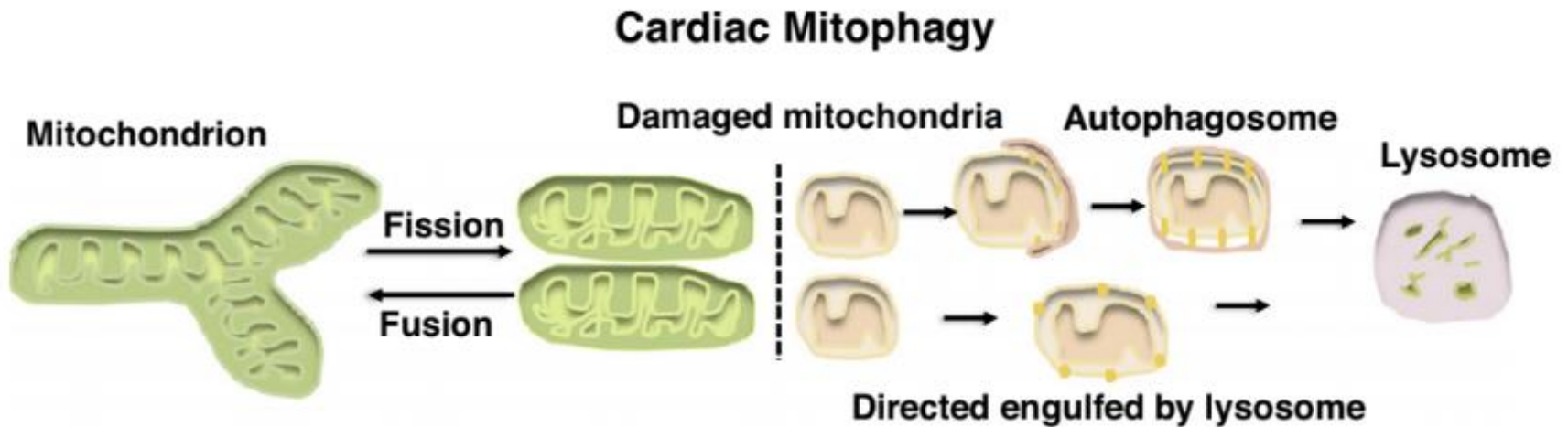
Mitophagy



Current Opinion in Genetics & Development

(Tong, M., and Sadoshima, J. 2016 Curr Opin Genet Dev.)

Mitophagy



Current Opinion in Genetics & Development

(Tong, M., and Sadoshima, J. 2016 *Curr Opin Genet Dev.*)

Mito-Keima



Lysosome



Green fluorescence

Isolation membrane



Mitophagosome



Red fluorescence

Objective

**To study the relationship
between ferroptosis and mitophagy
using Mito-Keima**

Methods

H9c2 cells cultured to 90% confluency and transferred to 12-well plates

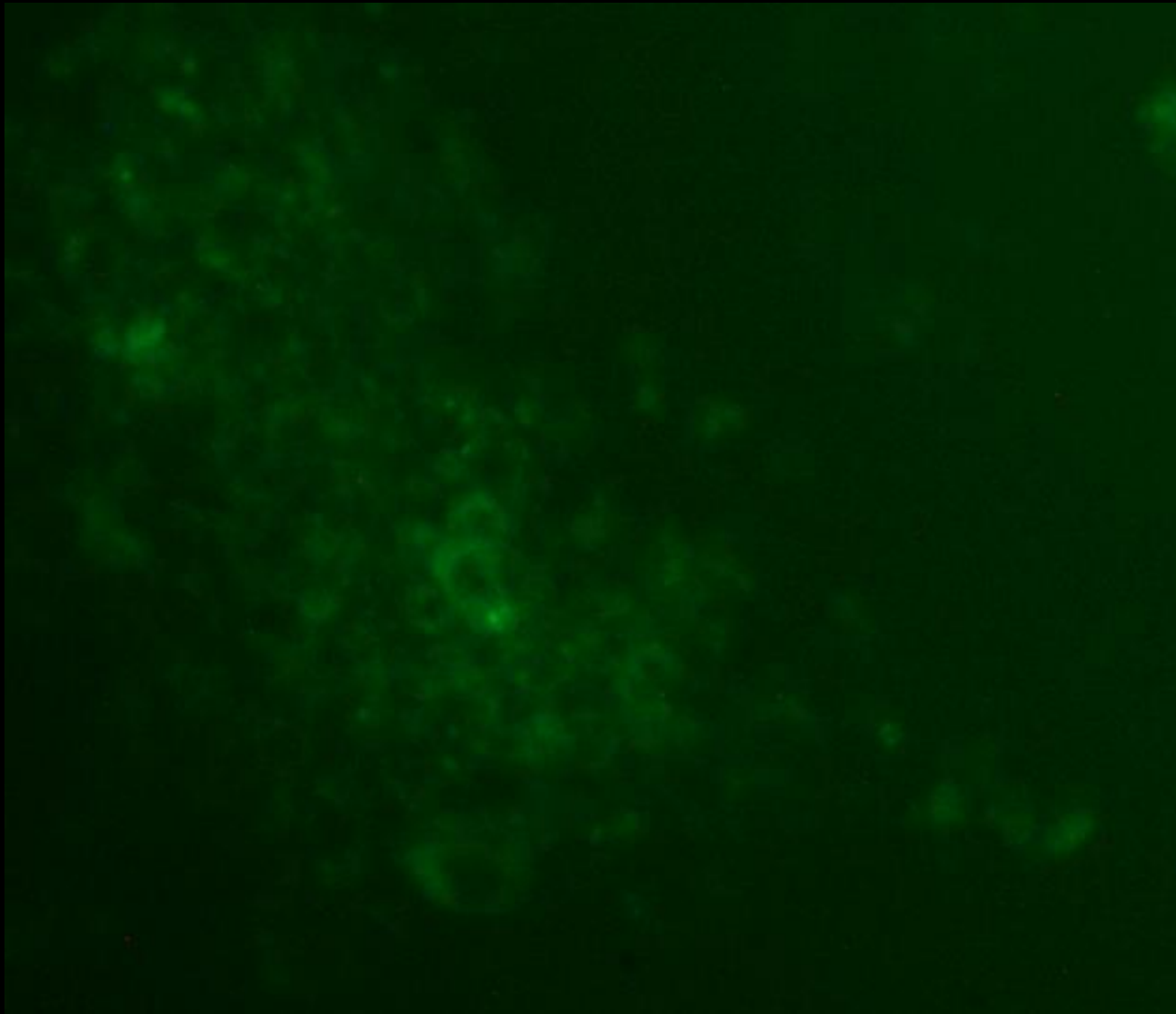
- Transfected with 10 μ L adenovirus carrying Mito-Keima for 24 hours

Induce cell death

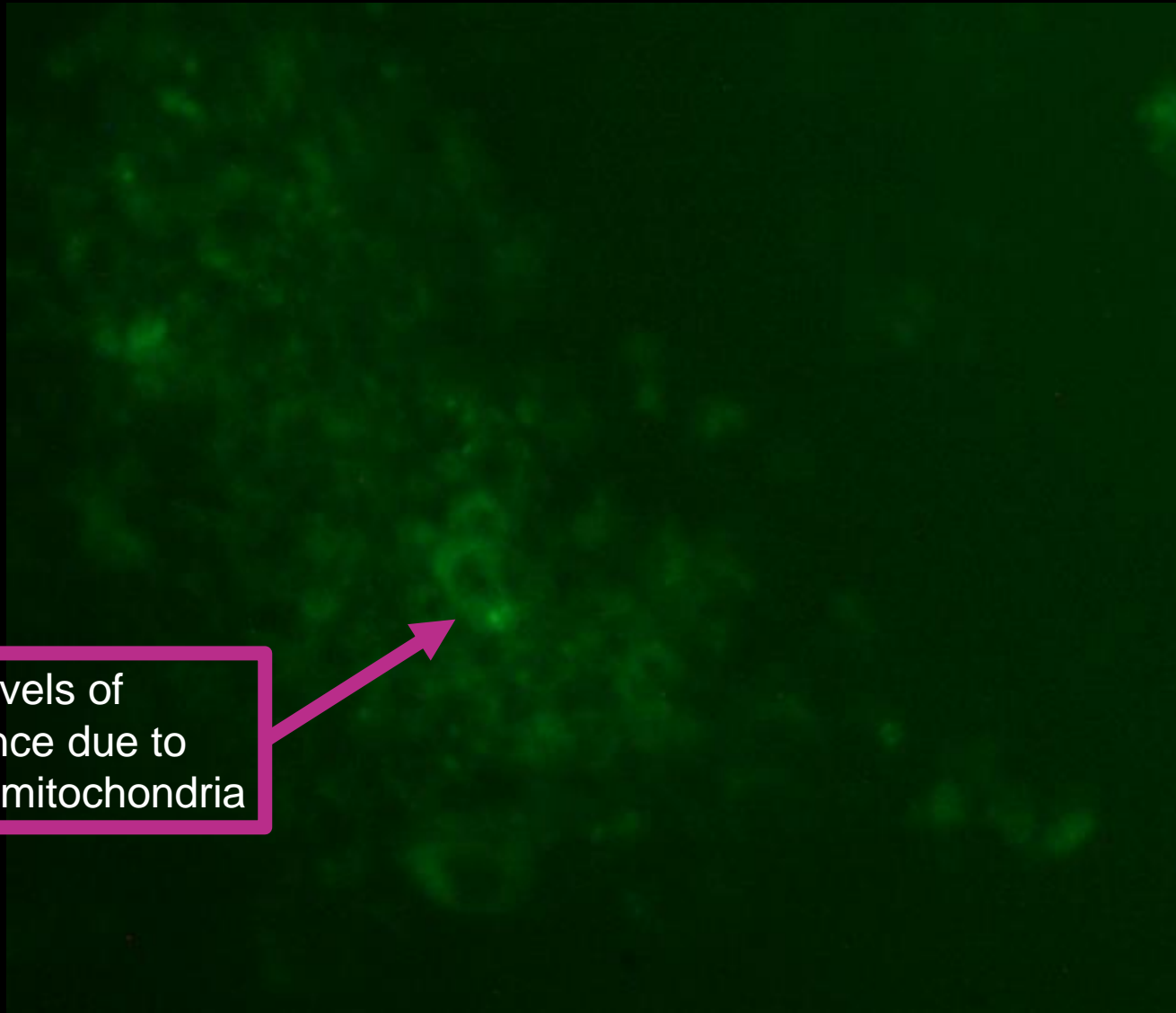
- Starvation for 24 hours (**mitophagy**)
- 100 ng/mL RSL3 for 5 hours (**ferroptosis**)
- 10 mM Fe (III)-citrate for 24 hours (**lipid ROS**)

Fluorescent microscopy used to detect occurrence of Mito-Keima

Mito-Keima Localized in Mitochondria

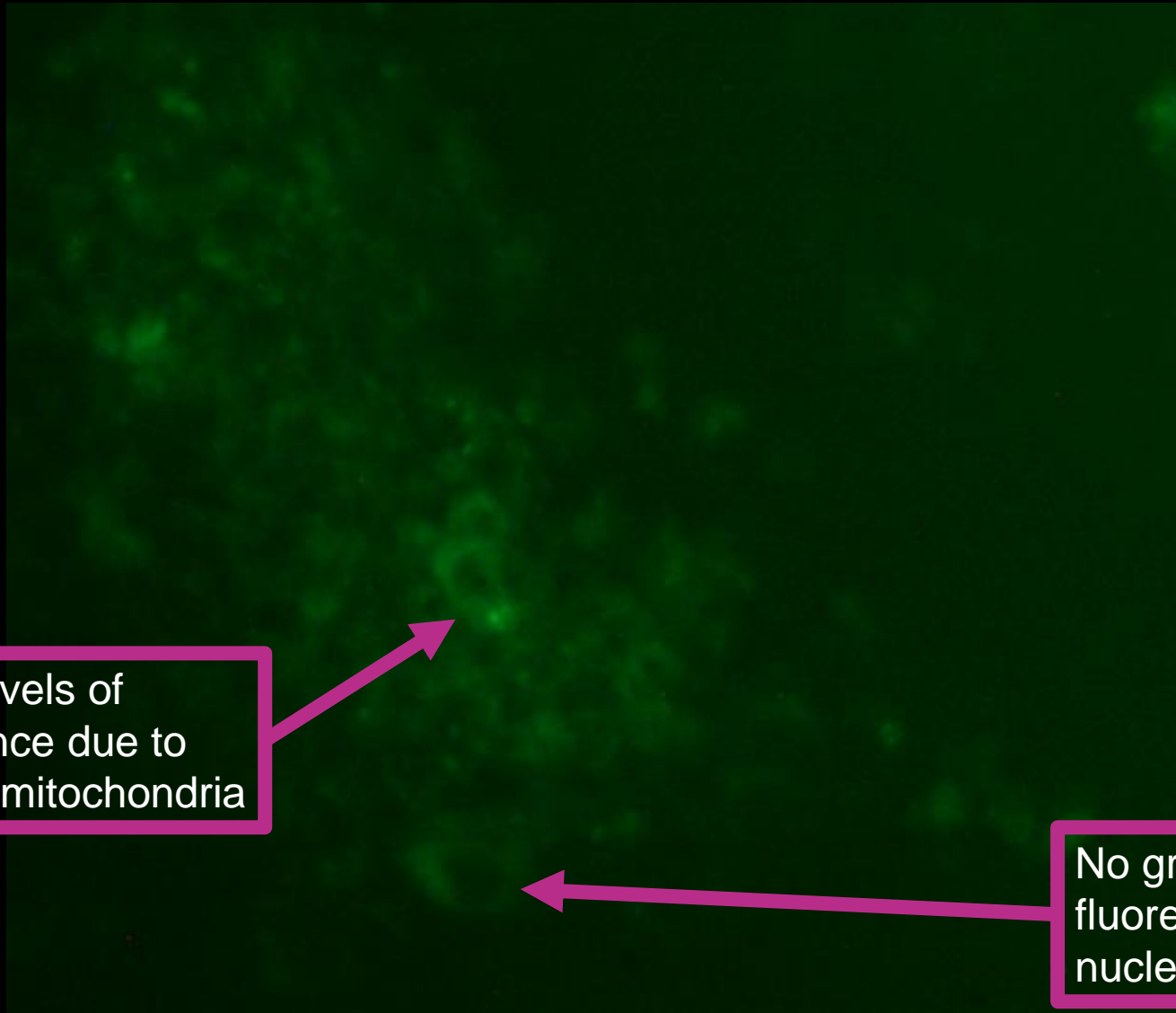


Mito-Keima Localized in Mitochondria



Varying levels of fluorescence due to scattered mitochondria

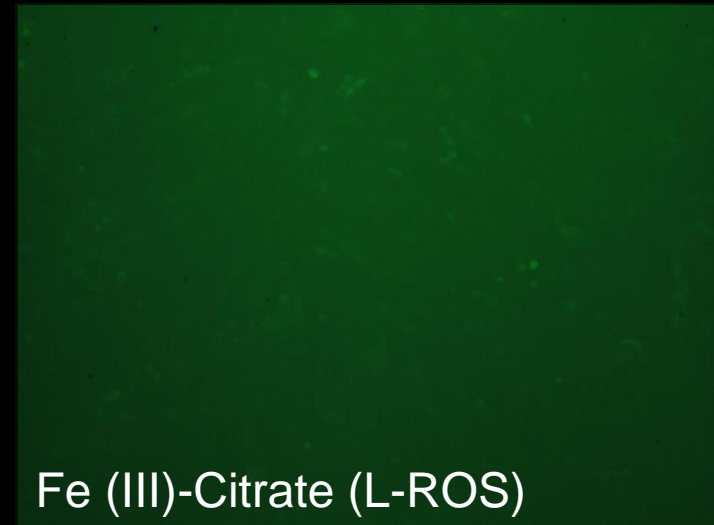
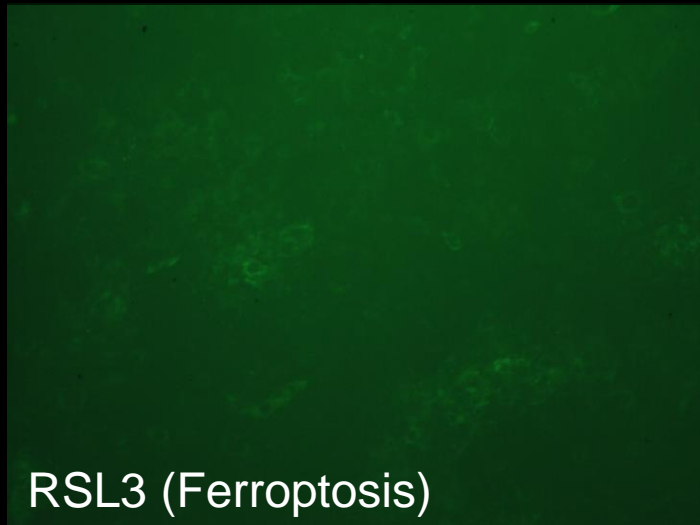
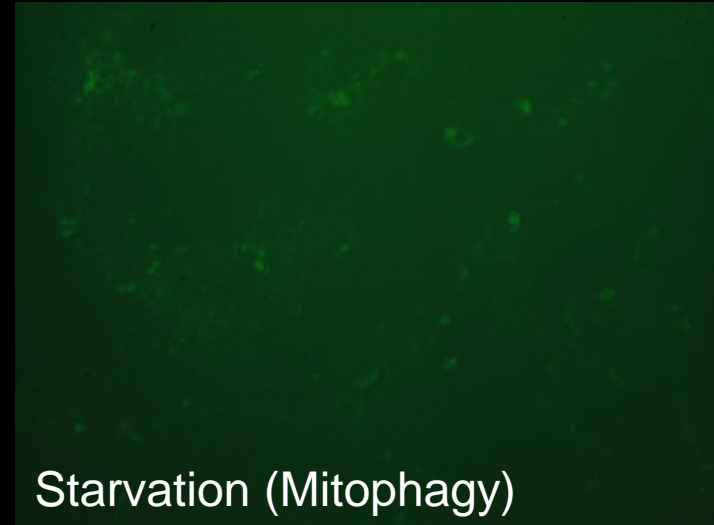
Mito-Keima Localized in Mitochondria



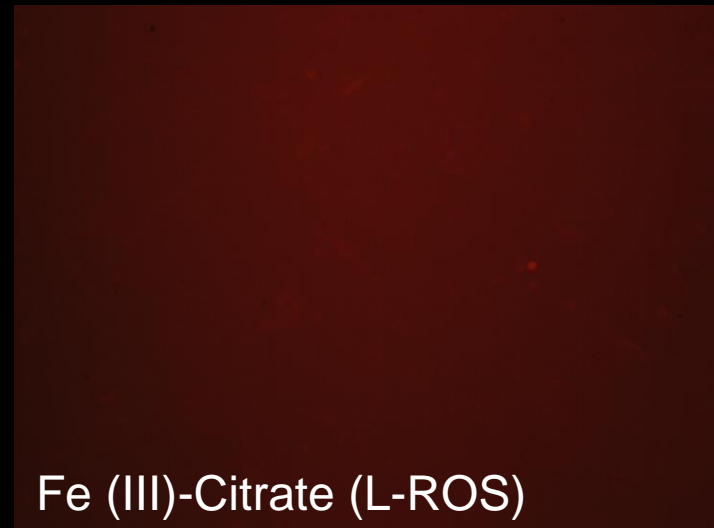
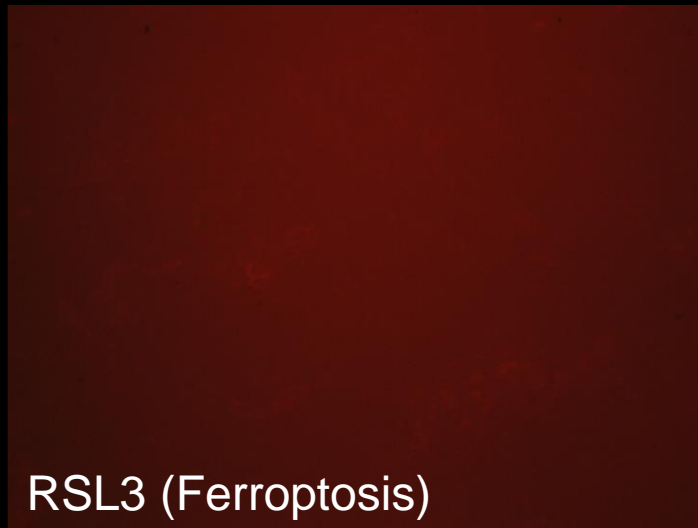
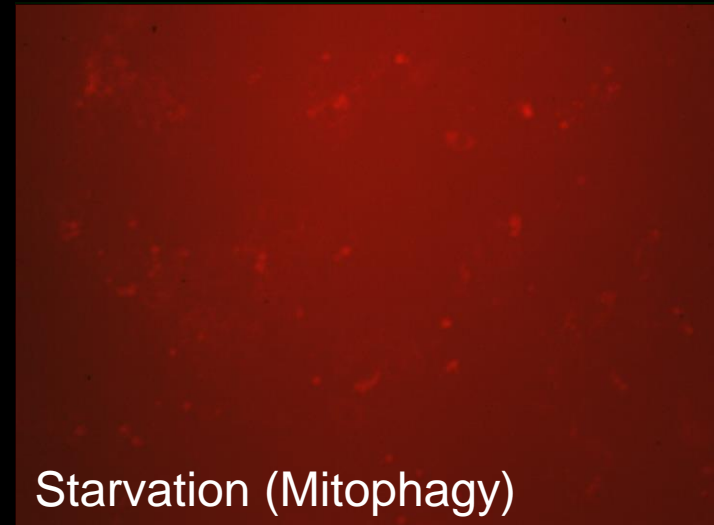
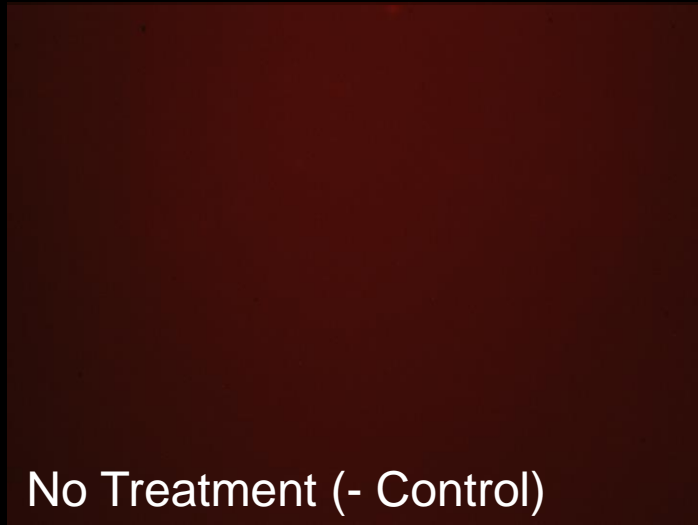
Varying levels of fluorescence due to scattered mitochondria

No green fluorescence in nucleus

Gene Transfer Status



Mitophagy Status



Results

Mito-Keima demonstrated treatment with RSL3 and Fe (III)-citrate induced **lower red fluorescence compared to treatment with starvation**

Conclusion

Mitophagy is a **distinct** process from ferroptosis

Further study of ferroptosis and mitochondria is required

Acknowledgements

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