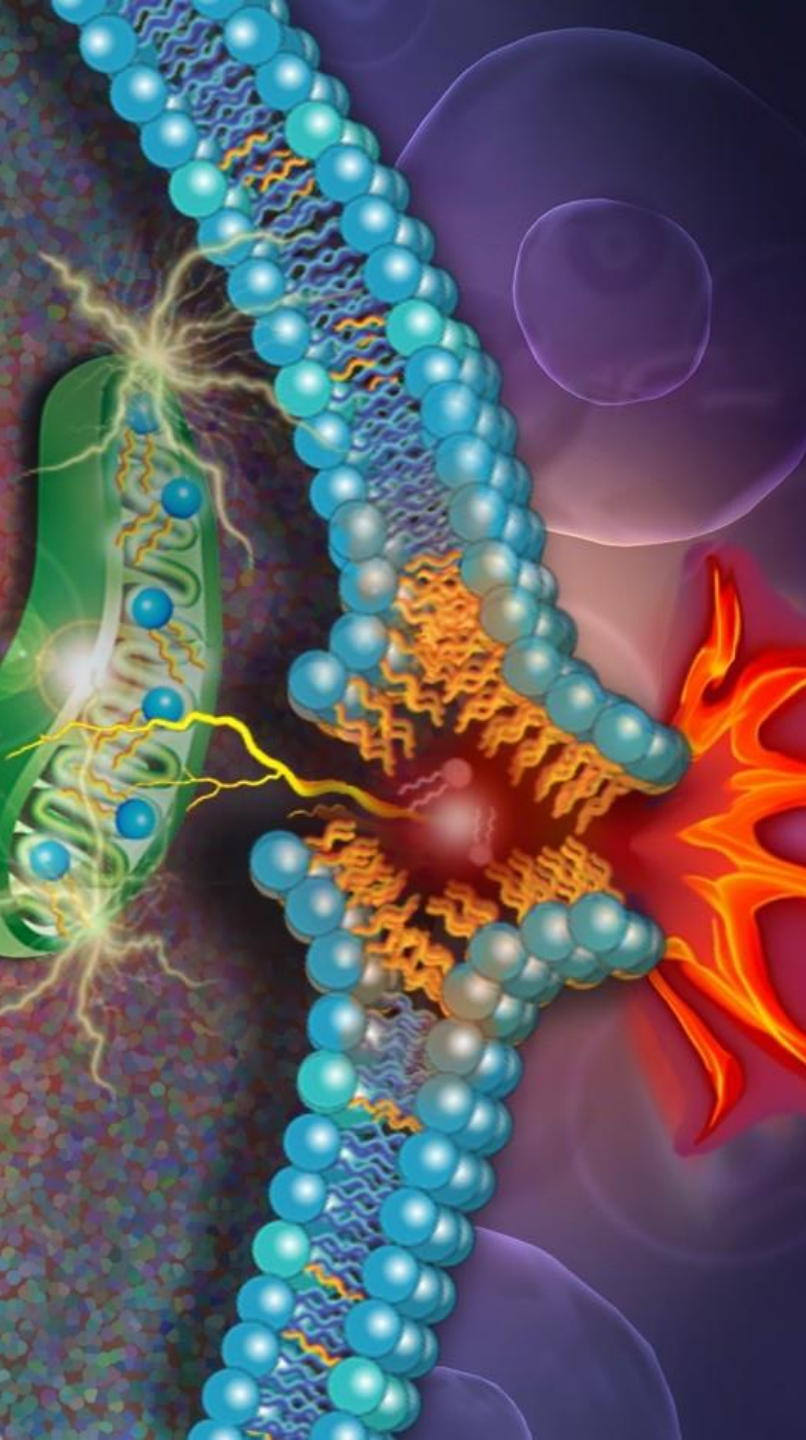




# **Ferroptosis**

## **Roles in Pathogenesis and Treatment**

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# Outline

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- **Overview of Thalassemia and Iron overload**
- **Principles of Ferroptosis**
- **The Evidence of Ferroptosis in Iron Overload Models**
- **Limitations and Future perspectives**

# β-thalassemia and Iron overload



- **β-thalassemia: iron overload is one of the most common disease-related complications**

- ↑ Morbidity and mortality
- ↓ Quality of life

## Cardiac T2\* MRI (ms)

### TDT

- <6-10 vs ≥6-10: increased risk of heart failure within 1 year (HR: 160-270)
- <20 vs ≥20: increased risk of arrhythmia within 1 year (HR: 4.6)
- <27 vs ≥27: increased risk of all-cause (adj HR: 8.6) and cardiovascular disease-related (adj HR: 8.6) death over 10 years
- <34 vs ≥34: increased risk of cardiac mortality or hospitalization (HR: 11.7)

## Serum ferritin (ng/mL)

### NTDT

- ≥800: 10-year morbidity free survival 0%
- ≤300: 10-year morbidity free survival 100%
- <800 vs ≥800: increased overall risk of death (adj HR: 9.8)

### TDT

- >1000 vs <1000: increased risk of heart failure (adj HR: 3.2) and death (adj HR: 2.6)
- >1700 vs ≤1700: increased risk of cardiac mortality or hospitalization (HR: 7.8)
- >2145 vs ≤2145: increased risk of all-cause death over 10 years (adj HR: 7.1)
- >2500 vs <2500: increased risk of heart failure (adj HR: 4.8) and death (adj HR: 4.4)
- >2500 vs ≤1000: increased odds of endocrine disease (adj OR: 2.75-3.53)
- >3265 vs ≤3265: increased risk of cardiovascular disease-related death over 10 years (adj HR: 37.2)
- Increase by >1339: increased risk of all-cause death over 10 years (adj HR: 21.5)
- Increase by >765: increased risk of cardiovascular disease-related death over 10 years (adj HR: 19.6)

## Liver iron concentration (mg/g)

### NTDT

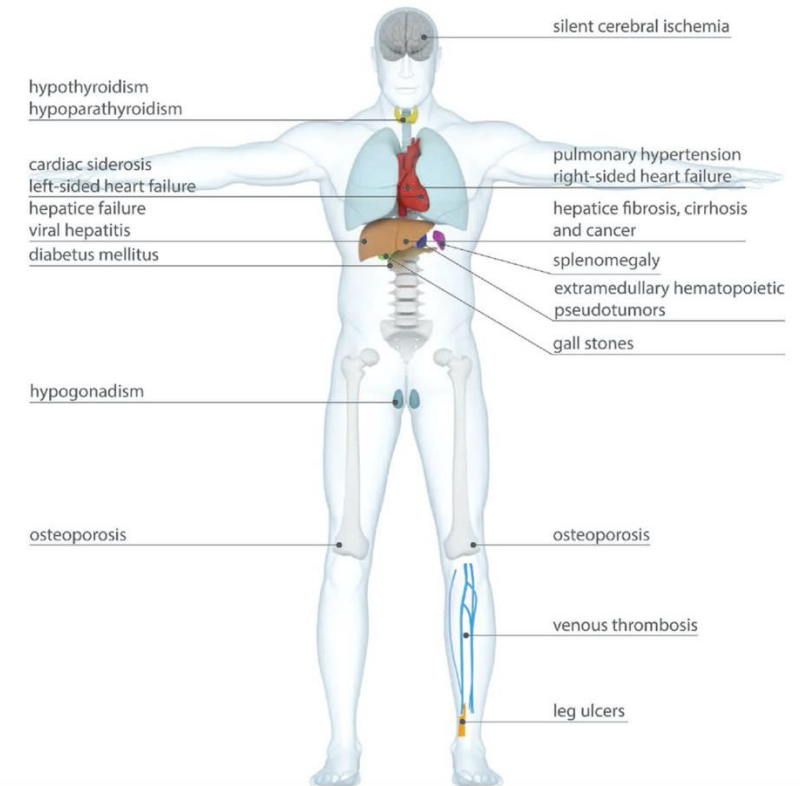
- ≥5 vs <5: increased odds of any morbidity (adj OR: 3.76)
- ≥6 vs <6: best predictor for increased odds of vascular disease (adj OR: 3.76)
- ≥7 vs <7: best predictor for increased odds of endocrine/bone disease (adj OR: 4.05)
- Increase by ≥1: increased odds of morbidity

### TDT

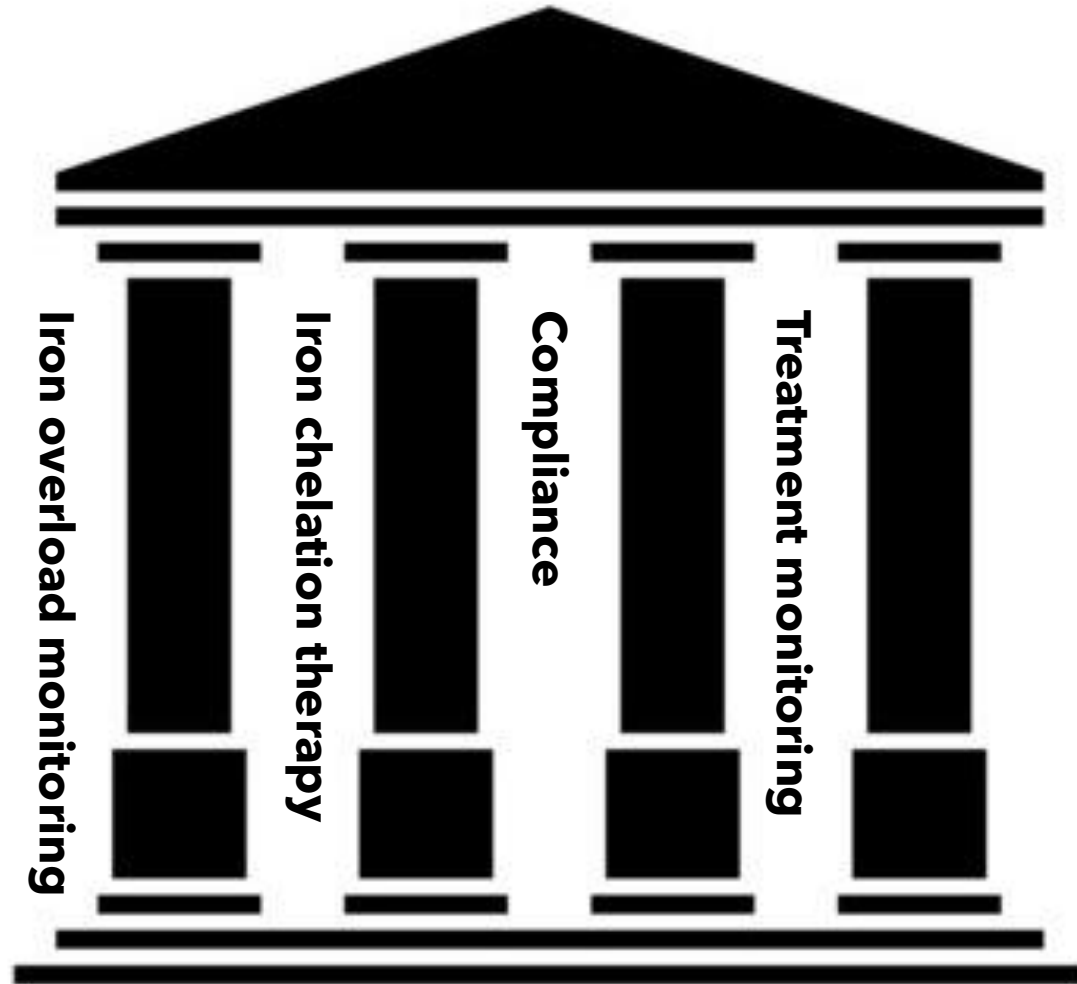
- >15 vs 7-15 vs <7: lower cardiac-disease free survival probability (50% vs 71.4% vs 93.3%)
- >8 vs ≤8: increased risk of all-cause (adj HR: 20.2) and cardiovascular disease-related (adj HR: 34.1) death over 10 years
- >16: predictor of hepatic fibrosis progression post bone marrow transplantation (HR for patients with values 12.8-40.6: 8.7)
- Increase by >1.4: increased risk of all-cause (adj HR: 27.6) and cardiovascular disease-related (adj HR: 20.3) death over 10 years

## TDT

## NTDT



# The Change in Philosophy of Iron Chelation Therapy

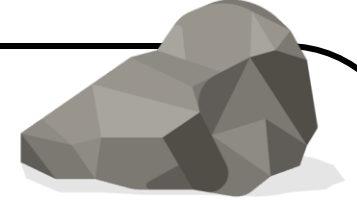


Preventive strategy

Maintenance of safe iron body stores

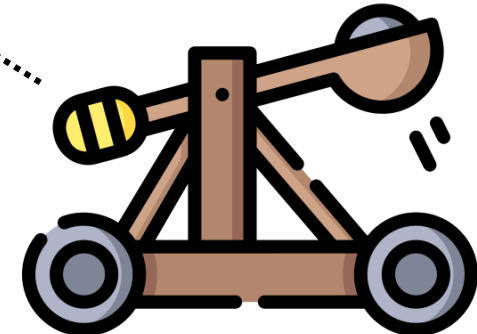
## Iron chelation therapy

- The non-responder
- Drug intolerance and contraindication
- Slow iron clearance from organ deposition



## Iron overload monitoring

- Accessibility
- Method variation and validation



# The Change in Philosophy of Iron Chelation Therapy



## Explore the new pathogenesis

1

### Novel therapies

- Adjunctive treatment

Iron chelator



Iron

Drug X



Cell death

2

### Novel biomarkers

- Novel iron overload monitoring
- Predicting the organ damage

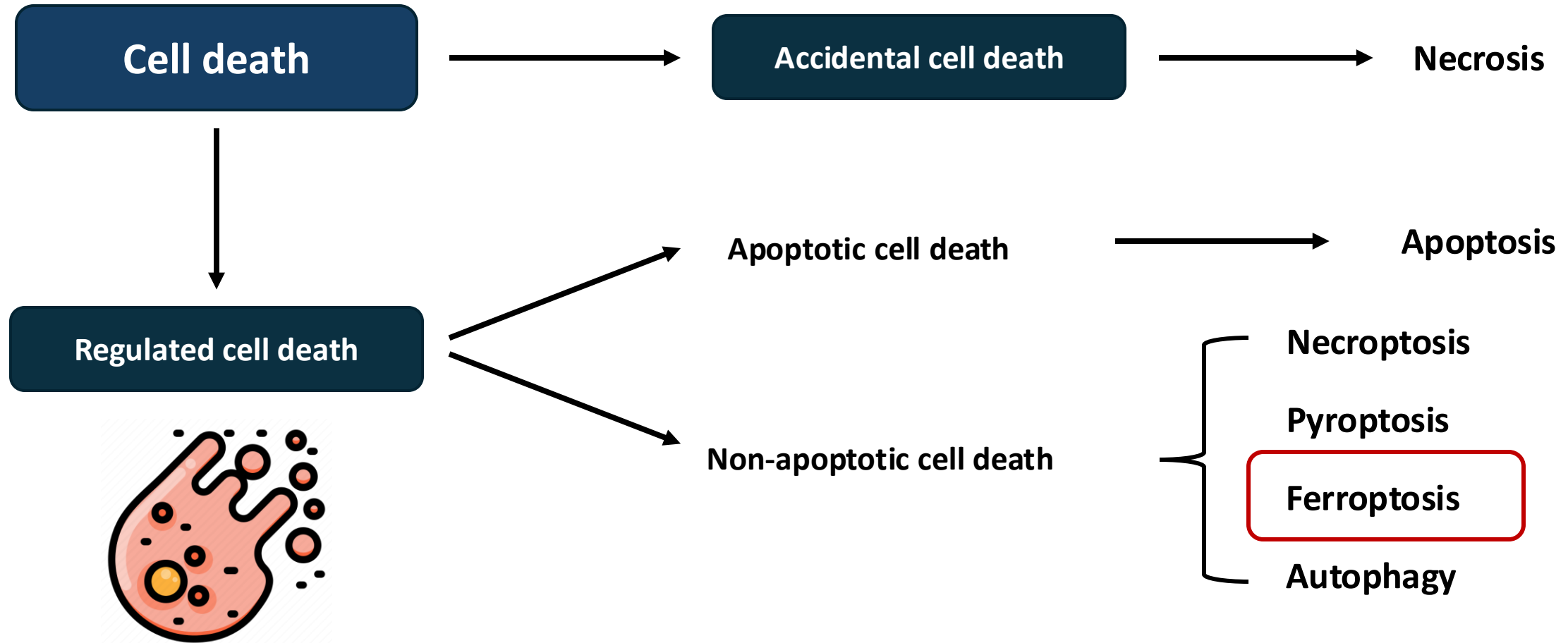
### Iron chelation therapy

- The non-responder
- Drug intolerance and contraindication
- Slow iron clearance from organ deposition

### Iron overload monitoring (MRI)

- Accessibility
- Method variation and validation

# Ferroptosis: Novel Mechanism of Iron-induced Organ Dysfunction

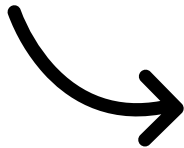


From several evidences: **ferroptosis** play a critical role in **iron overload-related organ dysfunction**

# Ferroptosis: Iron-Regulated Programmed Cell Death



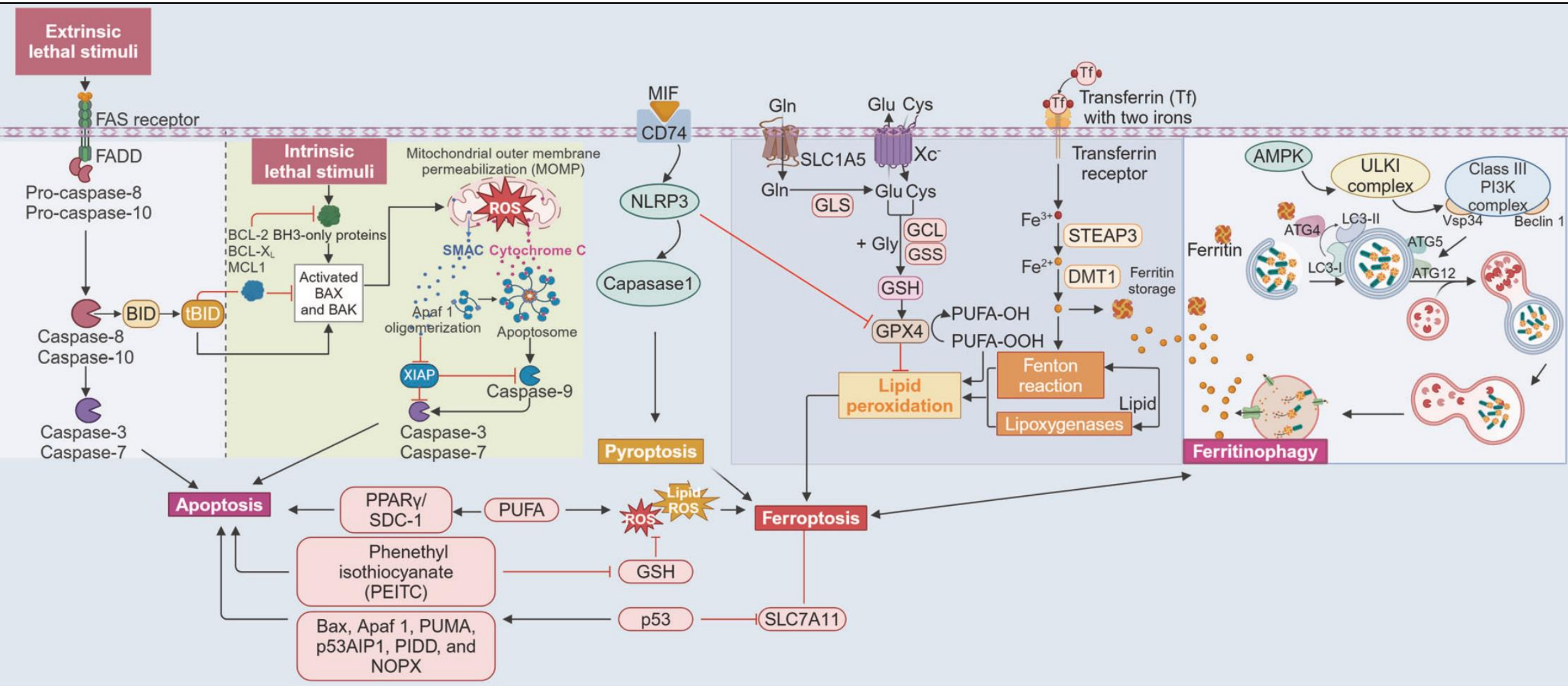
- **Non apoptotic form of iron-regulated programmed cell death**
- **Distinct characteristics compared to other programmed cell death**
  - **Biochemical features**, morphological features, and regulatory genes



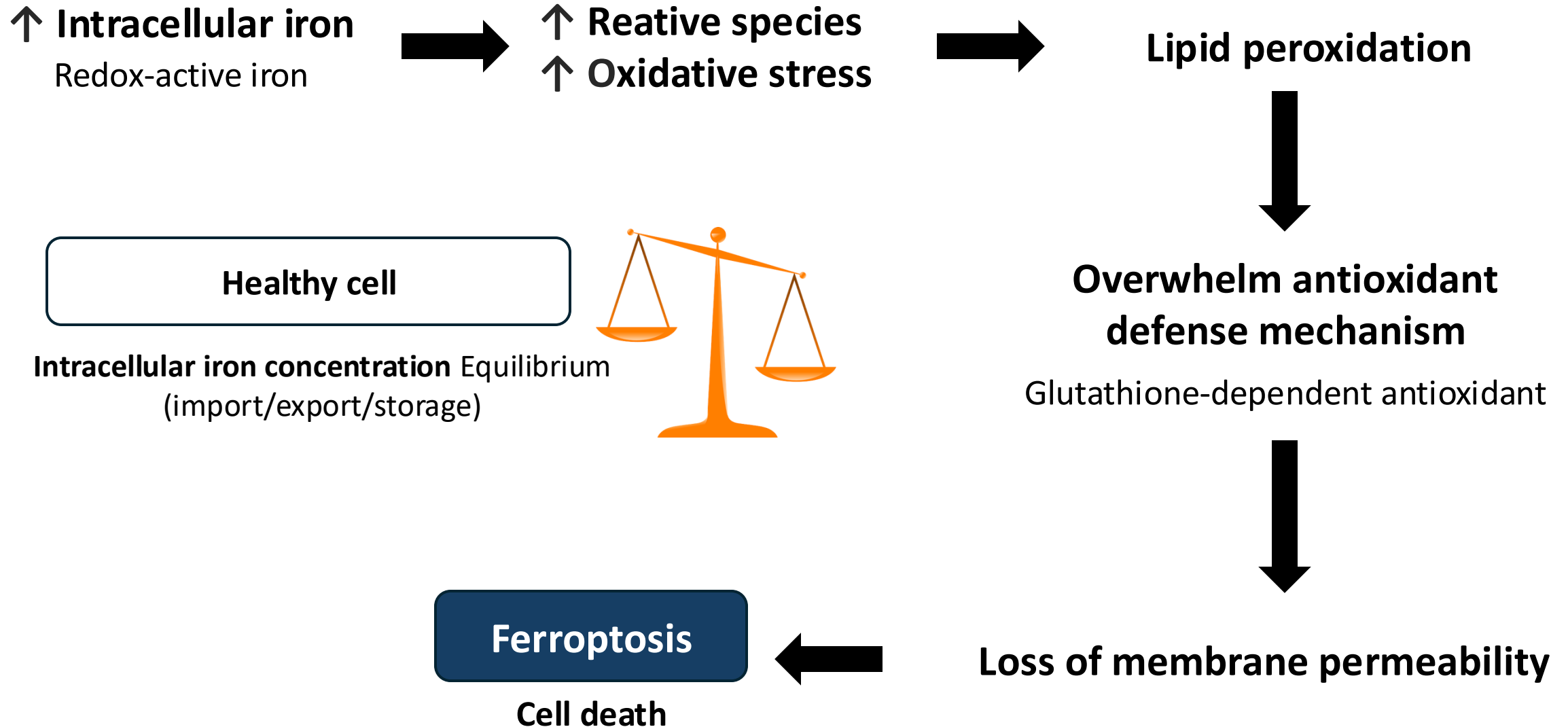
- **Dysregulation of intracellular iron**
- **Imbalance of antioxidant system**
  - ↓GPX4, ↓GSH
- **Accumulation of lipid peroxides**

- **Iron = essential part of driving lipid peroxidation and ferroptosis**
  - Associated with tissue damage driven by iron overload
  - Specifically rescued using an iron chelator

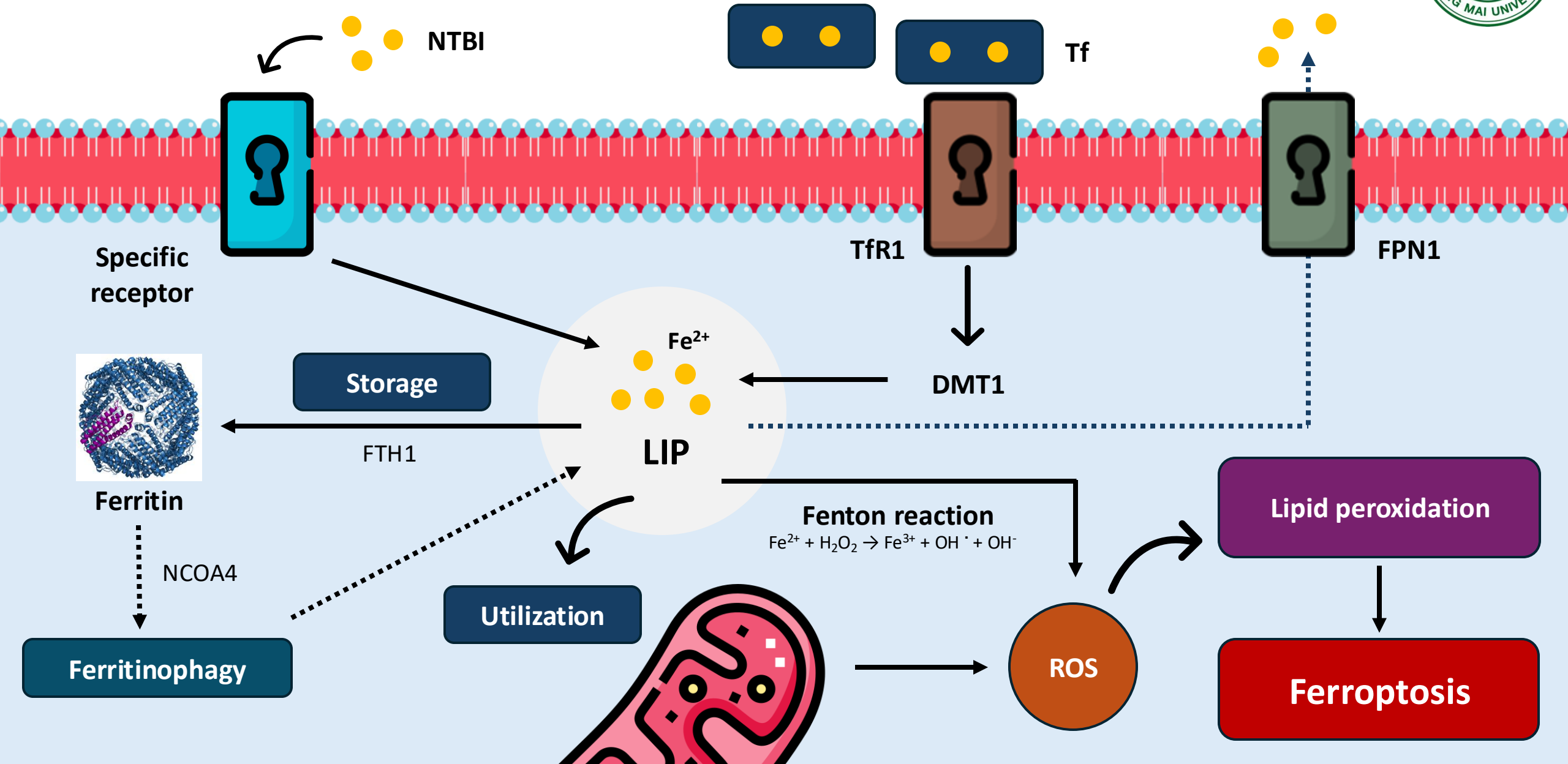
# The Crosstalk of Ferroptosis, Apoptosis, Autophagy, and Pyroptosis



# Ferroptosis: Iron-Regulated Programmed Cell Death



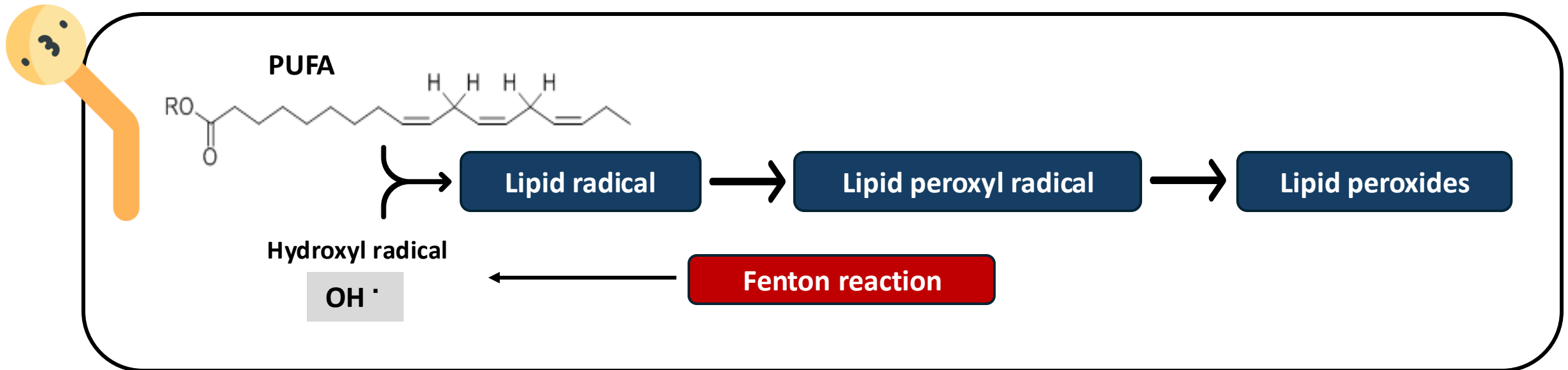
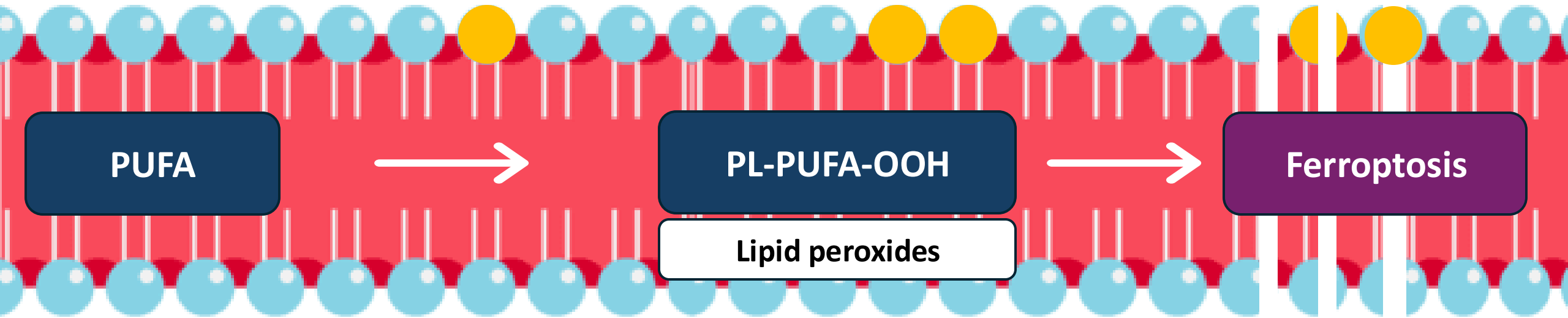
# Ferroptosis: ↑ Intracellular Iron and Oxidative Stress



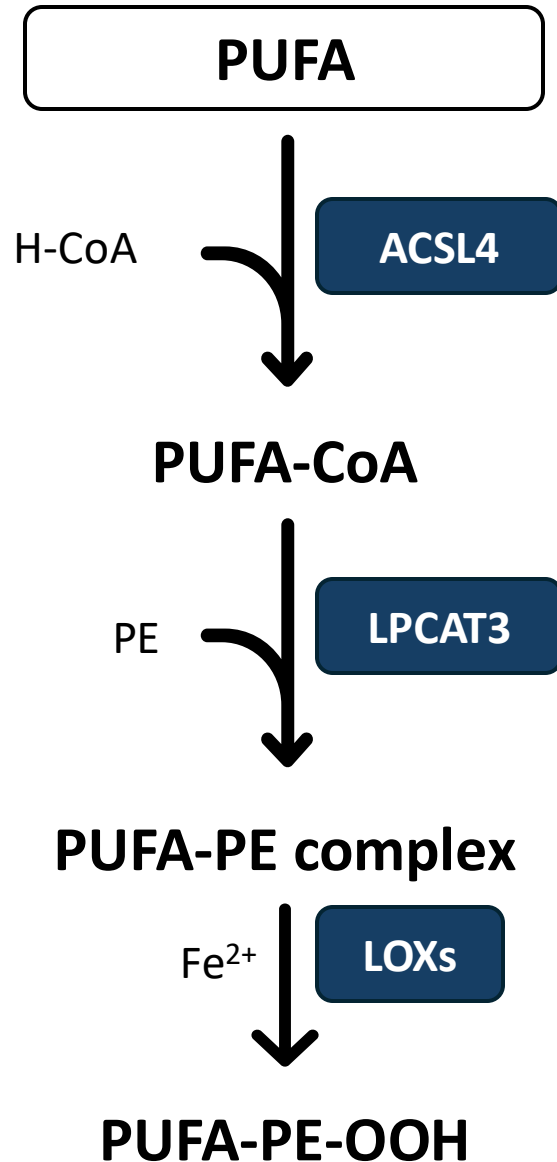
# Lipid Peroxidation: Non-Enzymatic Auto-Oxidation



PUFAs are the main substrate of lipid peroxidation during ferroptosis



# Lipid Peroxidation: Enzymatic Peroxidation



## Ferroptosis

- ↓ Membrane barrier function
- Change of membrane permeabilization

Lipid peroxides



# Ferroptosis: Lipid Peroxidation and Ferroptosis



Where are lipid peroxides form?

## Enzymatic reaction

- Lipoxygenases
- Cyclooxygenases
- P450 enzymes

PUFA  $\xrightarrow[\text{LPCAT3}]{\text{ACSL4}}$  PUFA-PE

$\xrightarrow[\text{Cofactor: Fe}^{2+}]{\text{Lipoxygenase}}$

Lipid peroxides

Toxic form

Lipid alcohol

Non-Toxic form

Glutathione Peroxidase 4  
(GPX4)

## Non-enzymatic reaction

- Iron: Fenton reaction

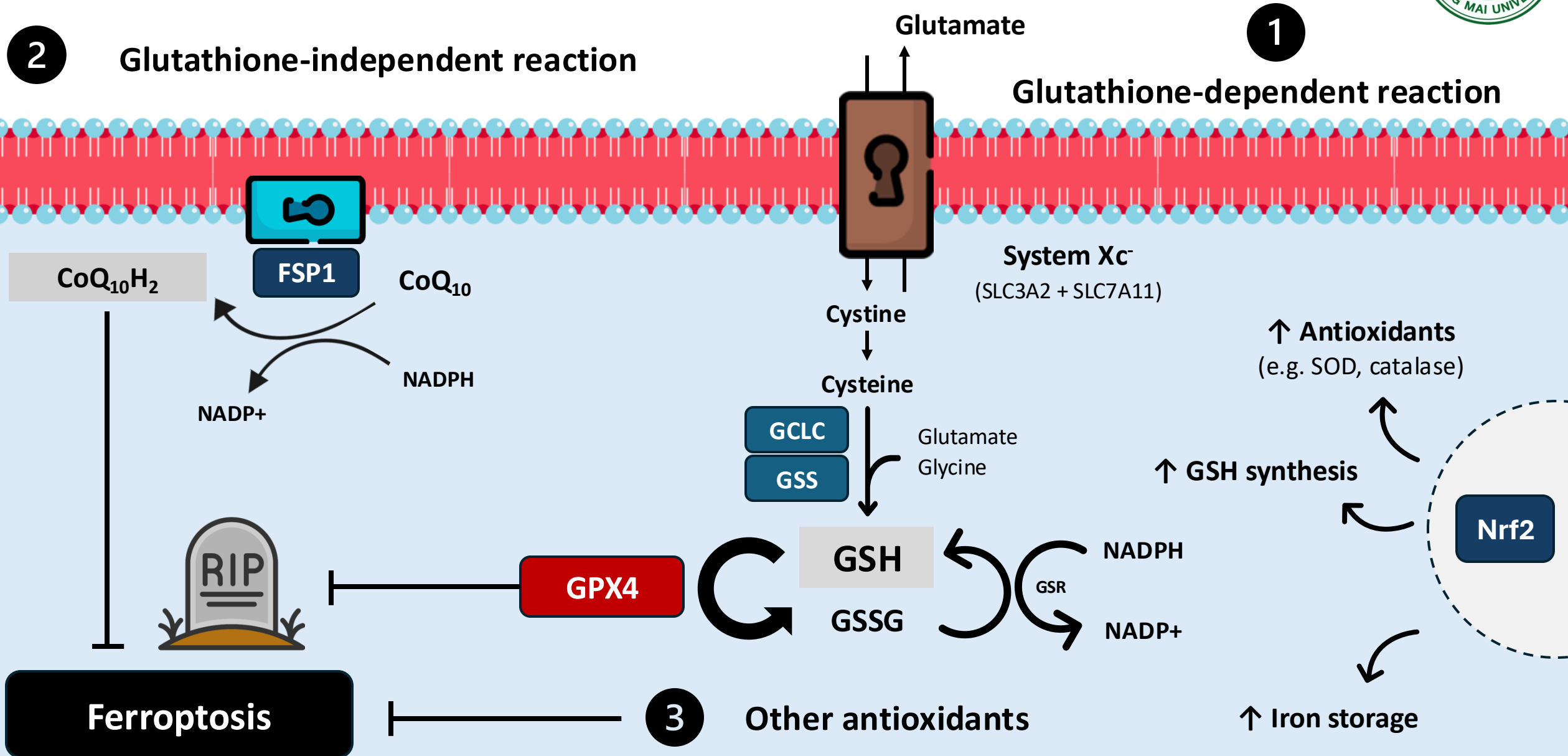


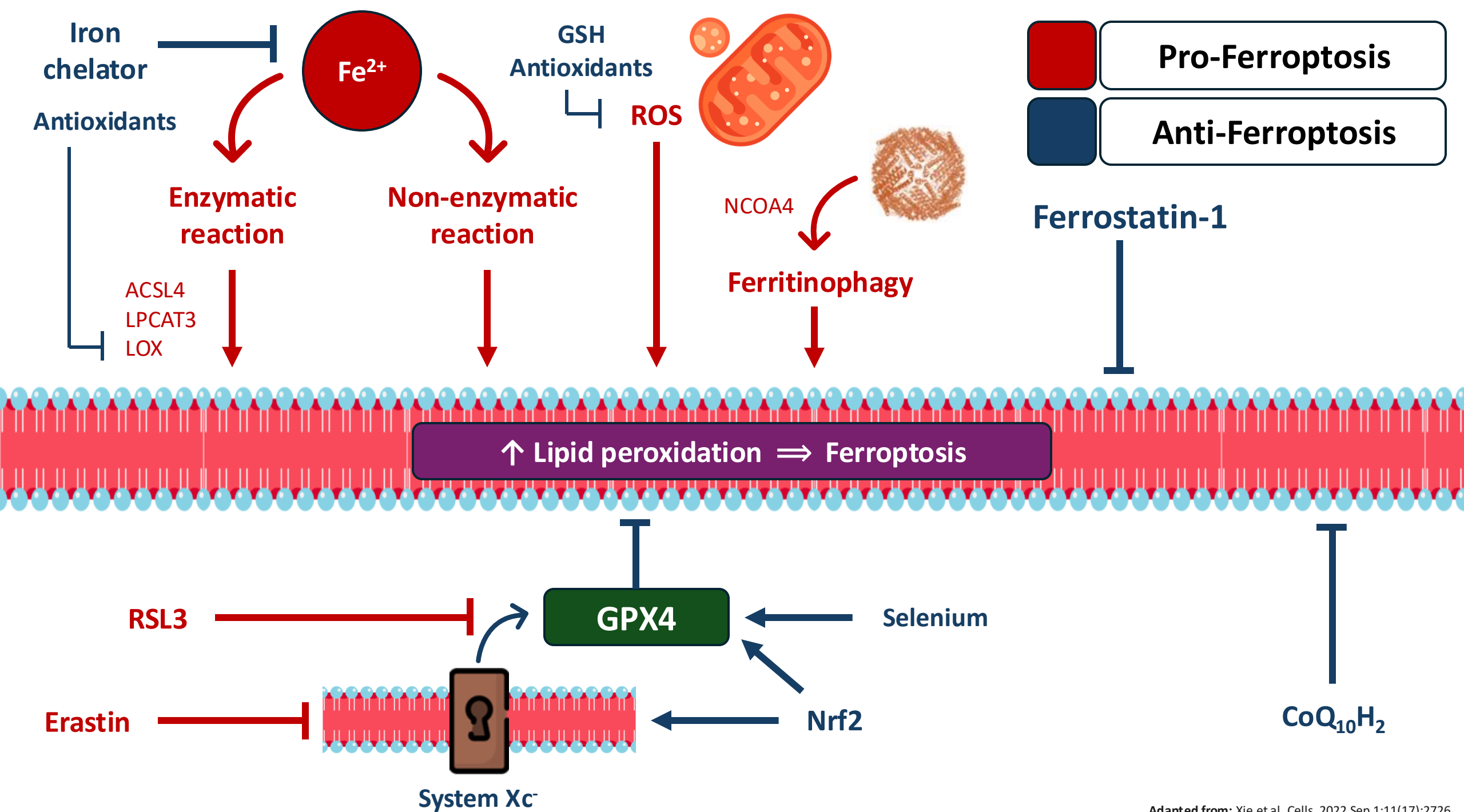
Loss of membrane permeability

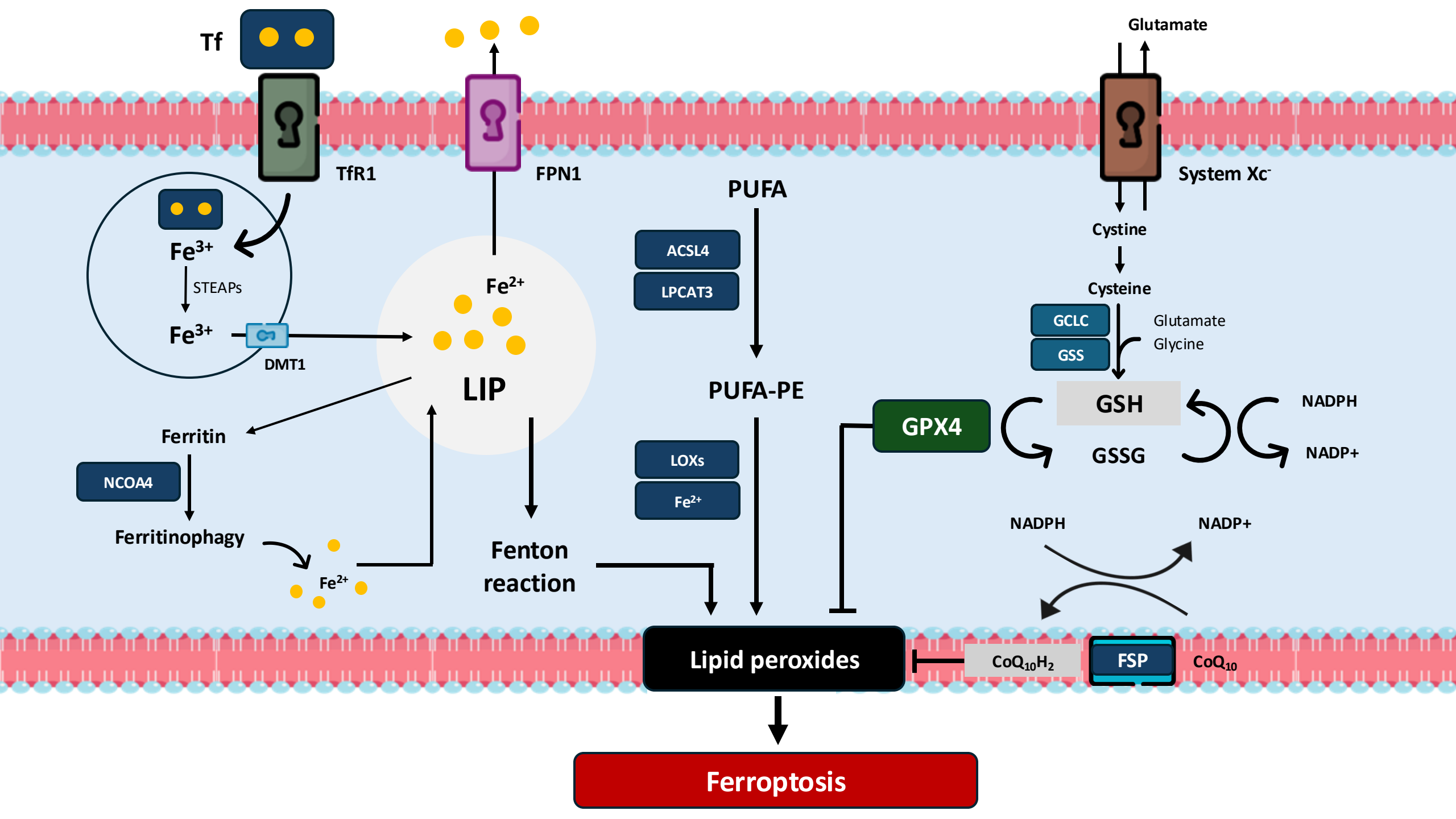
Cell death !

Ferroptosis

# Ferroptosis: The Anti-Ferroptosis Pathway







# Ferroptosis: Summary #1



- **Ferroptosis:** iron-regulated programmed cell death

## Hallmarks

- Iron accumulation
- Imbalance between oxidation and antioxidants
- Membrane lipid peroxidation

- **Roles of Iron in the ferroptosis pathway**

- Catalyze the reactions for ROS production via the Fenton reaction
- Serve as a cofactor for LOX enabling the enzyme to oxidize PUFAs

- **If lipid peroxidation overwhelm antioxidant defense mechanism**



**Ferroptosis**

```
graph TD; A[Free radical catalytic center<br/>Labile iron] --> B[Free radical and oxidative stress<br/>Superoxide, peroxide, hydrogen peroxide, hydroxyl radical]; B --> C[Molecular damage<br/>DNA, sugar, lipid, protein]; C --> D[Cell death<br/>Ferroptosis, apoptosis]; D --> E[Tissue damage<br/>Fibrosis, organ dysfunction]; E --> F[Release of labile iron and stored iron]; F --> A; F --> G((Vicious cycle)); G -.-> A;
```

**Free radical catalytic center**  
Labile iron

**Free radical and oxidative stress**  
Superoxide, peroxide, hydrogen peroxide, hydroxyl radical

**Molecular damage**  
DNA, sugar, lipid, protein

**Cell death**  
Ferroptosis, apoptosis

**Tissue damage**  
Fibrosis, organ dysfunction

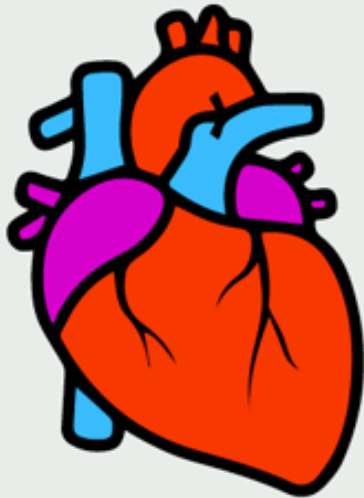
**Release of labile iron and stored iron**

**Vicious cycle**

# Iron Overload, Ferroptosis, and Organ Dysfunction

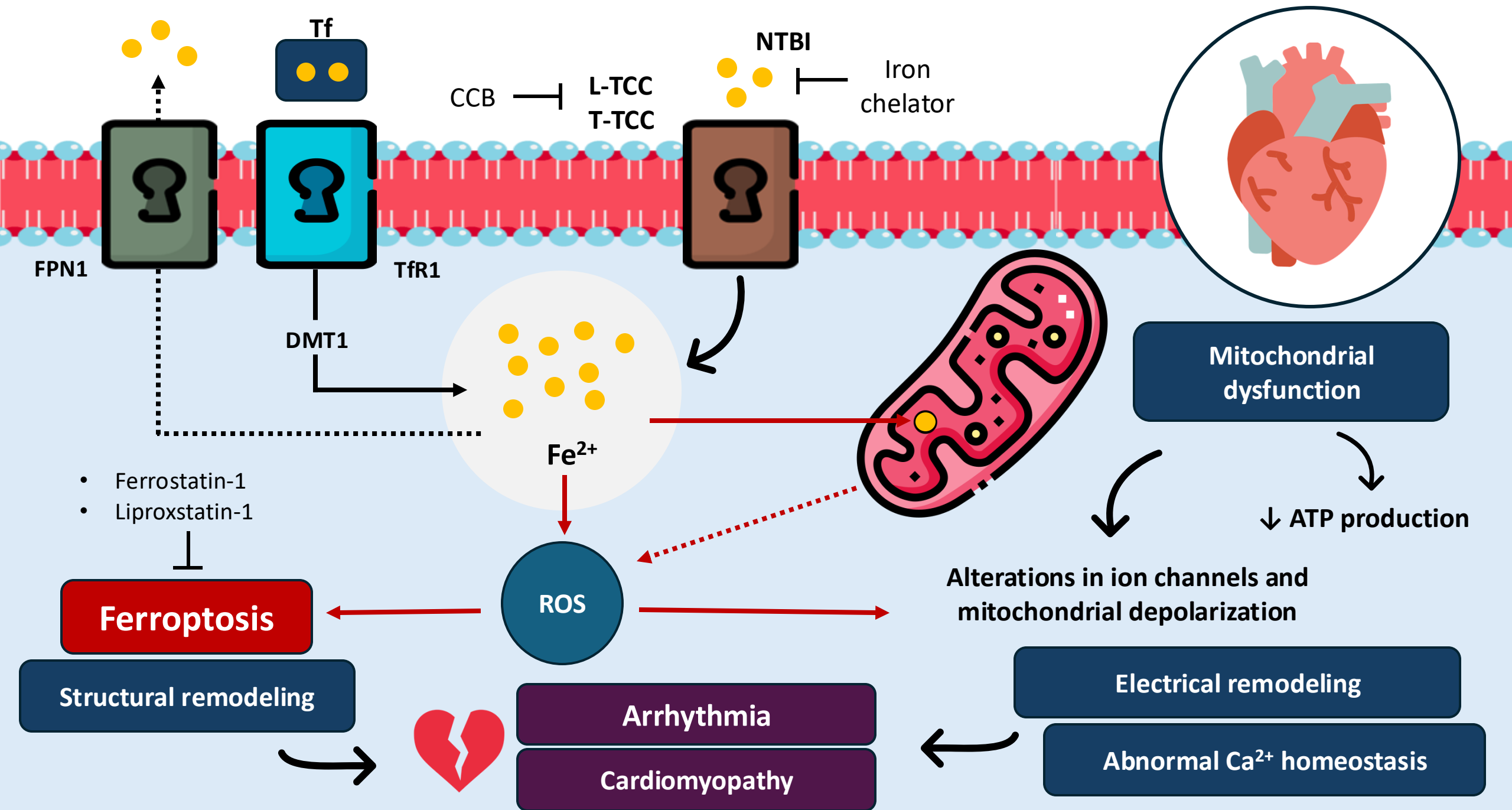


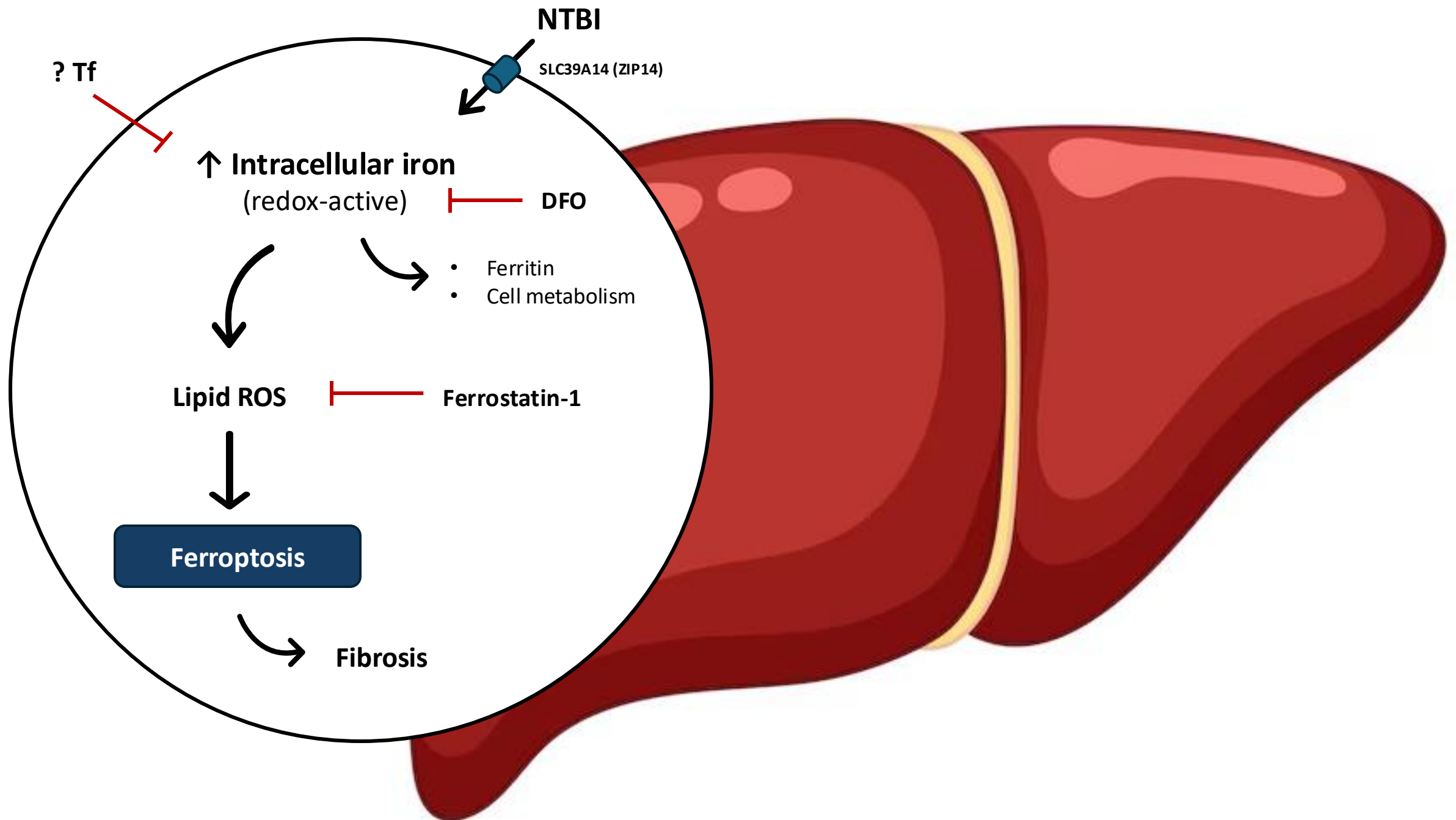
**FRAGILE**

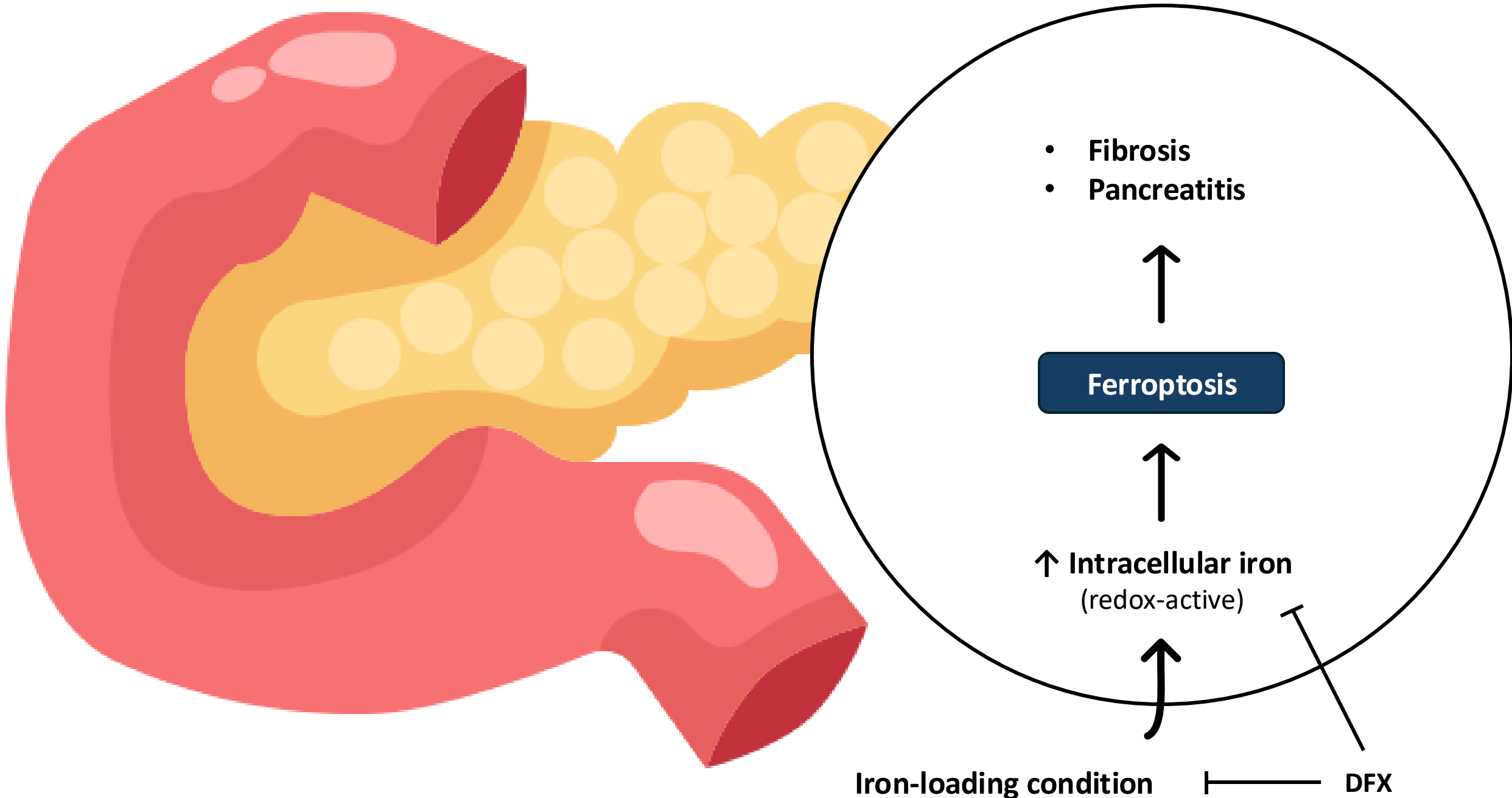


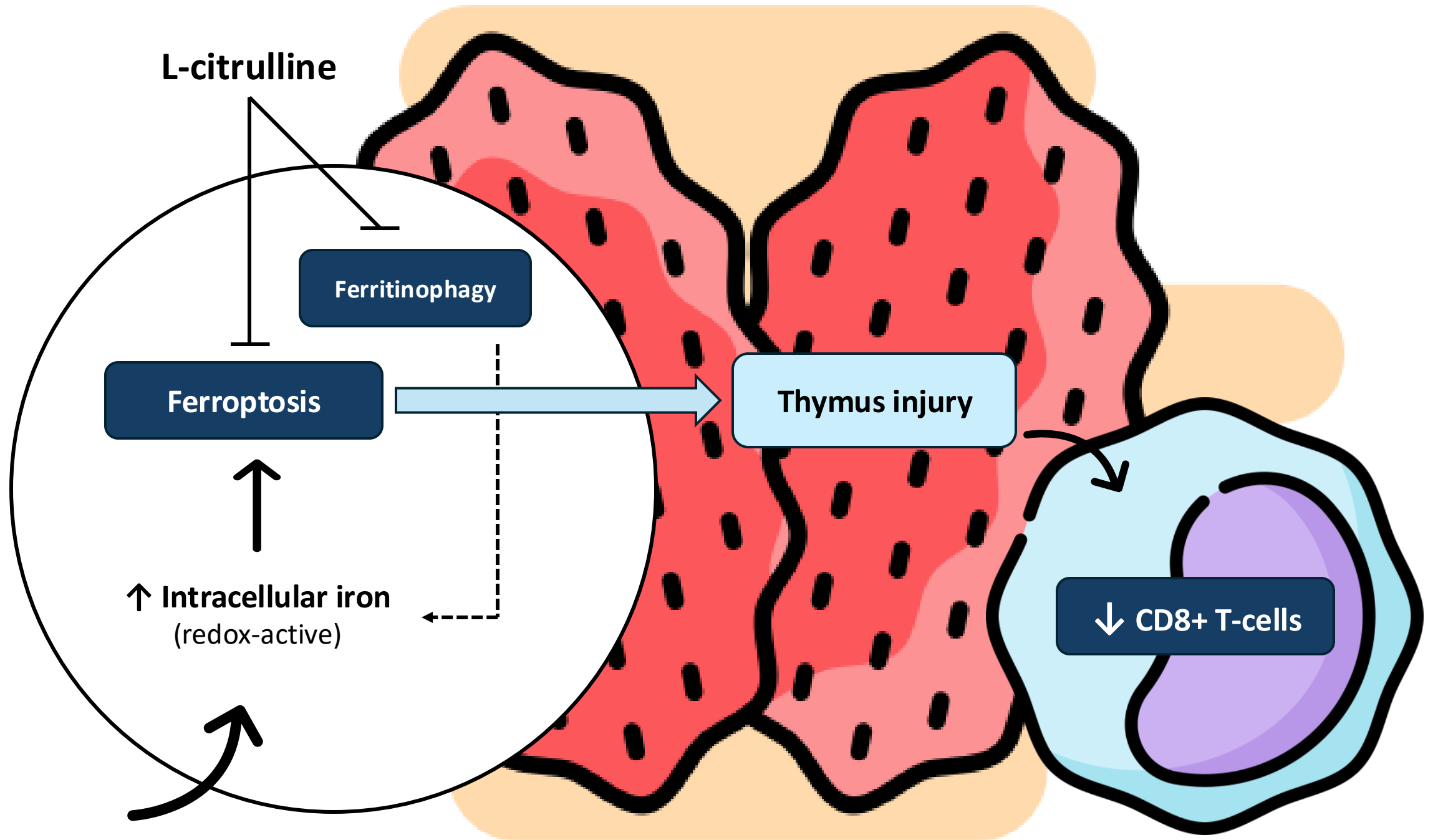
**HANDLE  
WITH CARE**

- Heart: one of the major target organs for iron deposition
- Myocardial hemochromatosis leading to morbidity and mortality in patients with iron overload
  - Cardiomyopathy
  - Cardiac arrhythmias
- Ferroptosis has recently been implicated in iron overload-induced cardiac cell death
- Myocardial dysfunction induced by ferroptosis
  - Inhibited by iron chelators and antioxidants

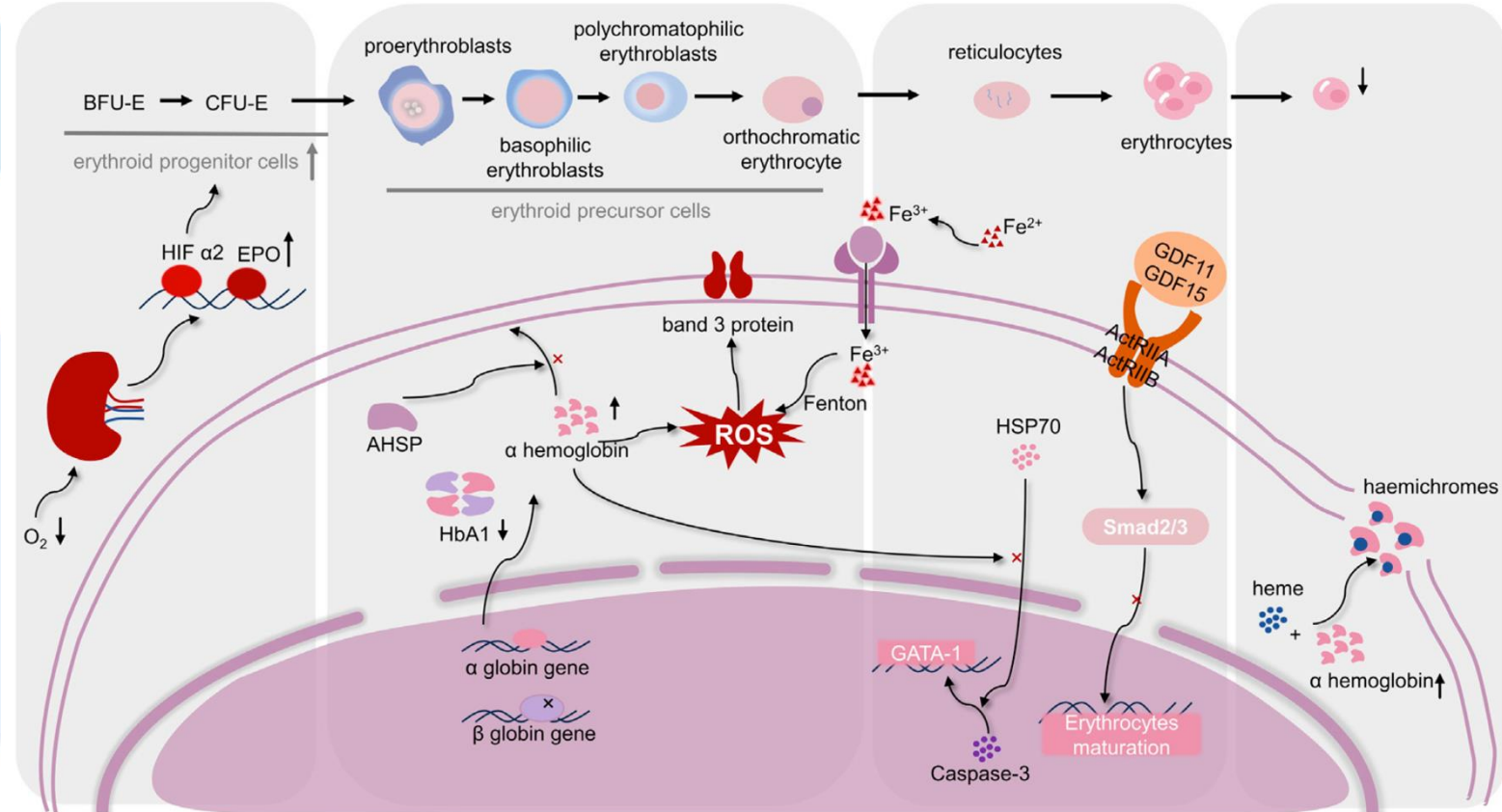
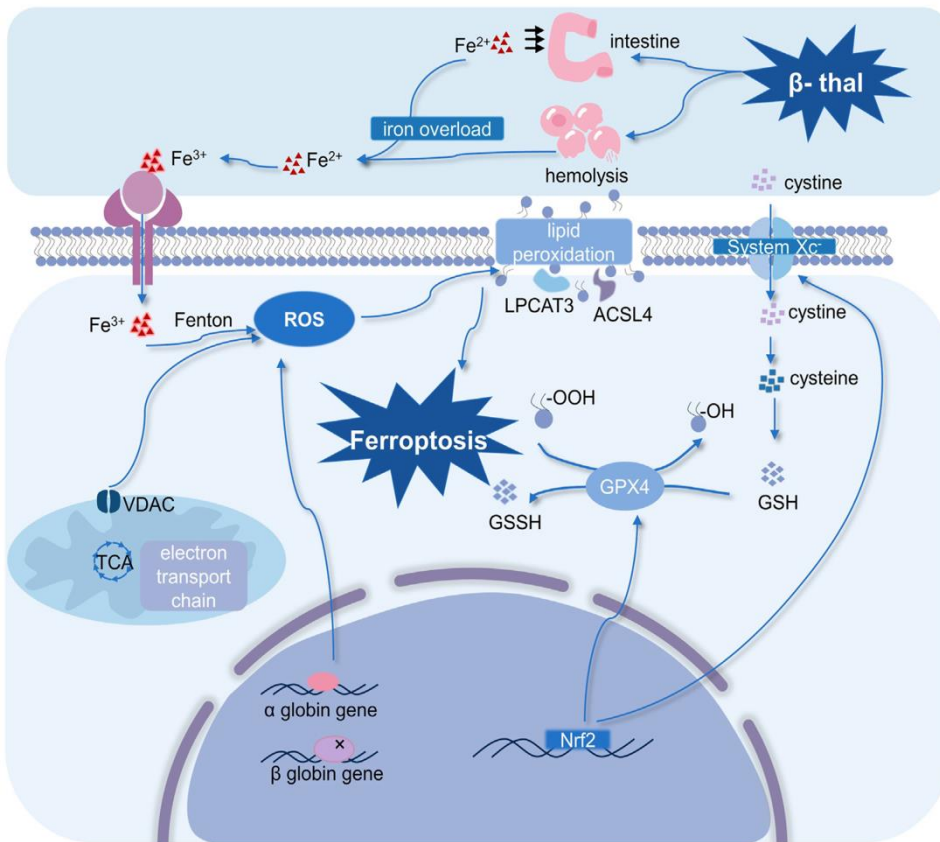






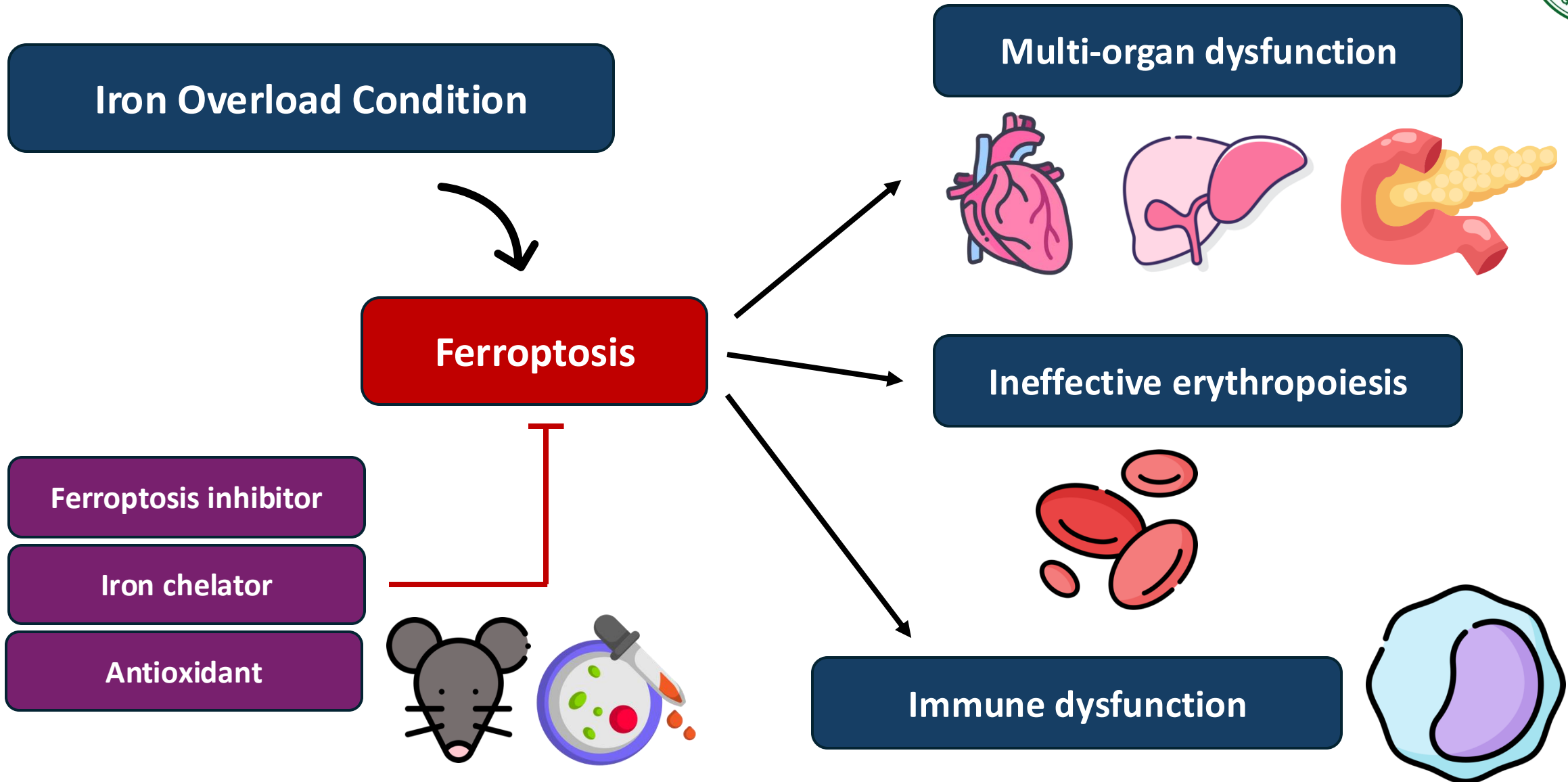


# Ferroptosis and Ineffective erythropoiesis in $\beta$ -thalassemia



The close interplay between ineffective erythropoiesis, iron overload, and ferroptosis in  $\beta$ -thalassemia

# Ferroptosis: Summary #2



# Knowledge Gaps and Limitations



- Different cell types or different conditions = Different ferroptosis susceptibility

$$\text{Fe Toxicity}_{\text{tissue}} = \sum^{\text{time}} \text{Tissue Reactive Iron} \times \text{Genetics} \\ \times \text{Environmental Factors} \times \Delta\text{Time}^8.$$

- Genetics
- Cellular redox status
- Disease & Chelation

- Crosstalk between ferroptosis and other forms of cell death in the context of iron overload remains unclear
- Gold standard markers to detect ferroptosis are still lacking
- Most studies = In vitro and in vivo studies
- ? The specificity of ferroptosis inhibitors:
  - Target pathological cell death without physiologic disturbance

# Future Perspectives

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## Novel biomarkers

- **Novel biomarkers for detecting ferroptosis at tissue and systemic level**
  - Early detection of organ damage (prevention), prognosis, treatment strategy
- **Precision medicine approaches:** conventional investigation + novel biomarkers

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## Therapeutic approaches

- **Novel targeted therapies**
- **Therapeutic strategy to mitigate iron overload-associated organ dysfunction**
  - **Combination therapy:** iron chelator, antioxidants, and ferroptosis inhibitors

# Potential Roles of Antioxidants in Beta-Thalassemia



| Antioxidant                                           | Outcomes                                                                  |
|-------------------------------------------------------|---------------------------------------------------------------------------|
| <b>• Transfusion-dependent thalassemia (TDT)</b>      |                                                                           |
| Vitamin C and E                                       | ↑ Antioxidant status                                                      |
| Flavanoids                                            | ↓ Serum oxidative stress, ↑ serum antioxidant capability                  |
| Curcuminoids with green tea                           | ↓ Redox-active iron                                                       |
| NAC                                                   | ↓ Serum oxidative stress                                                  |
| Alpha-lipoic acid                                     | ↓ Oxidative stress                                                        |
| Fermented papaya preparation                          | ↓ ROS, ↓ lipid peroxidation, ↓ PS externalization, ↑ GSH levels           |
| <b>• Non-transfusion-dependent thalassemia (NTDT)</b> |                                                                           |
| Vitamin E                                             | ↓ Lipid peroxidation in thalassemic RBCs, ↓ hemolysis, and ↑ RBC survival |
| Curcuminoids                                          | ↓ Oxidative stress, and ↓ iron overload                                   |
| Fermented papaya preparation                          | ↓ ROS, ↓ lipid peroxidation, ↓ PS externalization, ↑ GSH levels           |

# Take-Home Message

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- **Iron overload remains a major cause of multi-organ dysfunction in thalassemia**
- **Ferroptosis: non apoptotic form of iron-regulated programmed cell death**
  - Intracellular iron dysregulation
  - Imbalance of antioxidant system
  - Accumulation of lipid peroxides
- **Ferroptosis may play a critical role in the pathogenesis of iron overload-related organ dysfunction**
- **The discovery of ferroptosis as a central mechanism of iron-related toxicity organ dysfunction**
  - Novel therapeutic targets
  - Novel specific biomarkers