Ferroptosis: main features and regulatory pathways

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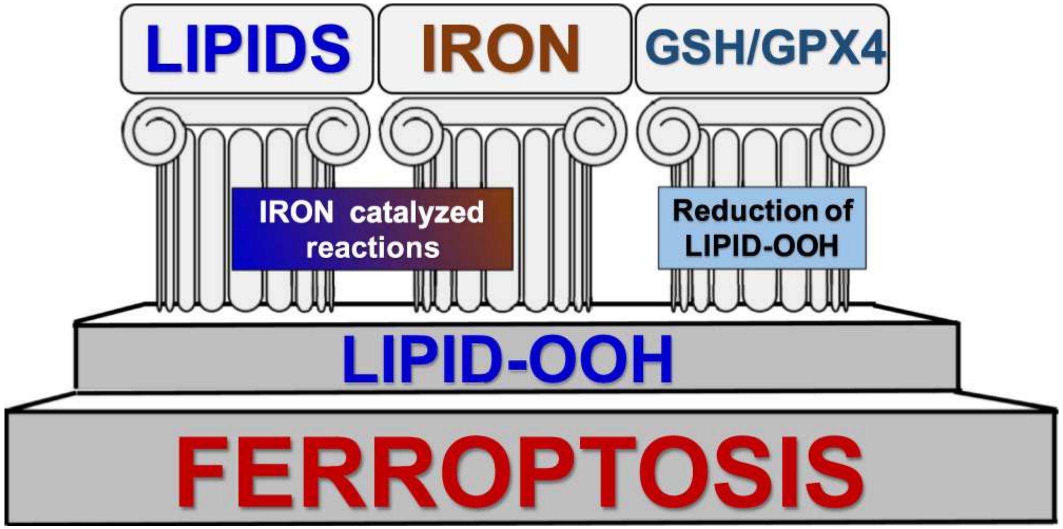
M2 GCD - UE Cell Dynamics – 10 november 2025

- Part 1: Hallmarks and regulation of ferroptosis
  - What is ferroptosis?
  - What are the hallmarks of ferroptotic cells?
  - How can we monitor / quantify ferroptosis?
  - How can we induce ferroptosis?
  - What are the main regulators of ferroptosis?

- Part2: Role of mitochondria in ferroptosis regulation
  - Regulation of mitochondrial shape and ferroptosis: role of OPA1

"**Ferroptosis** is defined as an iron-dependent form of regulated cell death, which occurs through the lethal accumulation of lipid-based reactive oxygen species (ROS) when glutathione (GSH)-dependent lipid peroxide repair systems are compromised."

From the review article by Hirschhorn and Stockwell. The development of the concept of ferroptosis (2019)

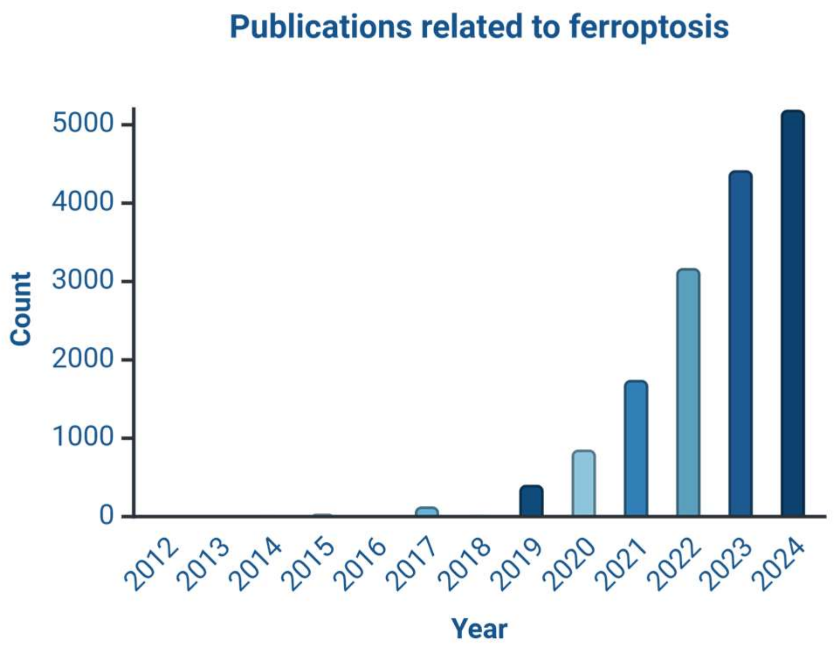


Three pillars of ferroptosis.
From Stoyanovsky *et al*, 2019

What is ferroptosis ?

- Ferroptosis is an **iron-dependent cell death** characterized by lipid peroxidation.
- **Lipid peroxidation** is a driver of ferroptosis.
- Ferroptosis is inhibited by iron chelators and antioxidants.
- Ferroptosis is morphologically and mechanistically different from apoptosis.
- Ferroptosis is a **caspase-independent** cell death.

An active field of research since the first description of ferroptosis

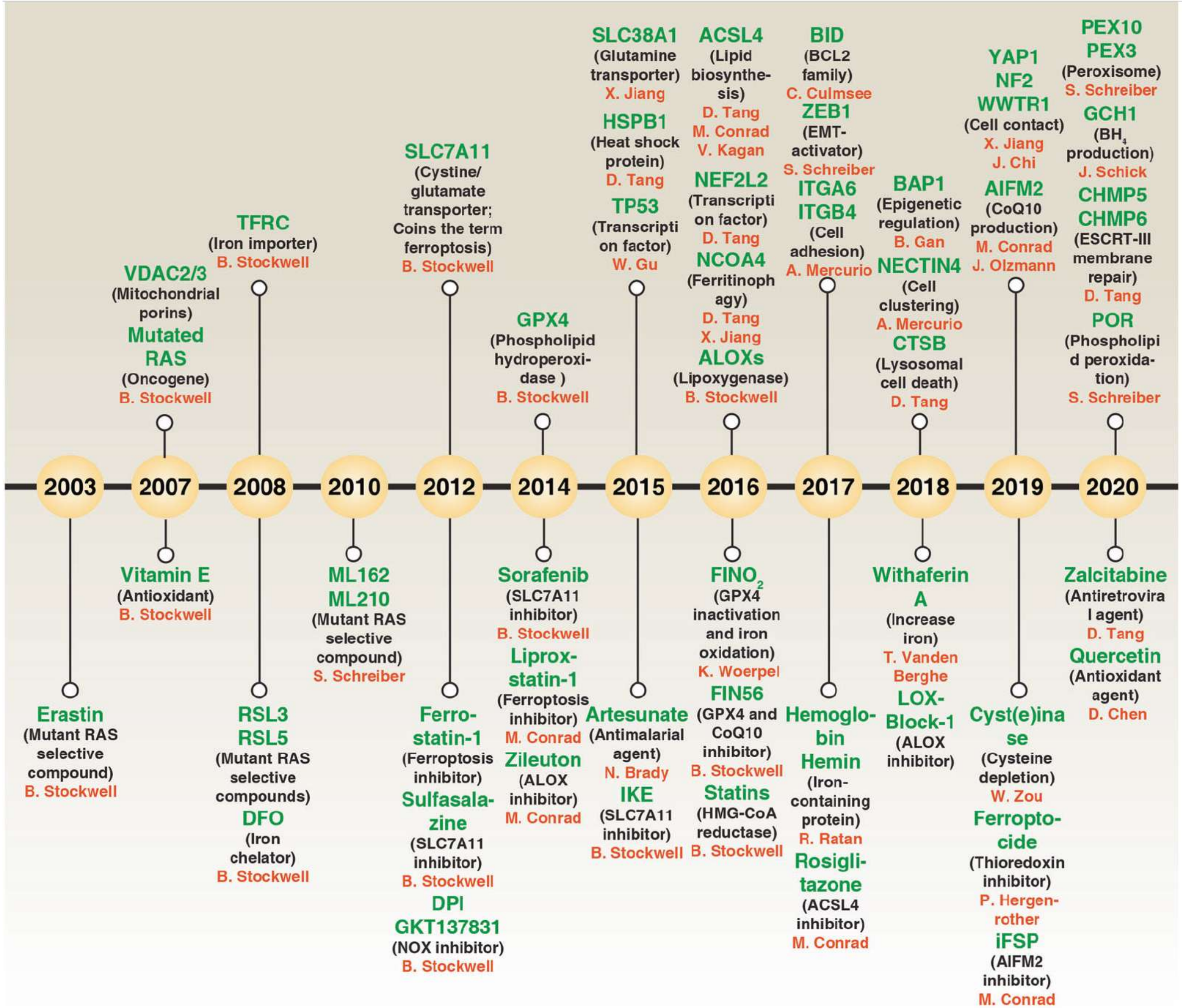


Articles retrieved in the Pubmed database using the keyword « ferroptosis »
Pubmed accessed on 11/01/24
Graph created in BioRender.com

Some of the major advances in the field

2012: first description of ferroptosis by Dixon and colleagues
2014: GPX4 is an essential regulator of ferroptotic cancer cell death
2016: identification of PUFAs as the most susceptible lipids to peroxidation
role of ACSL4 and ALOX enzymes
2019: identification of FSP1 as a ferroptosis inhibitor

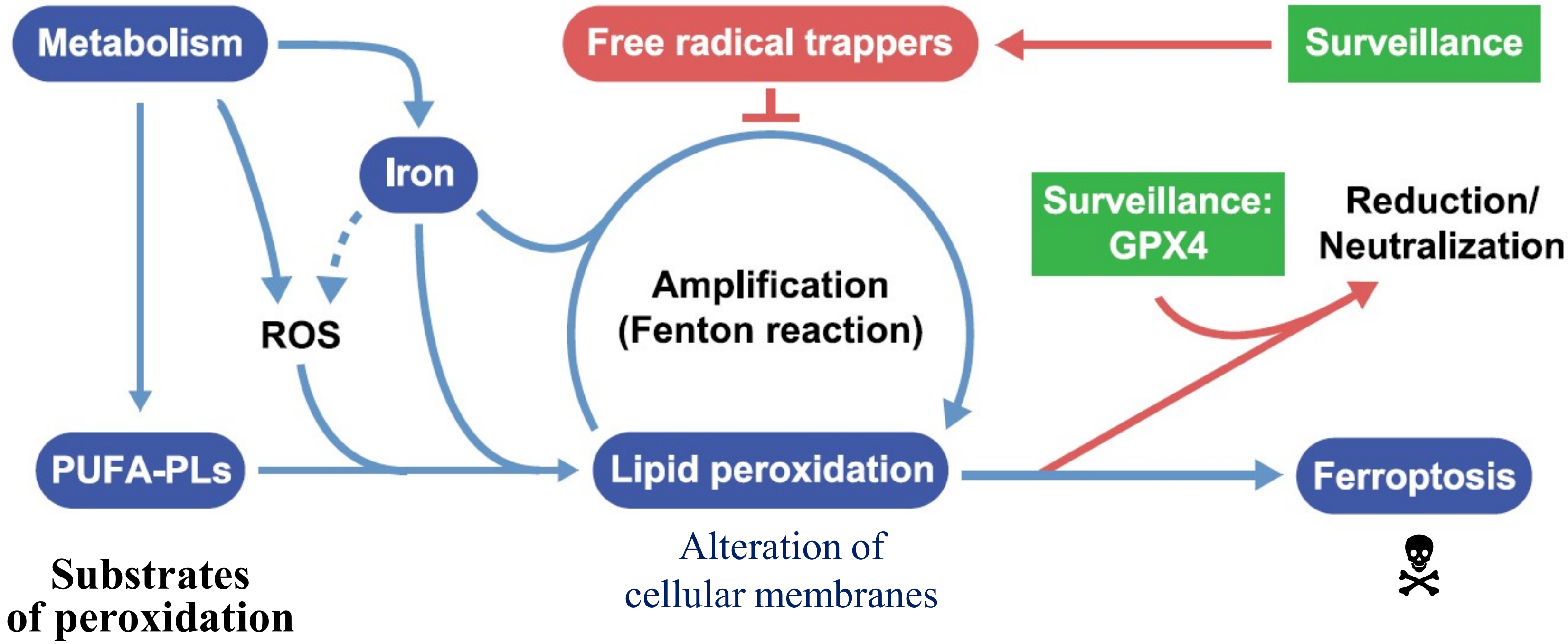
Timeline of ferroptosis research (2003-2020)



From Tang, D., Chen, X., Kang, R. *et al.* Ferroptosis: molecular mechanisms and health implications. *Cell Res* **31**, 107–125 (2021). https://doi.org/10.1038/s41422-020-00441-1

- Erastin is the first compound described to induce ferroptosis
- Highlights the numerous actors / pathways involved in ferroptosis regulation
- Highlights the pioneer work of Stockwell's lab to uncover ferroptosis regulation

Core features of ferroptosis



Molecular Cell

CellPress

Review

Ferroptosis at the intersection of lipid metabolism and cellular signaling

Deguang Liang,[1,3] Alexander M. Minikes,[1,2,3] and Xuejun Jiang[1,*]
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https://doi.org/10.1016/j.molcel.2022.03.022

https://doi.org/10.1016/j.molcel.2022.03.022

What are the morphological hallmarks of ferroptotic cells ?

Morphological features of ferroptotic cells

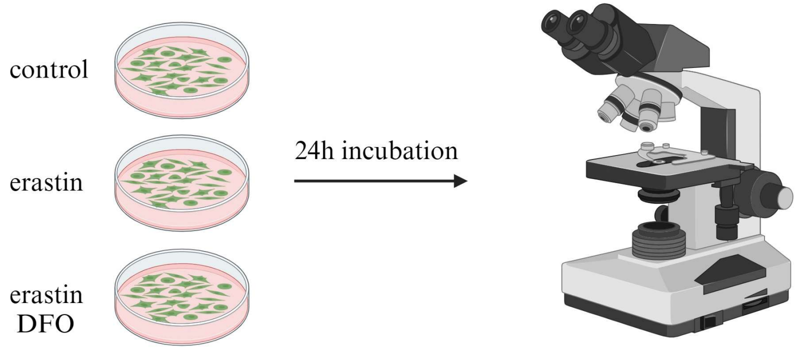
Morphological features of ferroptotic cells

- Loss of plasma membrane integrity
- Cytoplasmic swelling
- Swelling of organelles
- Moderate chromatin condensation

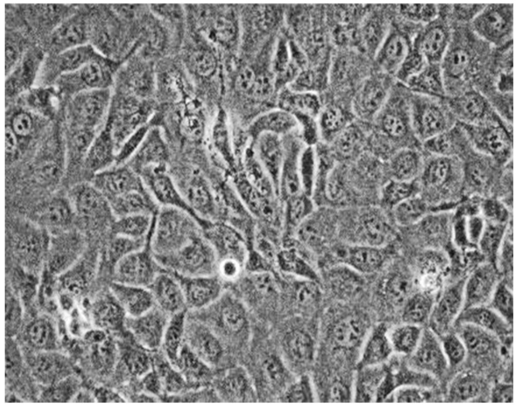
From Tang, D., Chen, X., Kang, R. *et al.* Ferroptosis: molecular mechanisms and health implications. *Cell Res* **31**, 107–125 (2021). https://doi.org/10.1038/s41422-020-00441-1

Morphological changes during ferroptosis

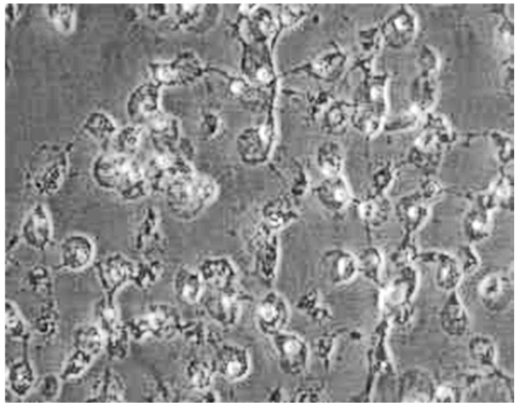
HT1080 cells
treated either with erastin
or with erastin plus DFO



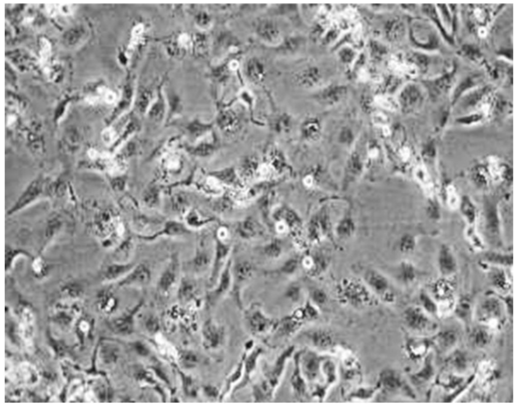
Vehicle



Erastin



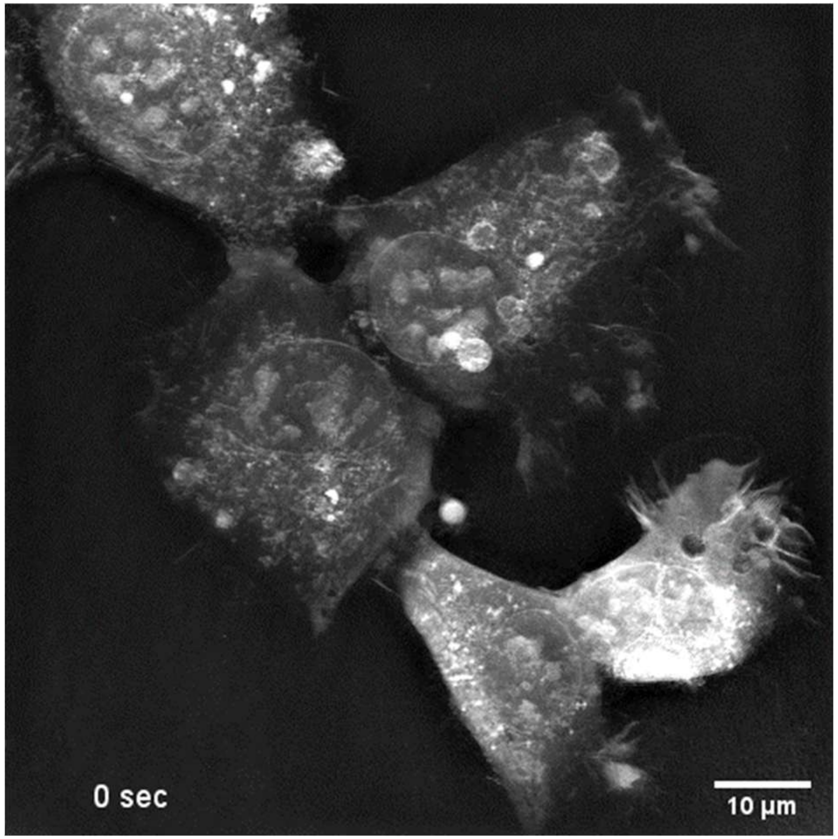
Erastin + DFO



Vehicle = DMSO
Erastin: ferroptosis inducer
DFO= deferoxamine, iron chelator

Emma Deleusse internship
(unpublished data, 2019)

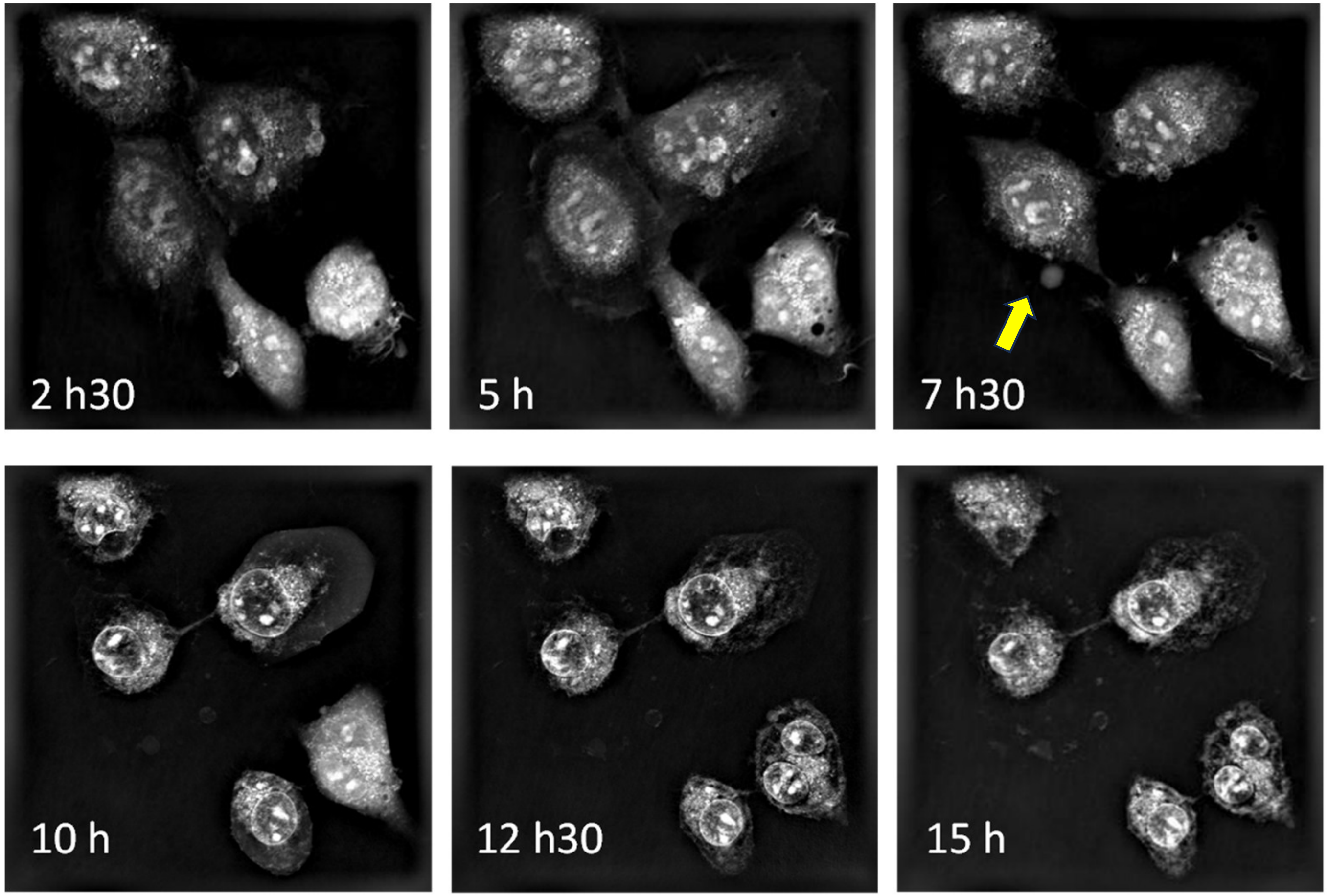
Morphological changes during ferroptosis



Movie of erastin-treated HT1080 cells (N. Le Floch-Leleu)
Obtained with the help of Thibault Courtheoux (Nanolive)

Morphological changes during ferroptosis

Erastin-treated HT1080 cells observed with the Nanolive technology



Unpublished results obtained with the help of Thibault Courtheoux (Nanolive)

- Membrane blebbing
  Altered membrane integrity
  visible after 7h30
- Changes in refractive index
  of the cytoplasm and nucleus
  are clearly visible after 10h
- Fast propagation to neighbor cells
- Dead cells remaining attached
  on the plate after 15 h

nature communications

Article

https://doi.org/10.1038/s41467-025-58175-w

Ferroptosis spreads to neighboring cells via plasma membrane contacts

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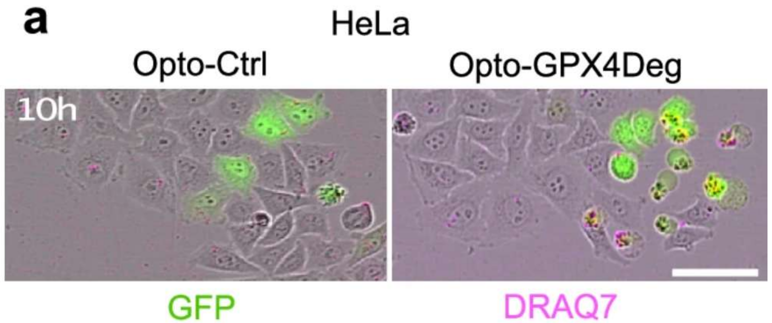
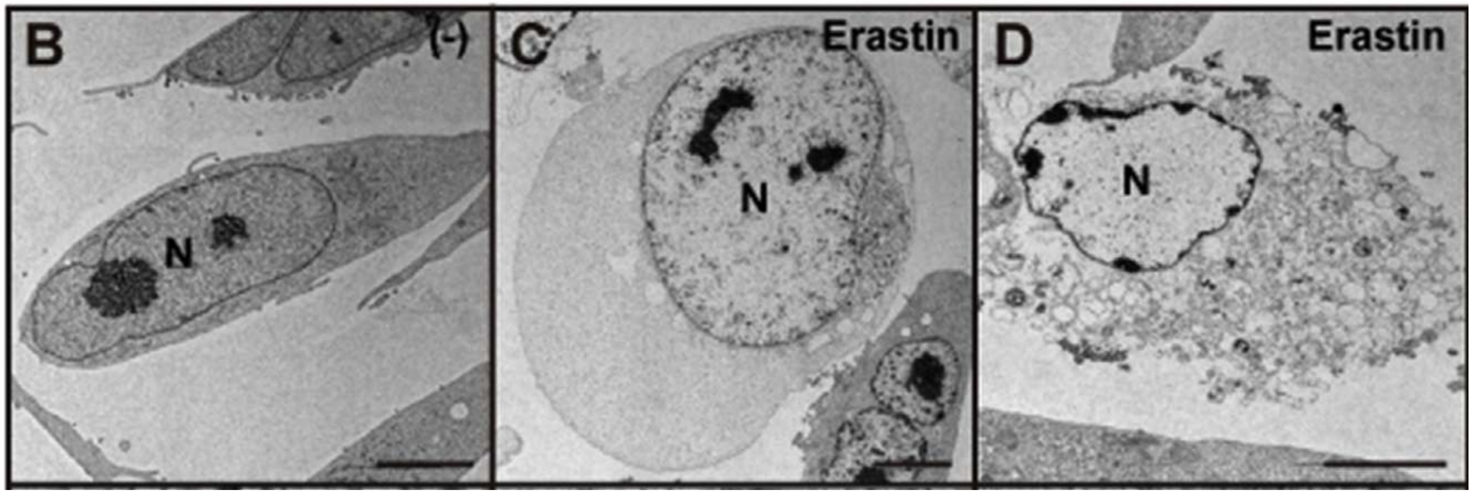
Bernhard F. Roeck[1,2], Sara Lotfipour Nasudivar[1,2], Michael R. H. Vorndran[1,2], Lena Schueller[1,2], F. Isil Yapici[2,3,4], Matthias Rübsam[2,4,5], Silvia von Karstedt[2,3,4], Carien M. Niessen[2,4,5] & Ana J. Garcia-Saez[1,2,6]



Morphological changes during ferroptosis

Transmission Electron Microscopy on HT1080 cells

control | Erastin-treated (10h)

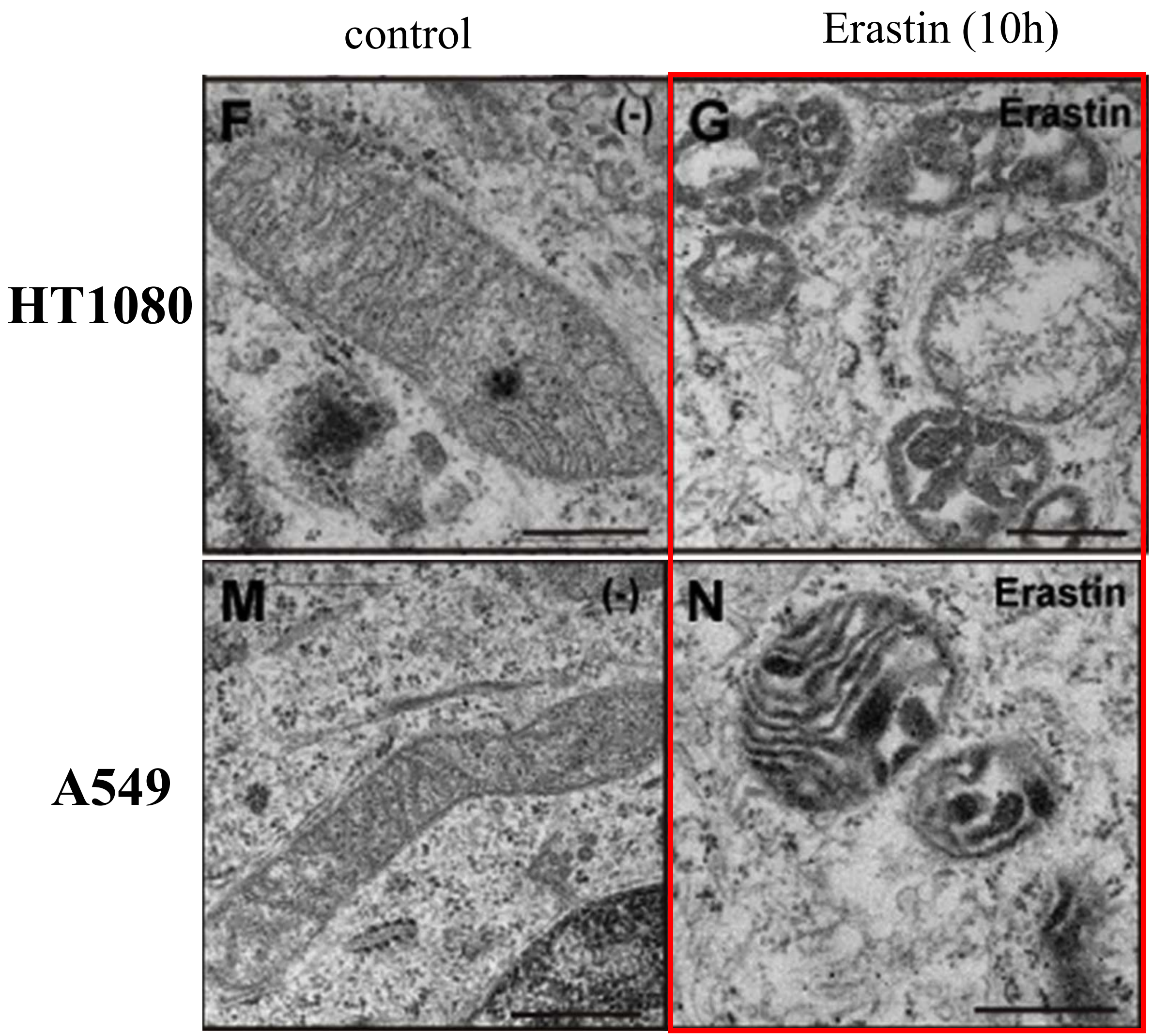


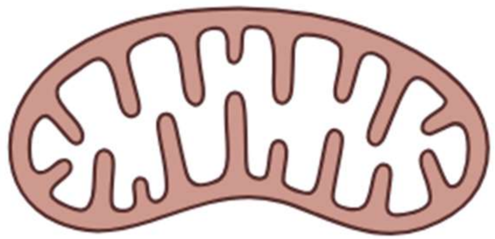
Erastin => decrease in the electronic density of both the nucleus and cytoplasm

Lucent nucleus and cytoplasm

Miyake *et al*. 2020

Morphological changes during ferroptosis





The mitochondrial structure
is altered in erastin-treated cells

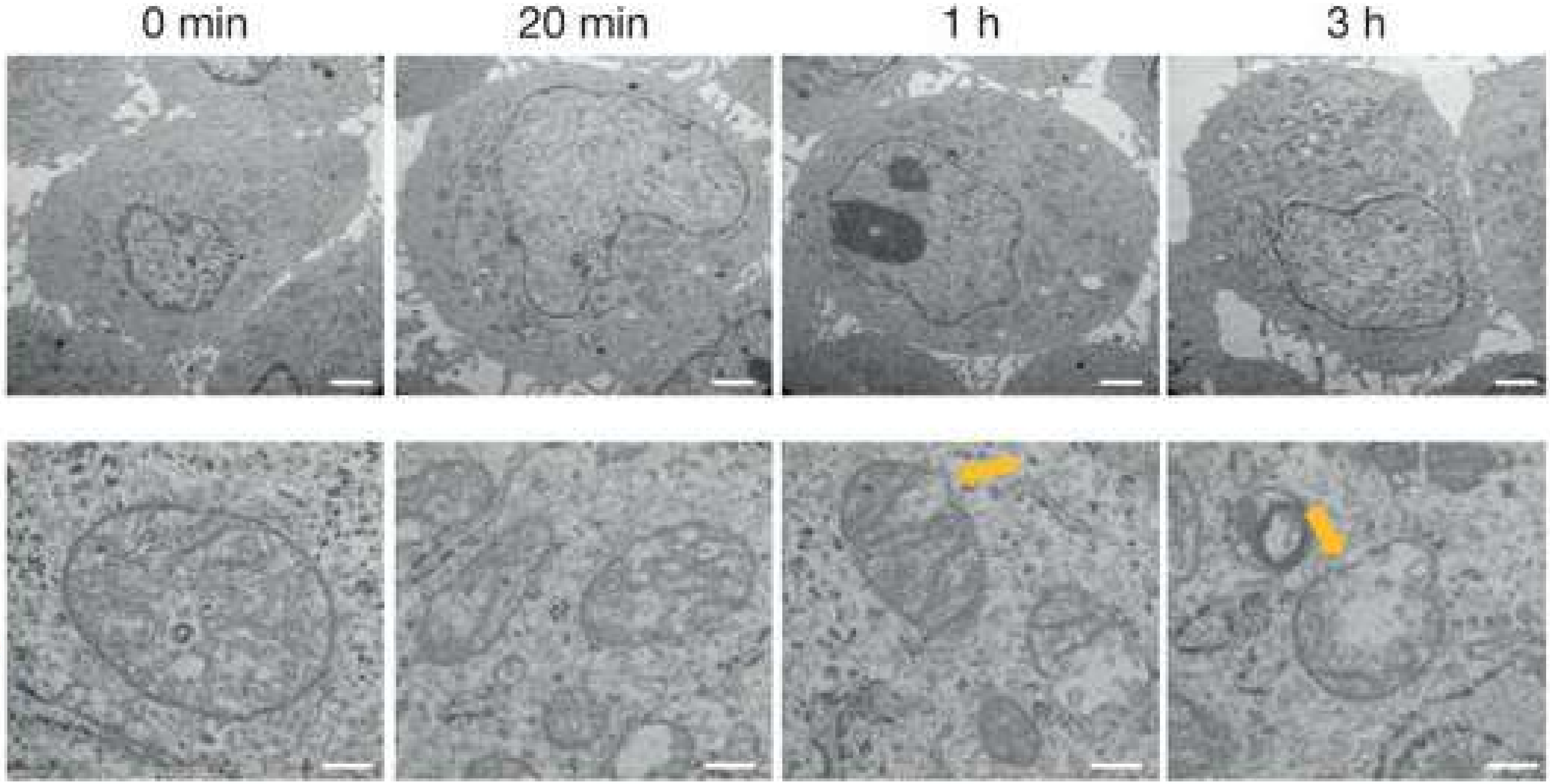
- decreased mitochondrial size
- reduced/absent mitochondrial cristae
- ruptured mitochondrial outer membrane

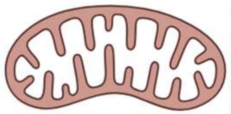
Miyake *et al*. 2020

Morphological changes during ferroptosis

RSL3 induces OMM rupture

The mitochondrial morphology is altered during ferroptosis

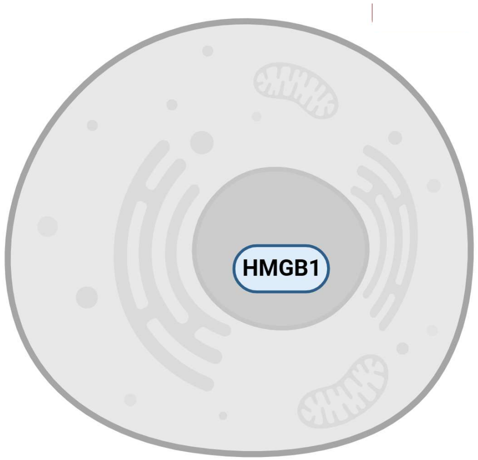


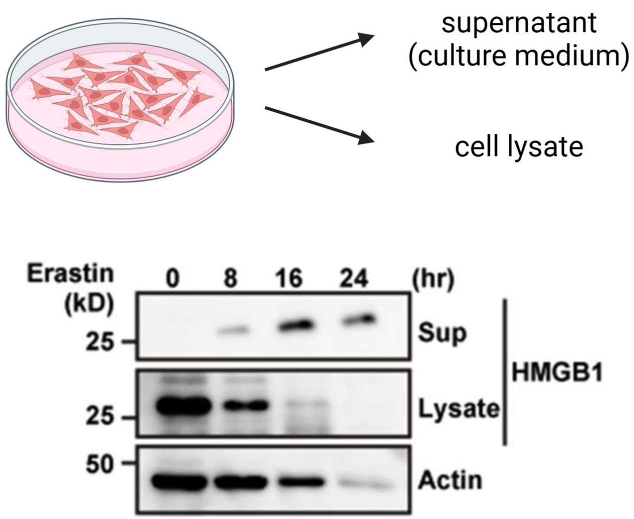


Electron micrographs showing a time-dependent OMM rupture (yellow arrows) on ferroptosis induction using RSL3 (50 nM; scale bars 2 µm top row, 200 nm bottom row)

Friedmann Angeli *et al*, 2014

HMGB1 is released from the nucleus during ferroptosis

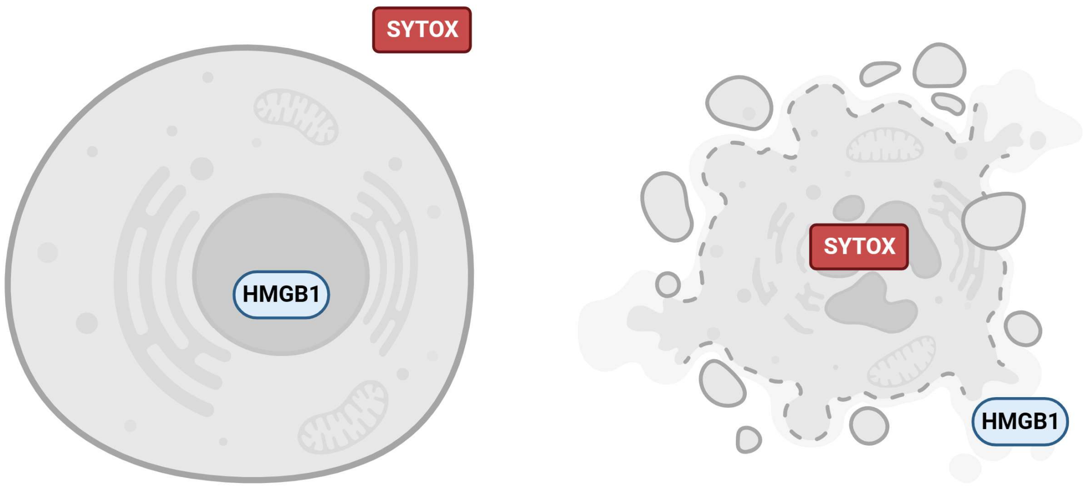




Detection of HMBG1 in the supernatant of erastin-treated cells => loss of membrane integrity

Miyake *et al*. 2020

HMGB1 is released from the nucleus during ferroptosis

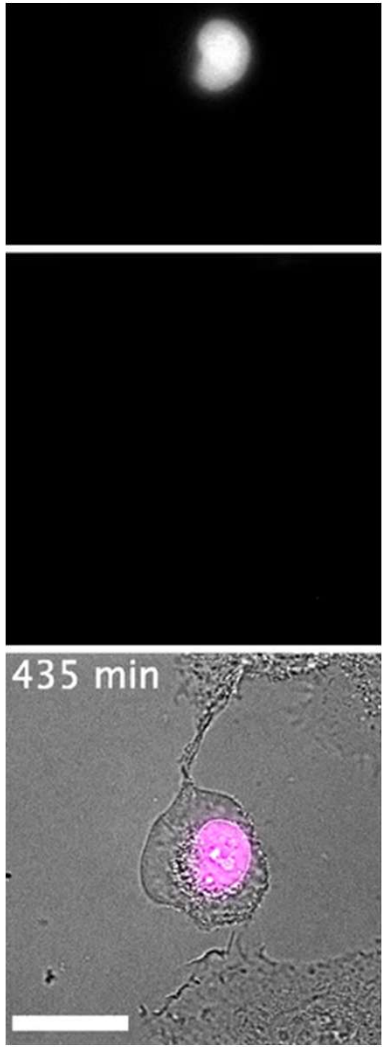


SYTOX enters cells only if membranes are damaged

HMGB1-mCherry

SYTOX

HMGB1-mCherry

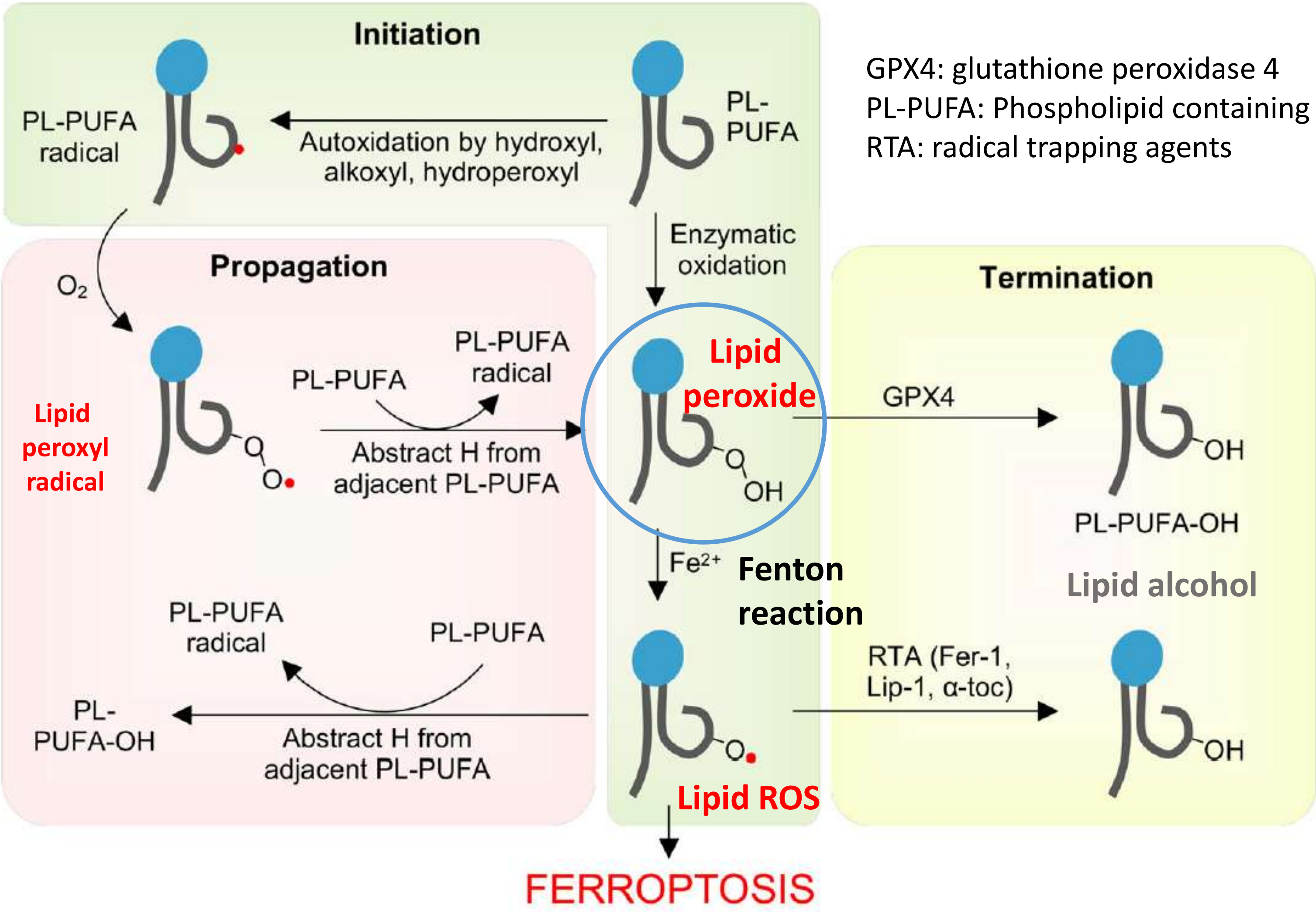


Miyake *et al*. 2020

These results suggest that nuclear membrane damage is induced prior to plasma membrane damage in ferroptosis.

Lipid peroxidation

Lipid peroxidation is a hallmark of ferroptosis

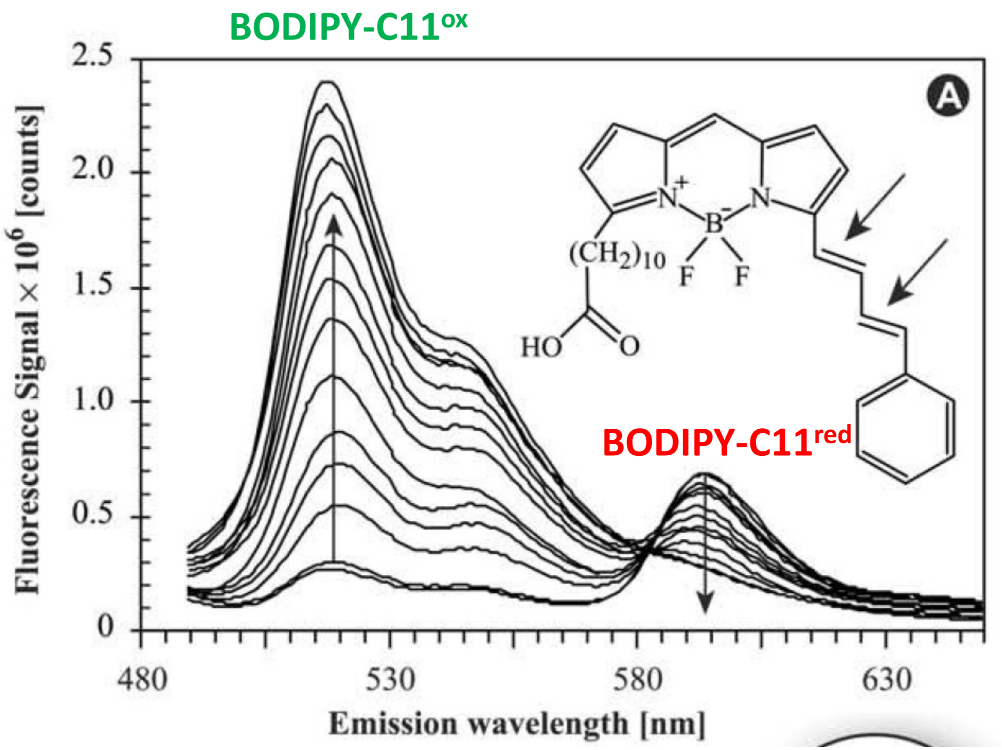


GPX4: glutathione peroxidase 4
PL-PUFA: Phospholipid containing poly-unsaturated fatty acid
RTA: radical trapping agents

Rodencal and Dixon *Proteomics* 2023, 23:2100308 https://doi.org/10.1002/pmic.202100308

How can we quantify lipid peroxidation ?

Lipid peroxidation can be detected using the C11-BODIPY581/591 fluorescent probe.



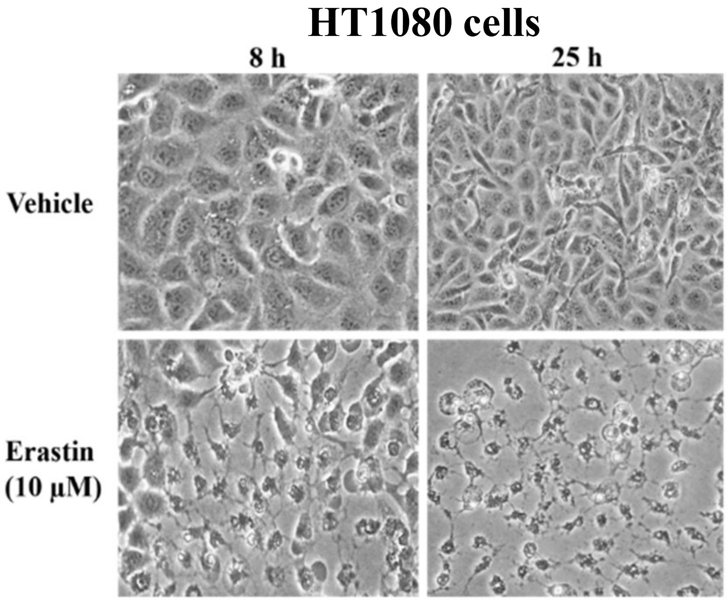
- Fatty acid analogue
- Fluorescence shift from red to green upon oxidation
- Oxidation of C11-BODIPY =>

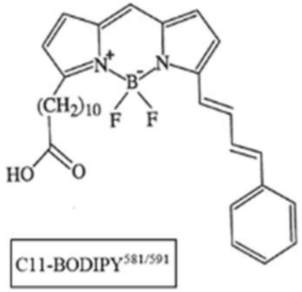
fluorescence at 520 nm ↗

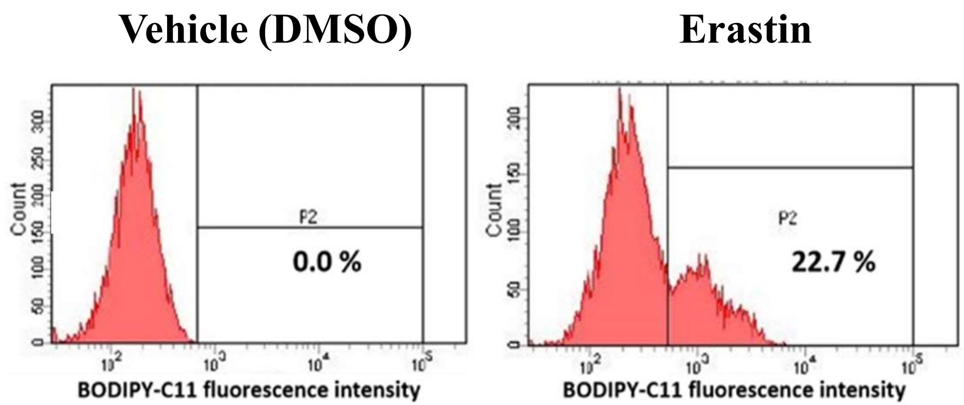
fluorescence at 595 nm ↘

Drummen, *et al.* 2002

How can we quantify lipid peroxidation ?





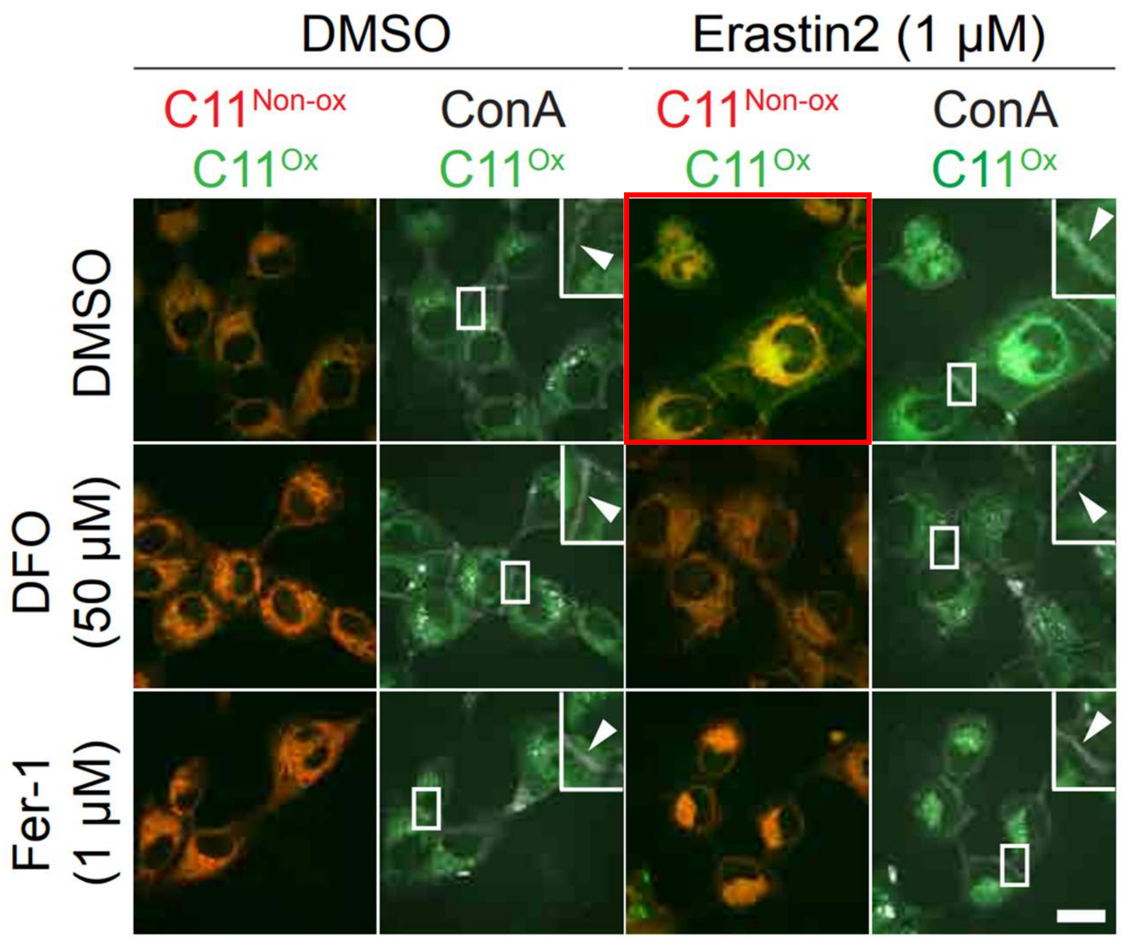


The increase in C11-BODIPY fluorescence intensity at 520 nm can be measured by flow cytometry

Unpublished data, N.Le Floch-Leleu

How can we quantify lipid peroxidation ?

Confocal microscopy

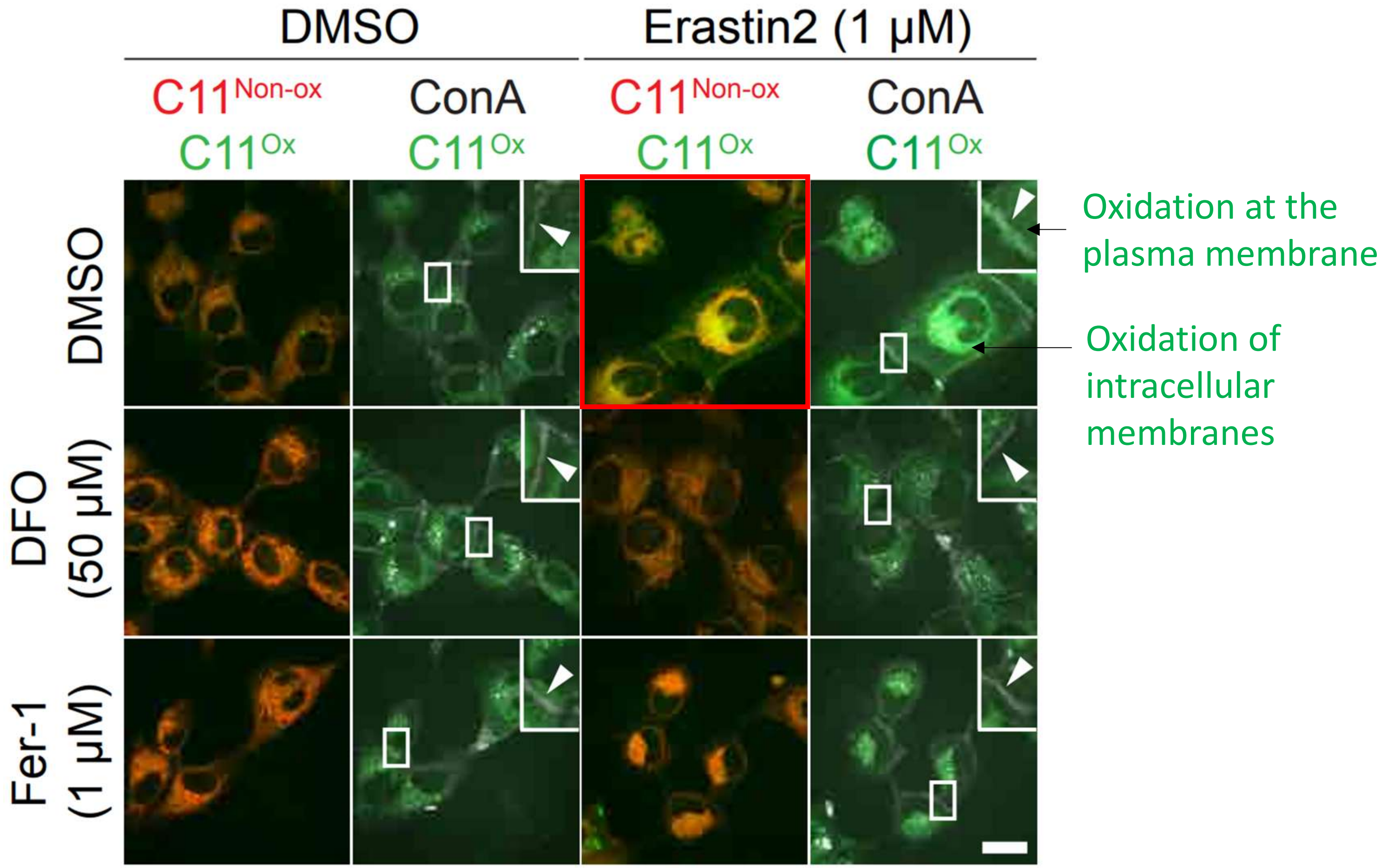


ConA: membrane staining
DFO and Fer-1 : ferroptosis inhibitors

Magtanong, *et al.* 2019

How can we quantify lipid peroxidation ?

Confocal microscopy

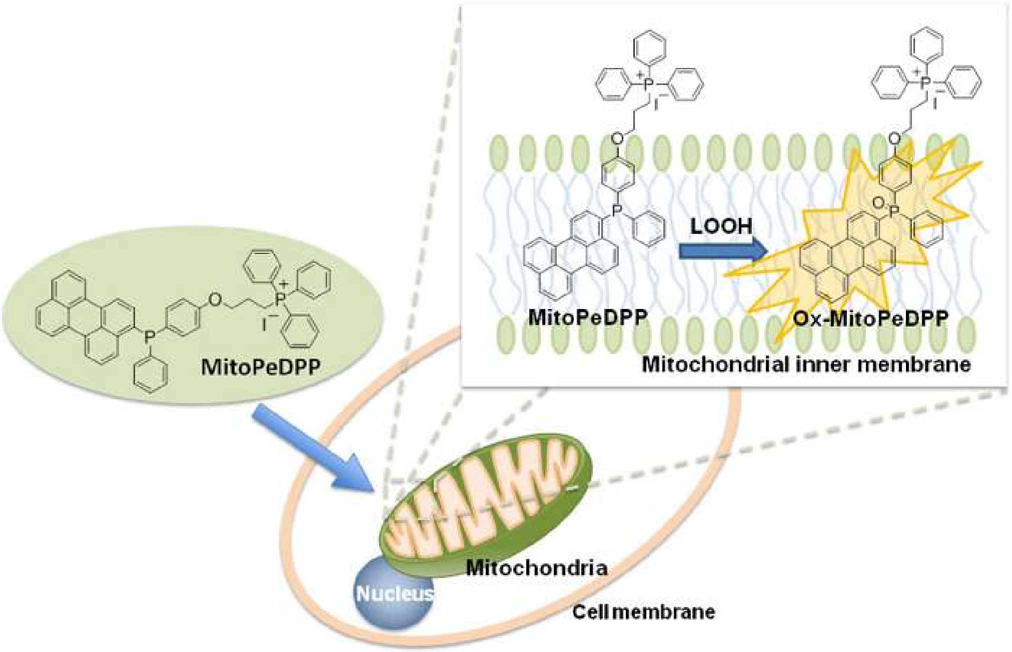


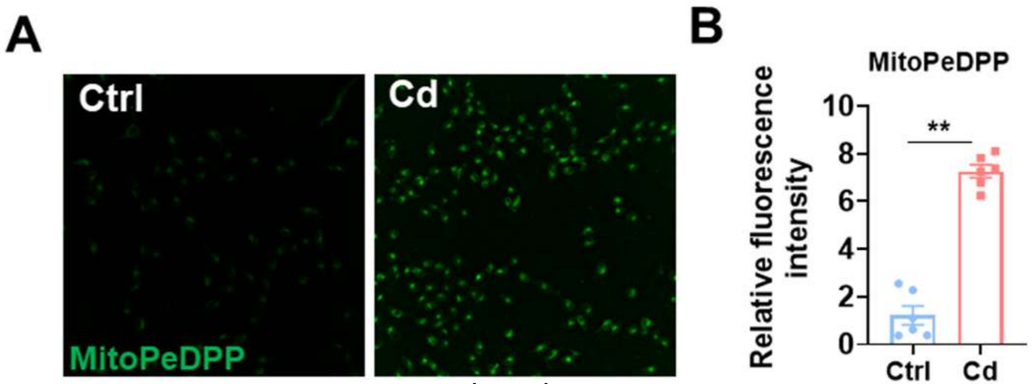
ConA: membrane staining
DFO and Fer-1 : ferroptosis inhibitors (iron chelator and RTA respectively)

Magtanong, *et al.* 2019

How can we quantify lipid peroxidation ?

Mitochondrial lipid peroxidation



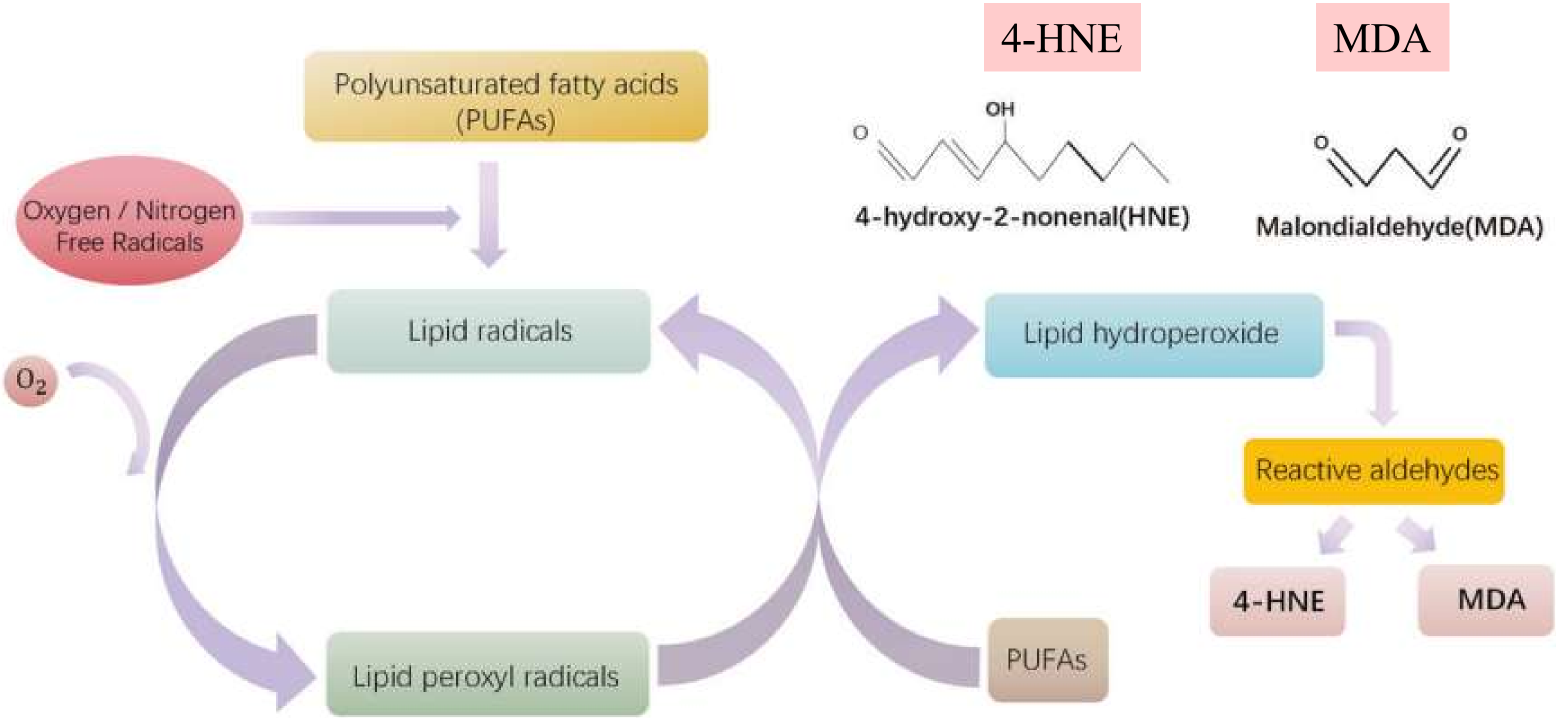


Cd: cadmium

https://www.dojindo.com/EUROPE/products/M466/

Guo *et al.*, 2024
https://doi.org/10.1016/j.redox.2024.103179

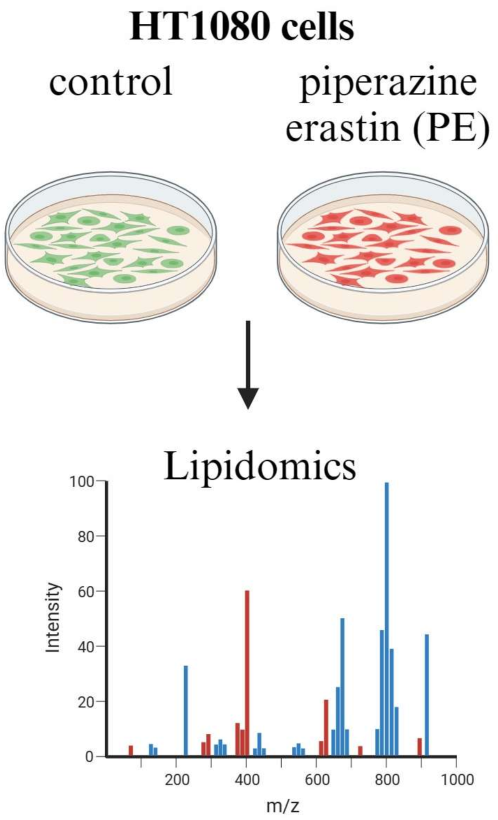
4-HNE and MDA are markers of lipid peroxidation



4-HNE and MDA are end products of lipid peroxidation

Zhang *et al*, 2023
https://doi.org/10.1038/s41420-023-01613-9

Peroxidation of PUFAs drives ferroptosis

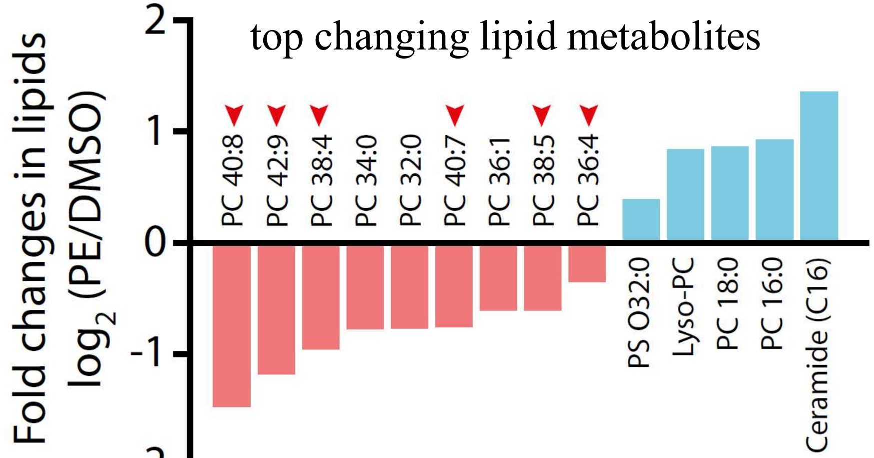


Peroxidation of polyunsaturated fatty acids by lipoxygenases drives ferroptosis

Wan Seok Yang[a,1], Katherine J. Kim[b], Michael M. Gaschler[c], Milesh Patel[d], Mikhail S. Shchepinov[e], and Brent R. Stockwell[b,c,1]

[a]Department of Biological Sciences, St. John's University, Queens, NY 11439; [b]Department of Biological Sciences, Columbia University, New York, NY 10027; [c]Department of Chemistry, Columbia University, New York, NY 10027; [d]Department of Medicine, New York University School of Medicine, New York, NY 10016; and [e]Retrotope, Inc., Los Altos, CA 94022

Edited by Benjamin F. Cravatt, The Scripps Research Institute, La Jolla, CA, and approved July 5, 2016 (received for review February 26, 2016)



Decreased levels of phospholipids containing PUFAs in cells undergoing ferroptosis

PE: a ferroptosis inducer

PC: phosphatidyl choline

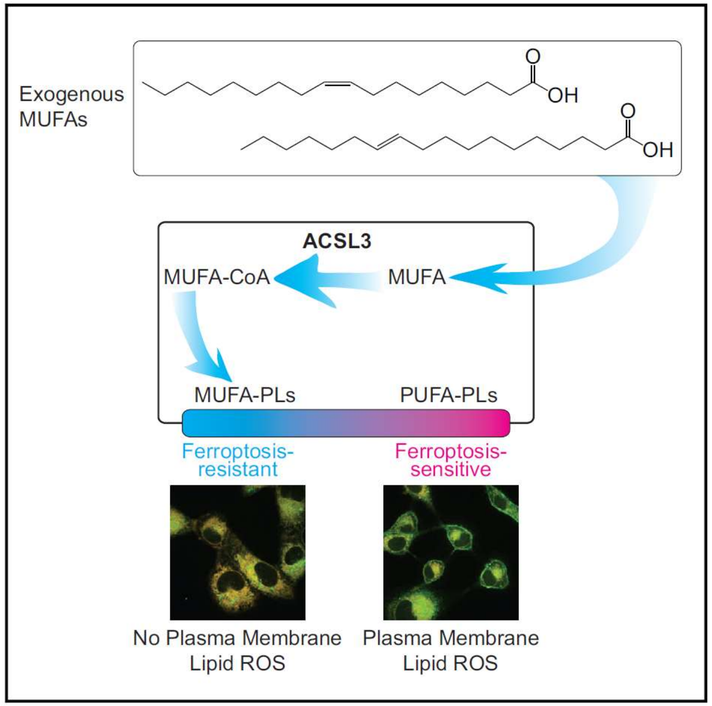
Yang *et al*, 2016

Article

Cell Chemical Biology

Exogenous Monounsaturated Fatty Acids Promote a Ferroptosis-Resistant Cell State

Graphical Abstract



Authors

Leslie Magtanong, Pin-Joe Ko, Milton To, ..., Daniel K. Nomura, James A. Olzmann, Scott J. Dixon

Correspondence

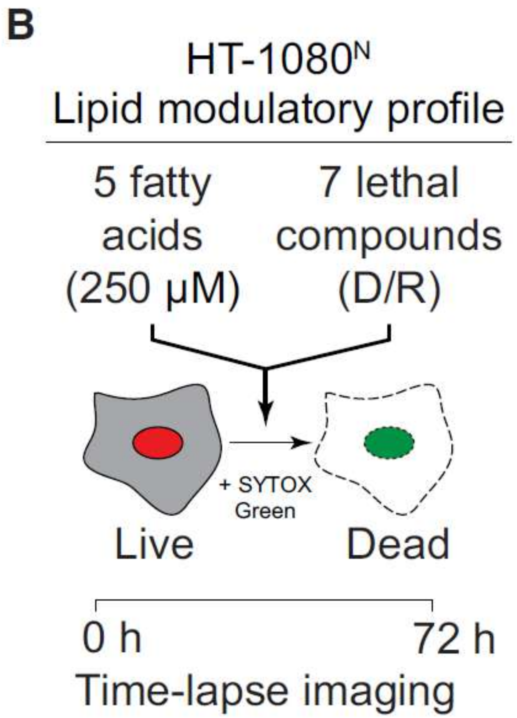
sjdixon@stanford.edu

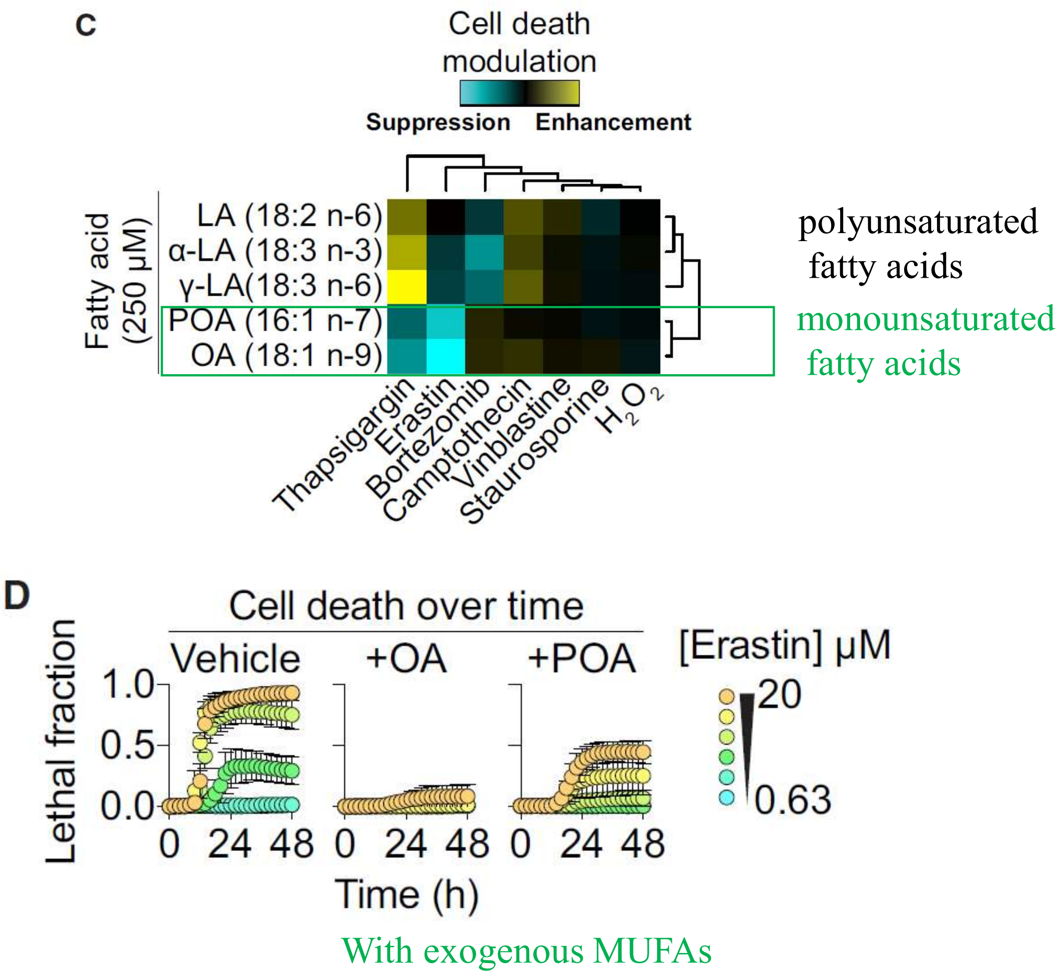
In Brief

Exogenous lipids can modulate both apoptotic and non-apoptotic cell death. Here we show that exogenous monounsaturated fatty acids can suppress the non-apoptotic process of ferroptosis by promoting the displacement of polyunsaturated fatty acids from plasma membrane phospholipids in an ACSL3-dependent manner.

MUFAs suppress erastin-induced cell-death

The experiment



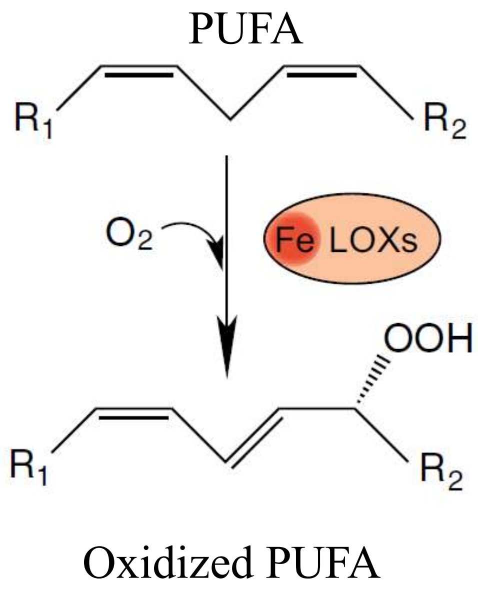


Magtanong *et al*, 2016

Role of lipoxygenases in ferroptosis ?

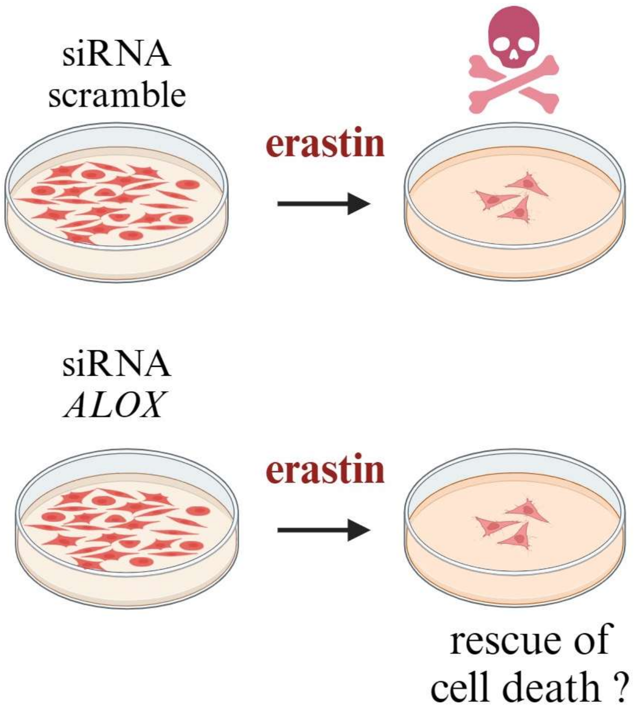
Lipoxygenases (**LOX)**

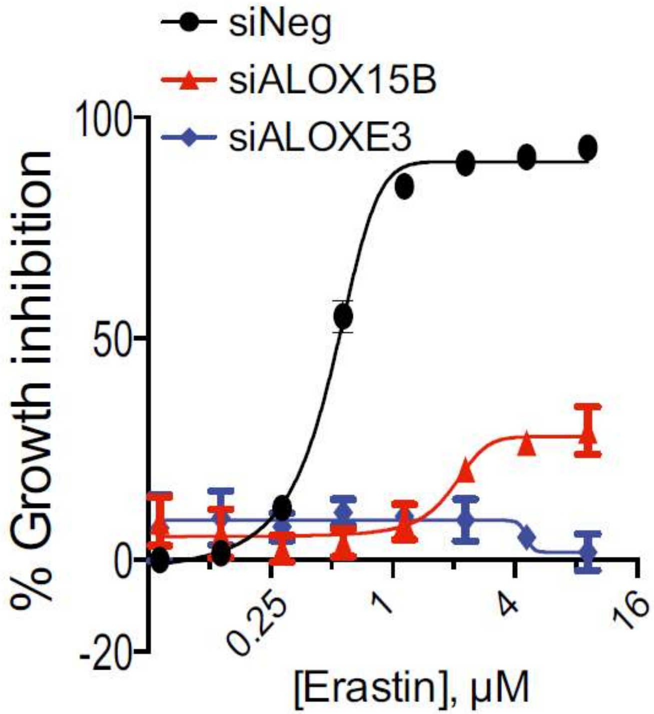
- Iron-containing enzymes that catalyze the oxidation of PUFAs
- Can promote ferroptosis



Yang *et al*, 2016

Role of lipoxygenases in ferroptosis ?

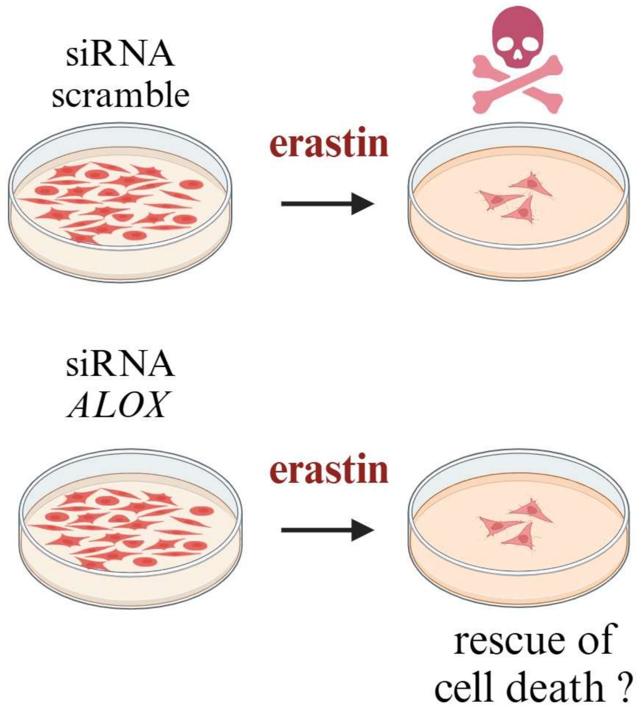


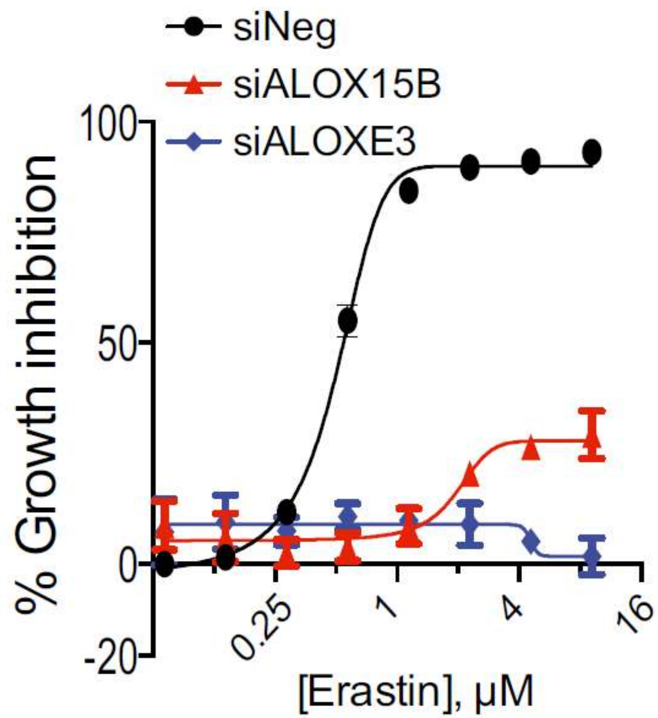


Conclusion ?

Yang *et al*, 2016

Role of lipoxygenases in ferroptosis ?





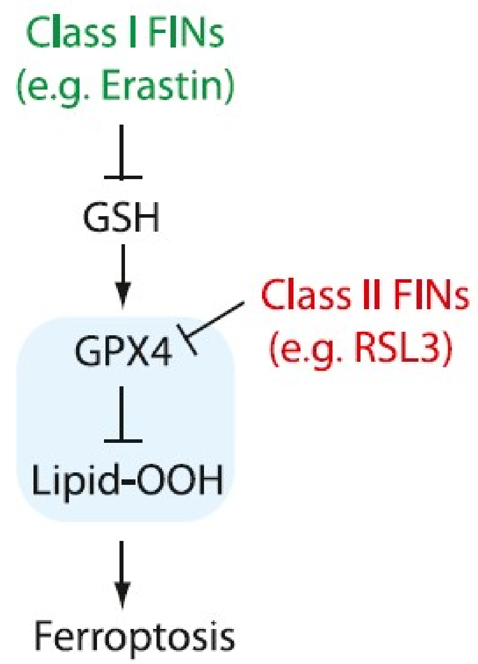
Erastin-induced cell-death is rescued by the silencing of ALOX15B and ALOXE3

Yang *et al*, 2016

Ferroptosis inducers and their mode of action

Ferroptosis inducers (FIN)

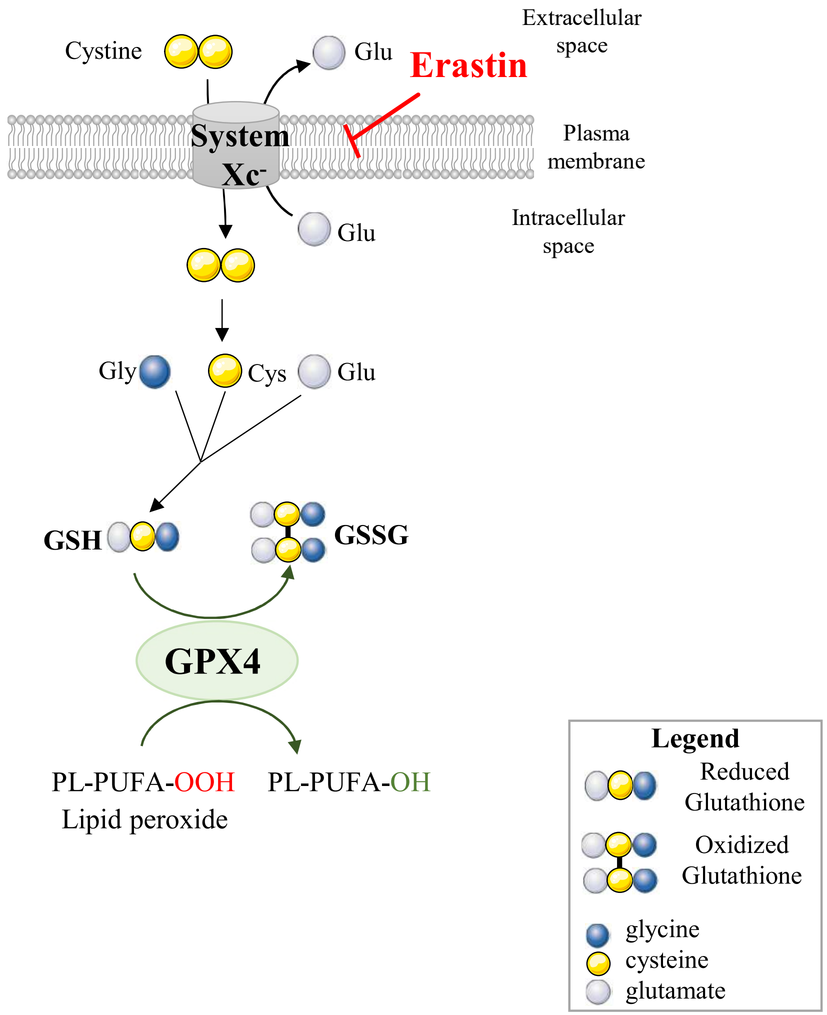
- Class I FINs involve cellular glutathione (GSH) depletion, class II FINs lack this characteristic.
- Class II FINs trigger ferroptosis through inhibition of glutathione peroxidase 4.



Yang *et al*., Cell, 2013. http://dx.doi.org/10.1016/j.cell.2013.12.010

Erastin inhibits cystine uptake

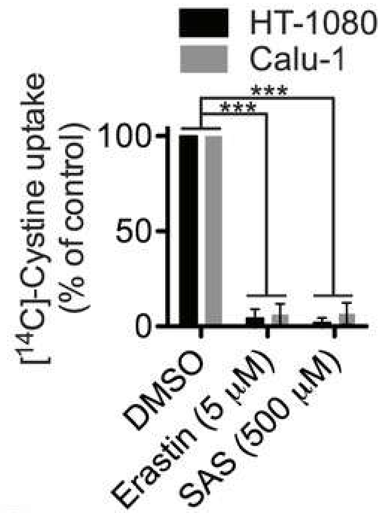
- Discovered in 2003
- Inhibits cystine/glutamate antiporter (system Xc-)
- Triggers GSH depletion by inhibiting the entry of cystine



Erastin inhibits cystine uptake

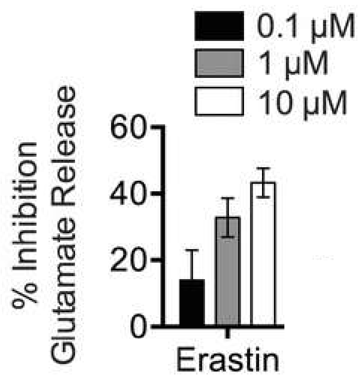
How to show the inhibition of the system Xc^-?

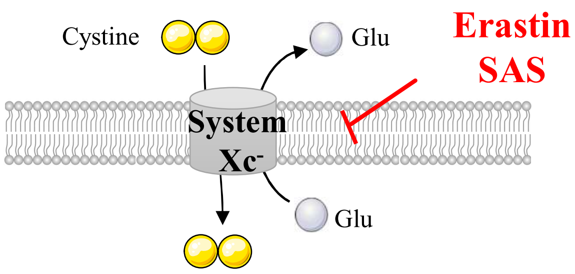
Assay for cystine uptake



Assay for glutamate release

enzyme-coupled fluorescent assay



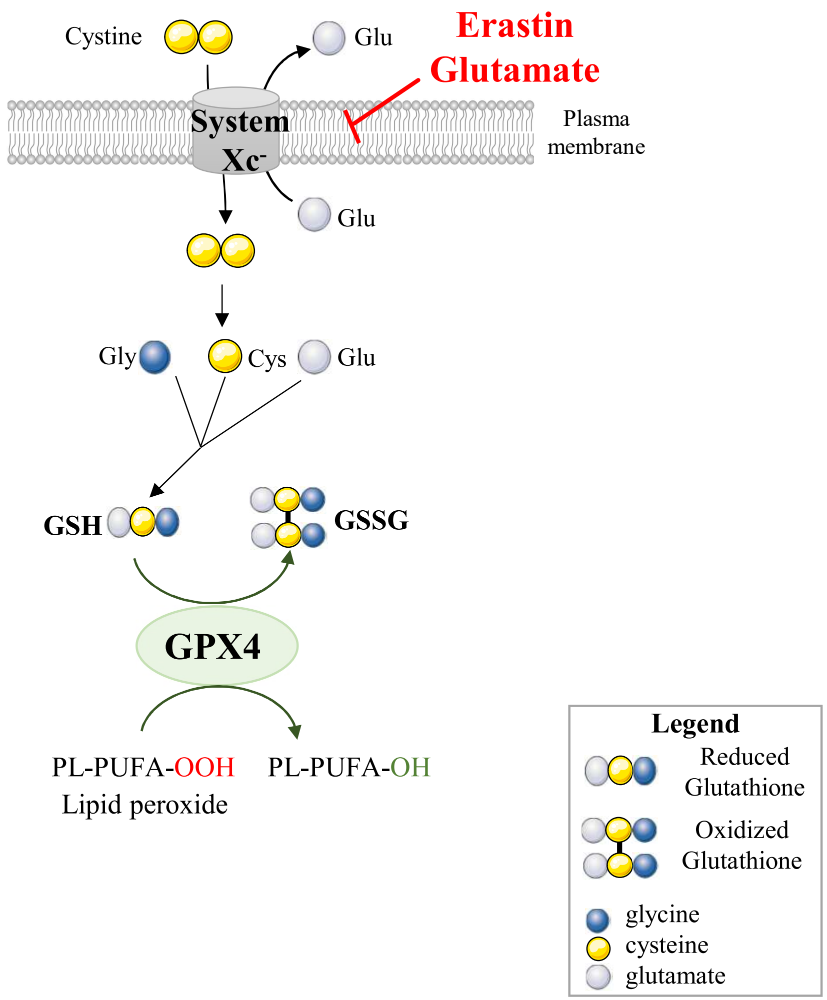


SAS: sulfasalazine (system Xc^- inhibitor)

Dixon, *et al*. 2014

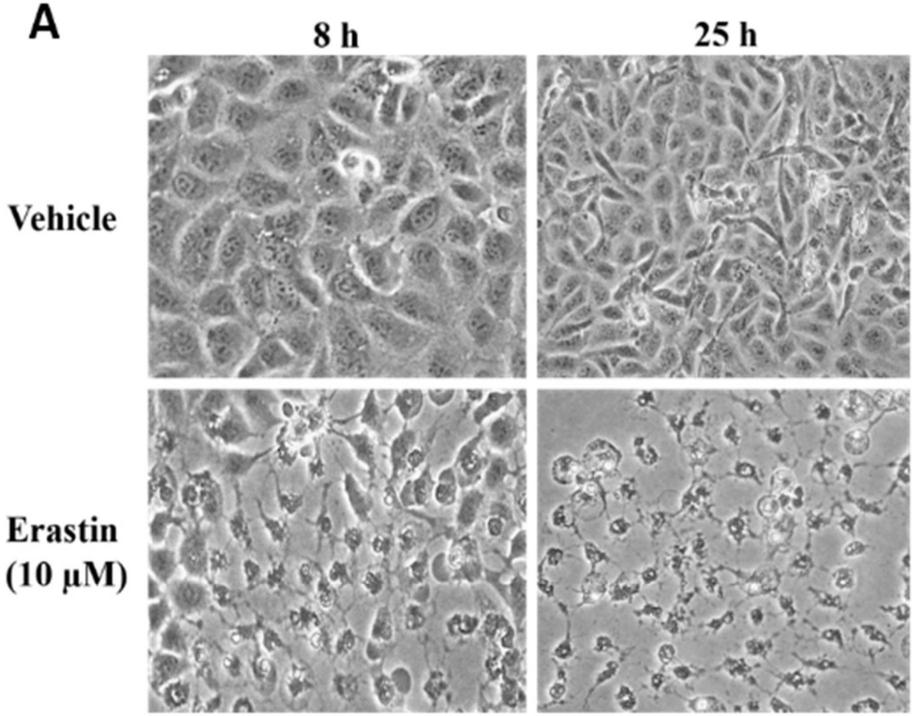
Glutamate can induce ferroptosis

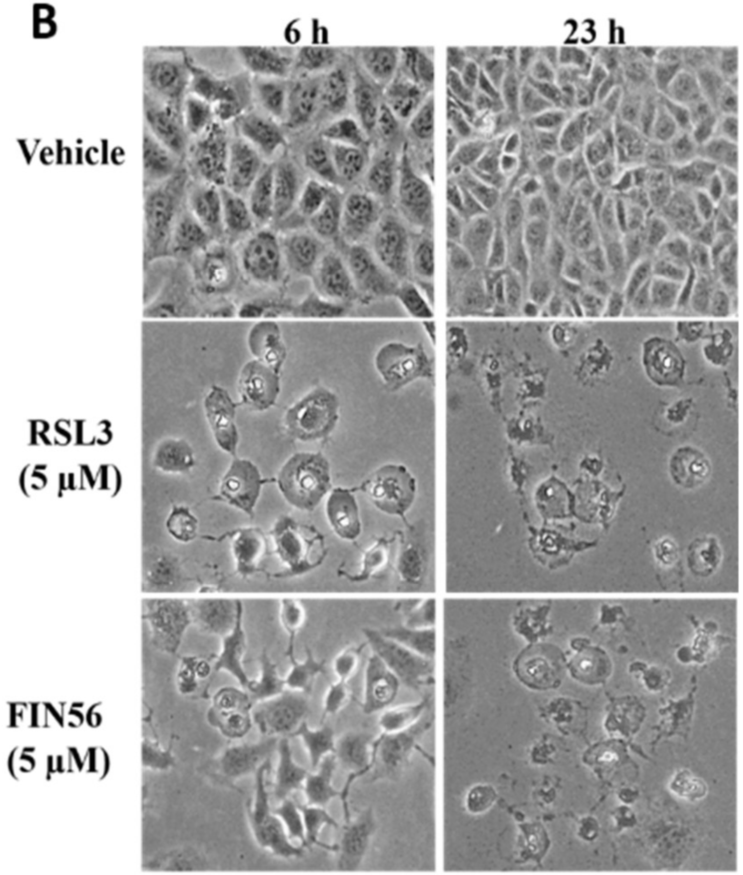
- Glutamate excitotoxicity in neurons
- Glu in excess inhibits the system Xc^-



RSL3 and FIN56 are ferroptosis inducers

HT1080 cells

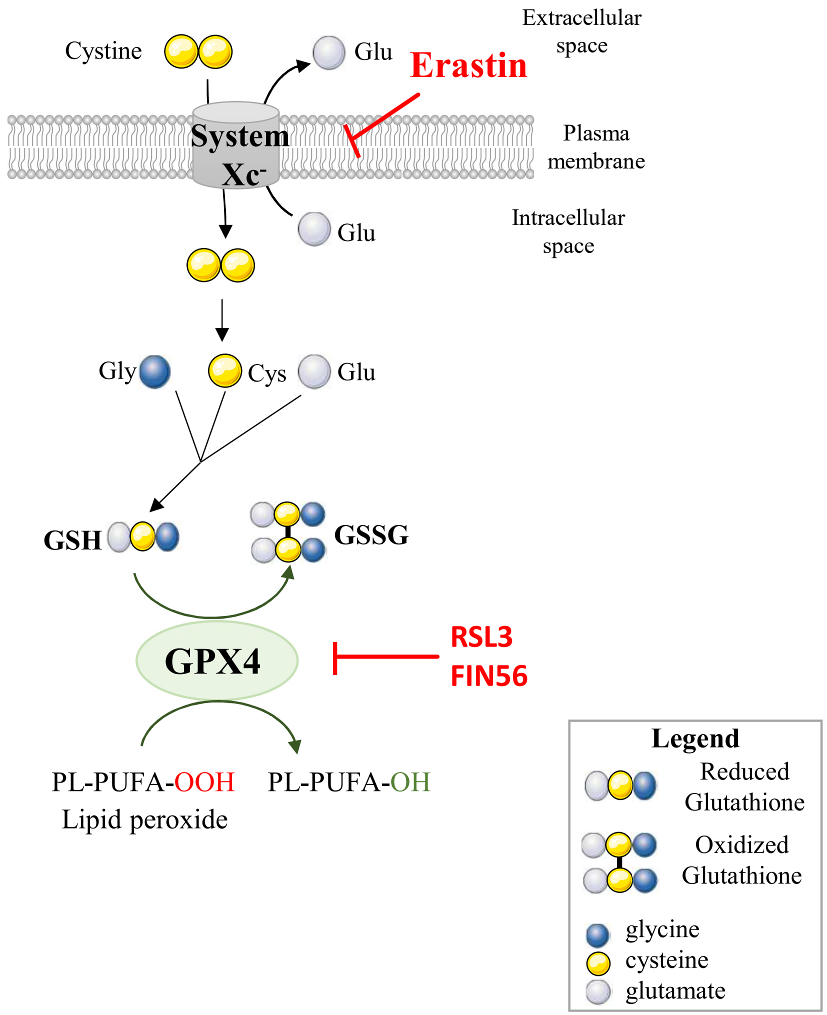




N. Le Floch-Leleu, unpublished data

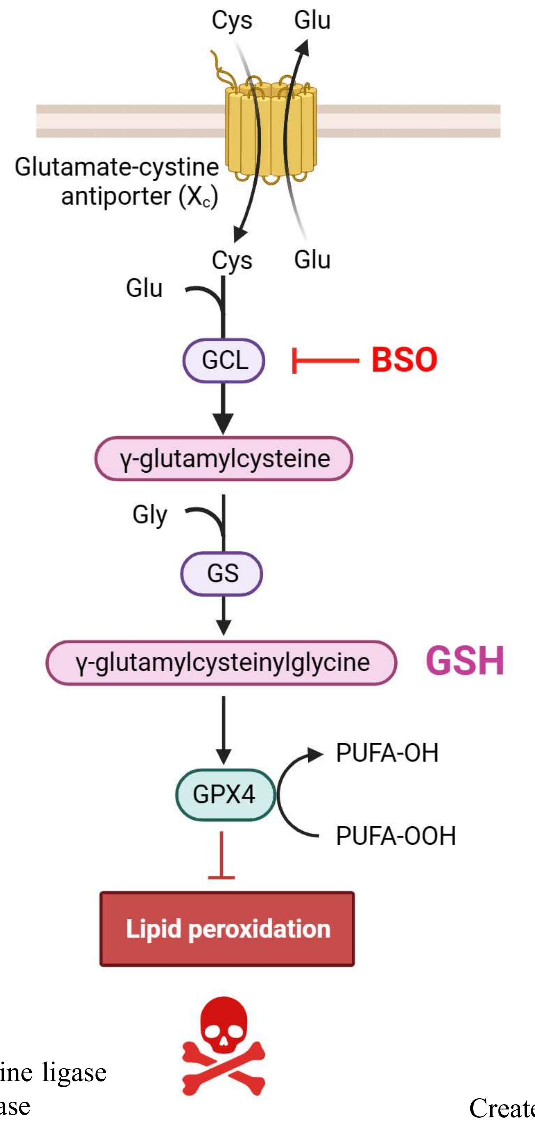
RSL3 and FIN56 are class II FINs

- RSL3 and FIN56 do not deplete GSH
- RSL3 directly inhibits GPX4
- FIN56 decreases GXP4 stability



BSO induces ferroptosis by depleting GSH

BSO inhibits glutamate-cysteine ligase leading to decreased GSH synthesis



GCL: glutamate-cysteine ligase
GS: glutathione synthase

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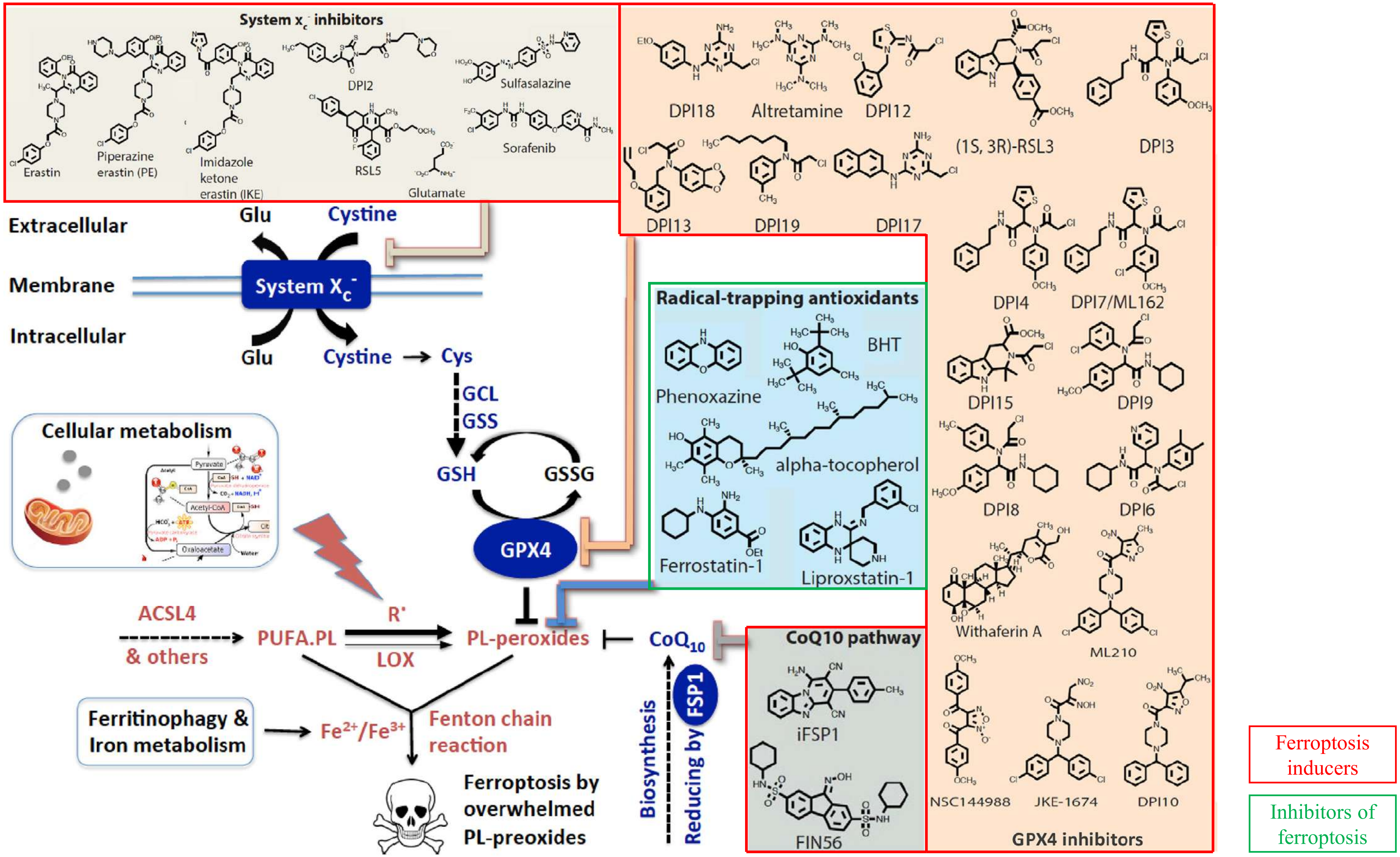


Figure 1. The Ferroptosis Pathway and Relevant Chemical Probes

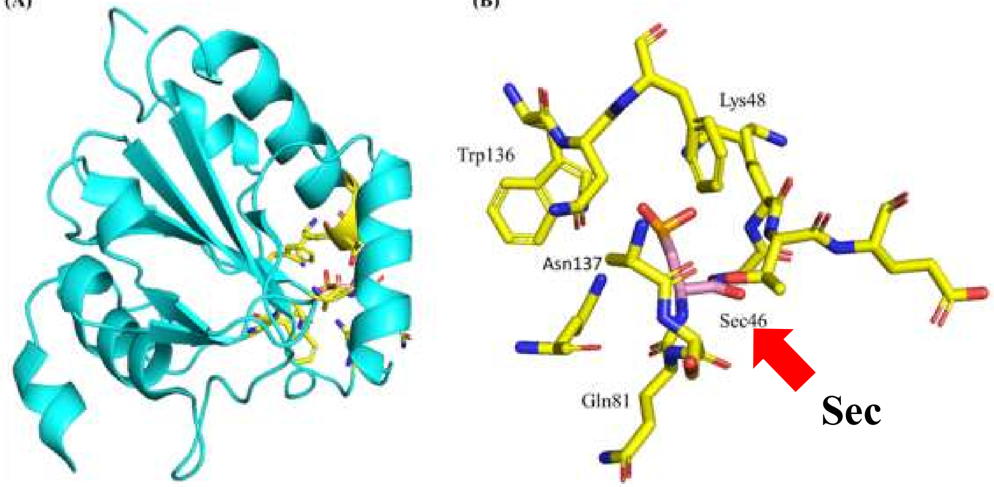
Adapted from Stockwell and Jiang, 2019. https://doi.org/10.1016/j.chembiol.2020.03.013

Glutathione Peroxidase 4 (GPX4) is the central regulator of ferroptosis

GPX4 is a central regulator of ferroptosis

- Glutathione Peroxidase 4 is a member of the GPX family (8 members)
- Oxidoreductase activity
- Sole member of the GPX family able to eliminate complex hydroperoxides such as lipid peroxides
- Requires glutathione for its activity (GSH)
- Its catalytic site contains a selenocysteine (Sec)

GPX4 structure — Active site



Adapted from Weaver and Skouta, 2022
https://doi.org/10.3390/biomedicines10040891

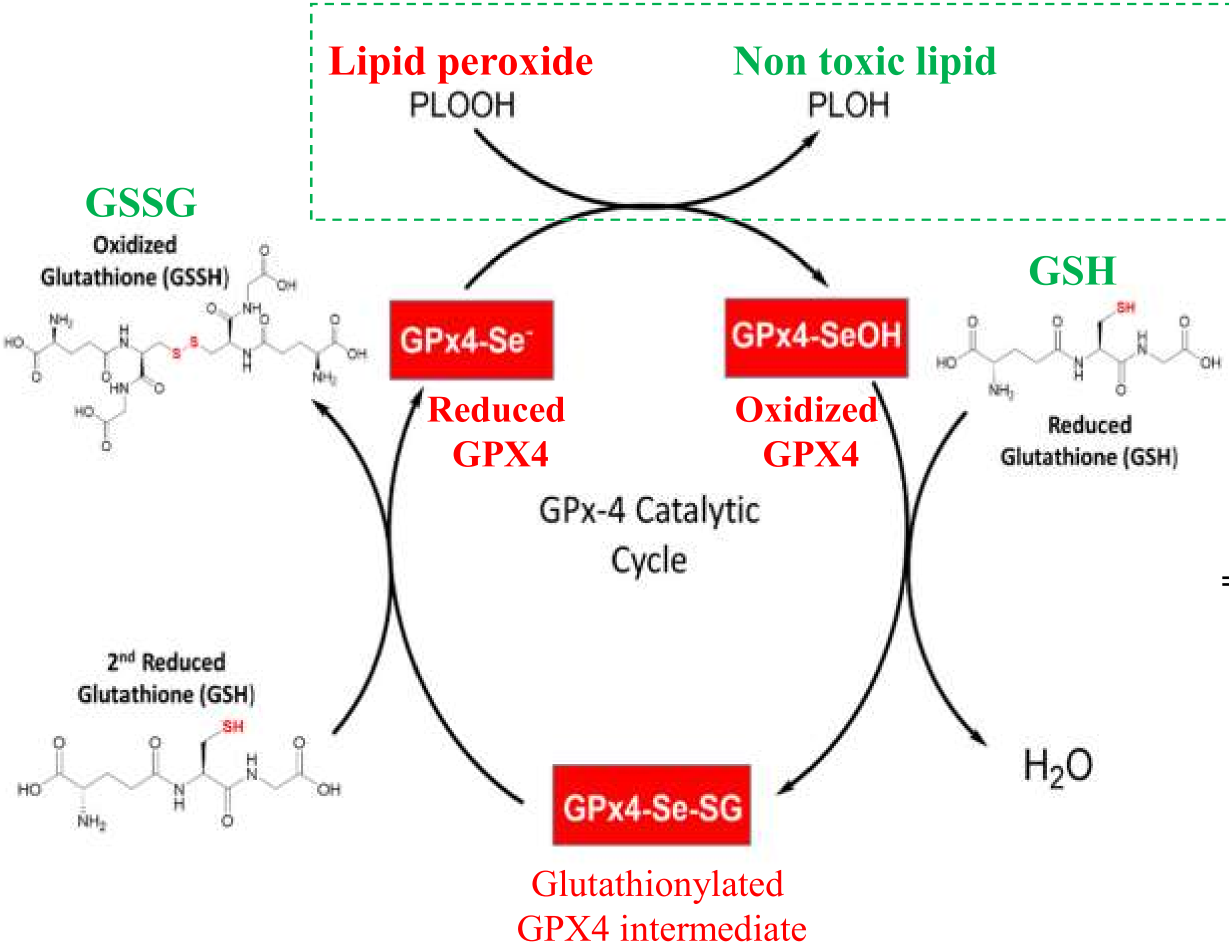
biomedicines MDPI

Review

The Selenoprotein Glutathione Peroxidase 4: From Molecular Mechanisms to Novel Therapeutic Opportunities

Kamari Weaver and Rachid Skouta *

GPX4 peroxidase reaction cycle



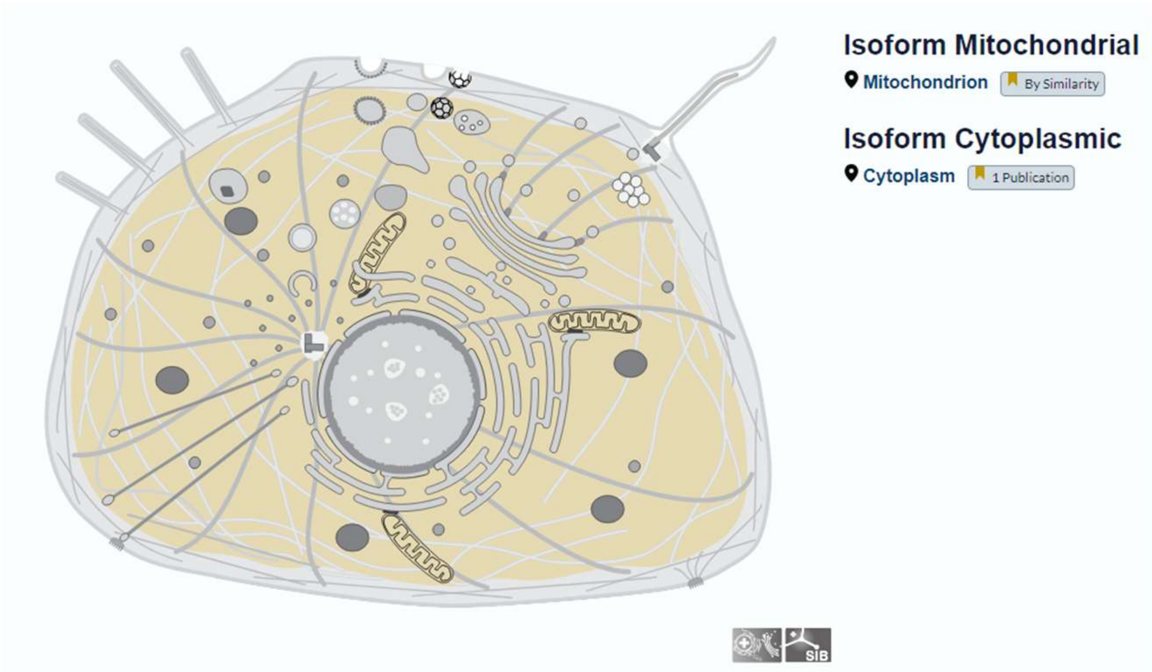
Reduction of toxic lipid peroxides (detoxification)

2 equivalents of GSH are required to reduce Se at the active site

=> GSH is an essential co-factor for GPX4

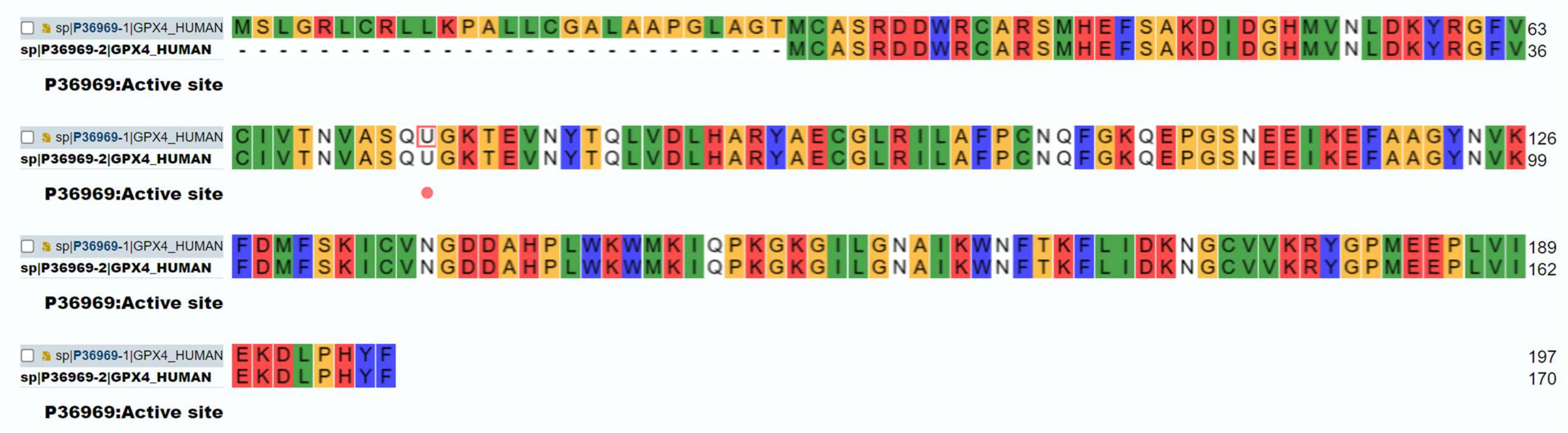
Adapted from Weaver and Skouta, 2022
https://doi.org/10.3390/biomedicines10040891

GPX4 : two isoforms with different subcellular locations



https://www.uniprot.org/uniprotkb/P36969/entry#subcellular_location

GPX4 : two isoforms with different subcellular locations

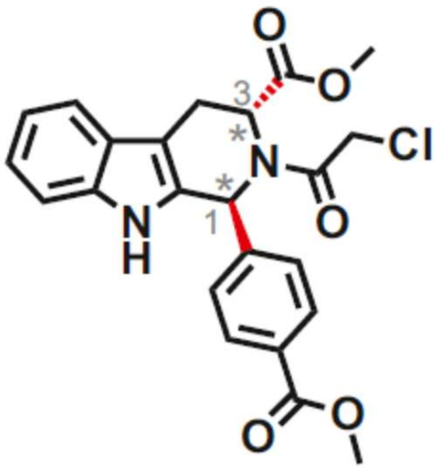


Alignment of the two GPX4 isoforms produced by alternative initiation of translation

Upper lane : mitochondrial isoform
Lower lane: cytosolic isoform (27 aa shorter)
Alignment done using the Align 2 sequences tool from Uniprot (accessed on 01 november 2023)

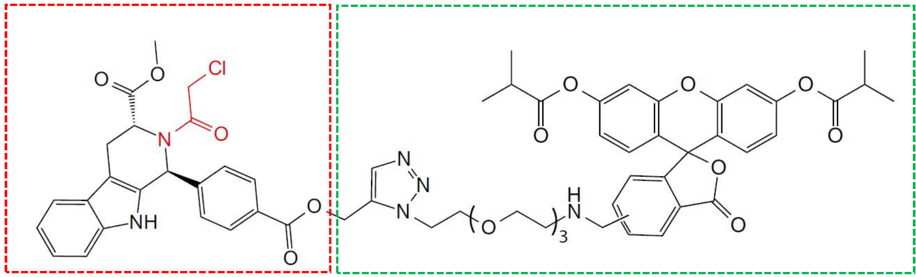
RSL3 inhibits GPX4 through binding to its active site

- RSL3 was the first GPX4 inhibitor to be identified
- RSL3 interacts with GPX4 active site

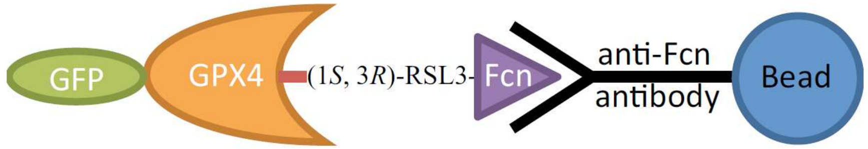


(1*S*,3*R*)-RSL3

Pull-down assay using a (1S, 3R)-RSL3-fluorescein probe



(*1S, 3R*)-RSL3-Fcn



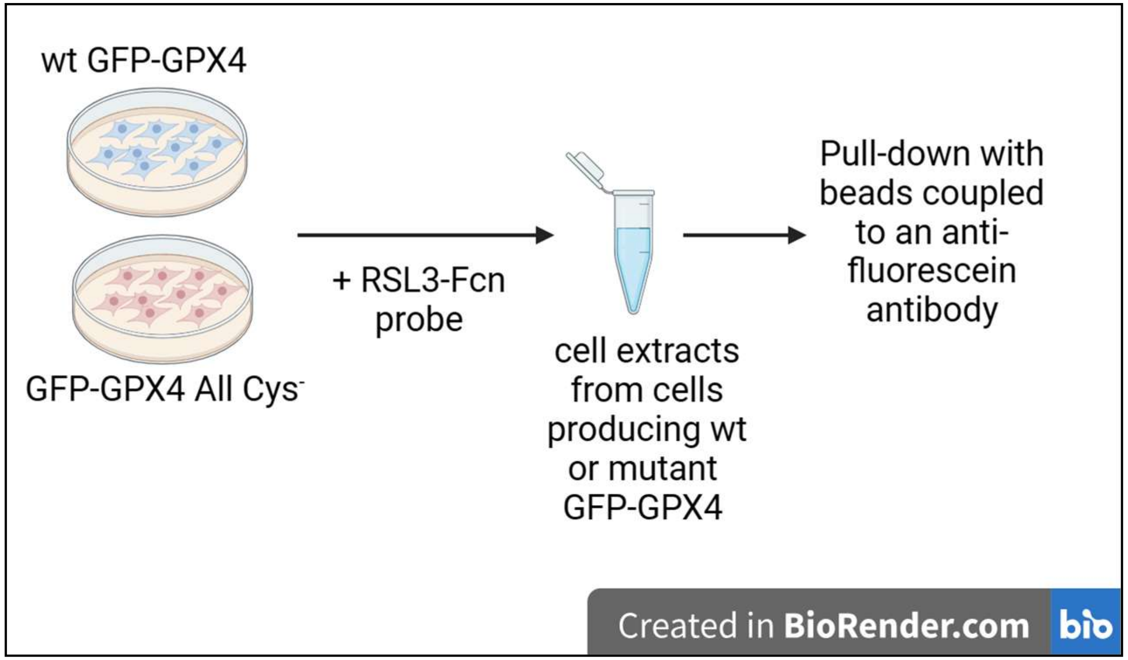
Affinity complex formed during the pull-down

Adapted from Yang *et al*, PNAS, 2016

RSL3 inhibits GPX4 through binding to its active site

- RSL3 was the first GPX4 inhibitor to be identified
- RSL3 interacts with GPX4 active site

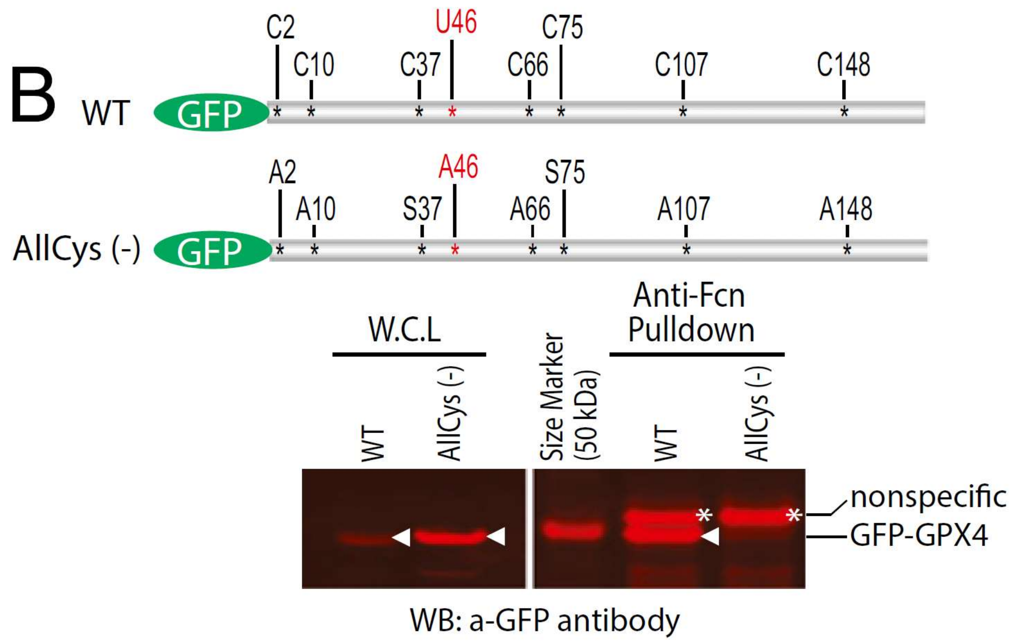
Pull-down assay using a
(1S, 3R)-RSL3-fluorescein probe



Adapted from Yang *et al*, PNAS, 2016

RSL3 inhibits GPX4 through binding to its active site

- RSL3 was the first GPX4 inhibitor to be identified (2008)
- RSL3 interacts with GPX4 active site

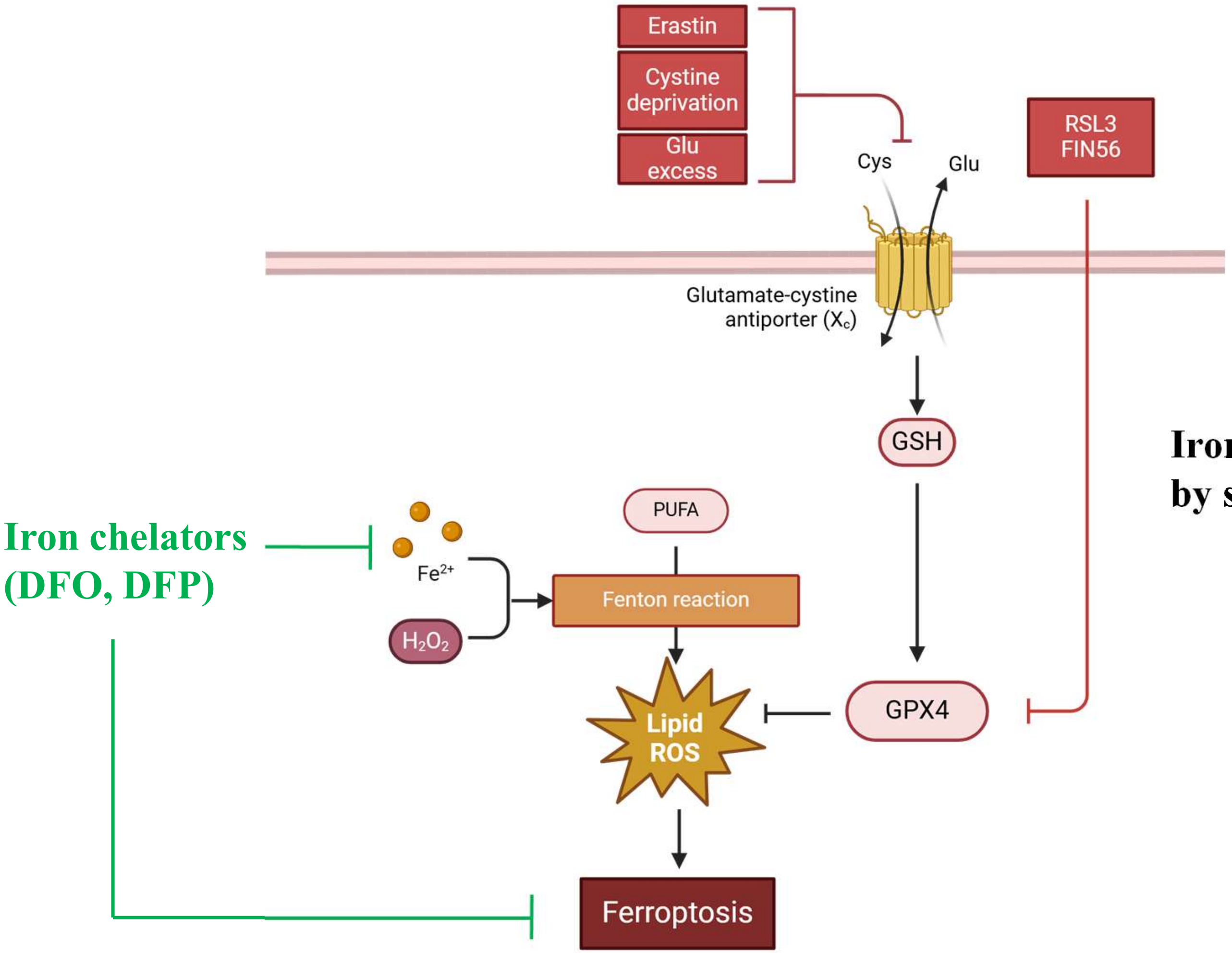


RSL3 does not bind to a mutant GPX4 where the active-site selenocysteine and all other cysteines were replaced with either alanine or serine

Adapted from Yang *et al*, PNAS, 2016

The role of iron in ferroptosis

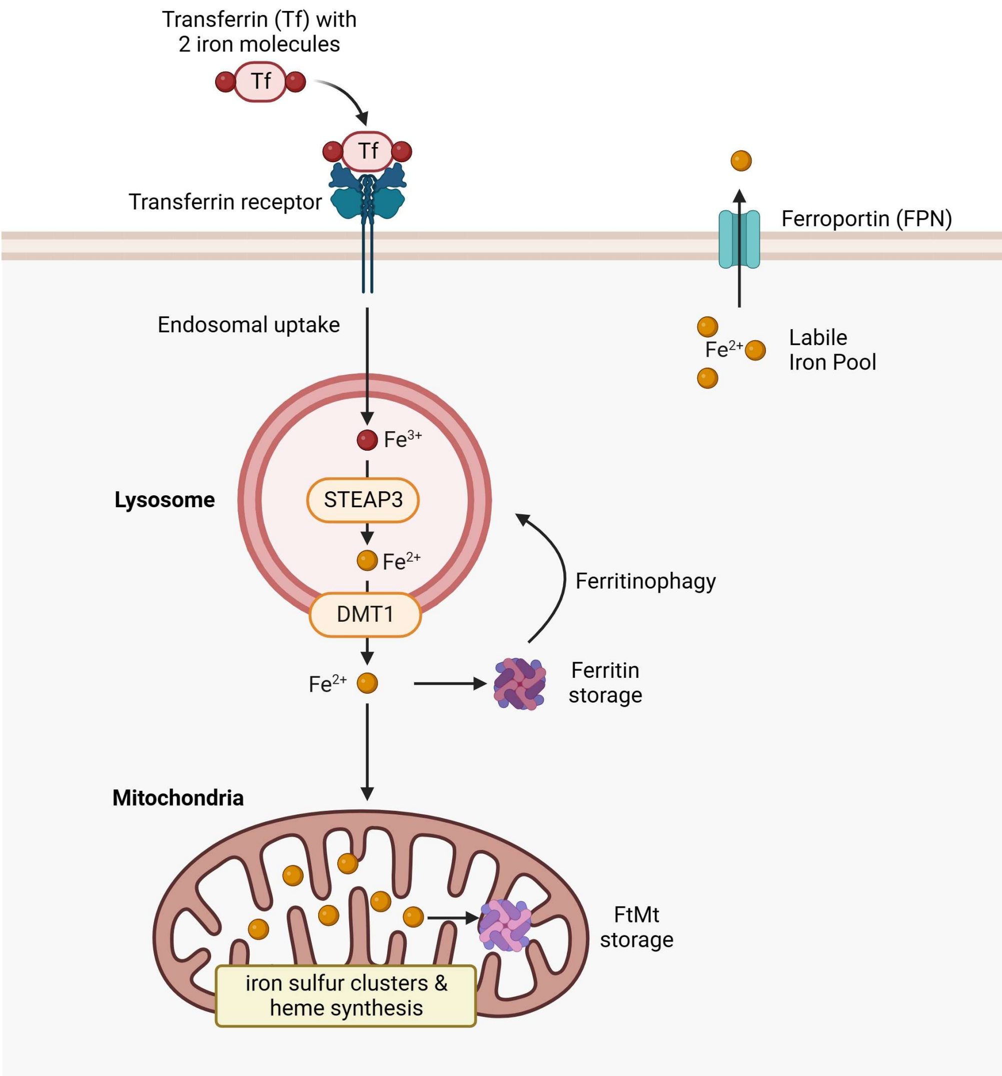
High levels of iron are required for ferroptosis



Iron chelators inhibit ferroptosis whether induced by system Xc- inhibition or direct GPX4 inhibition

Created with BioRender.com

Iron metabolism

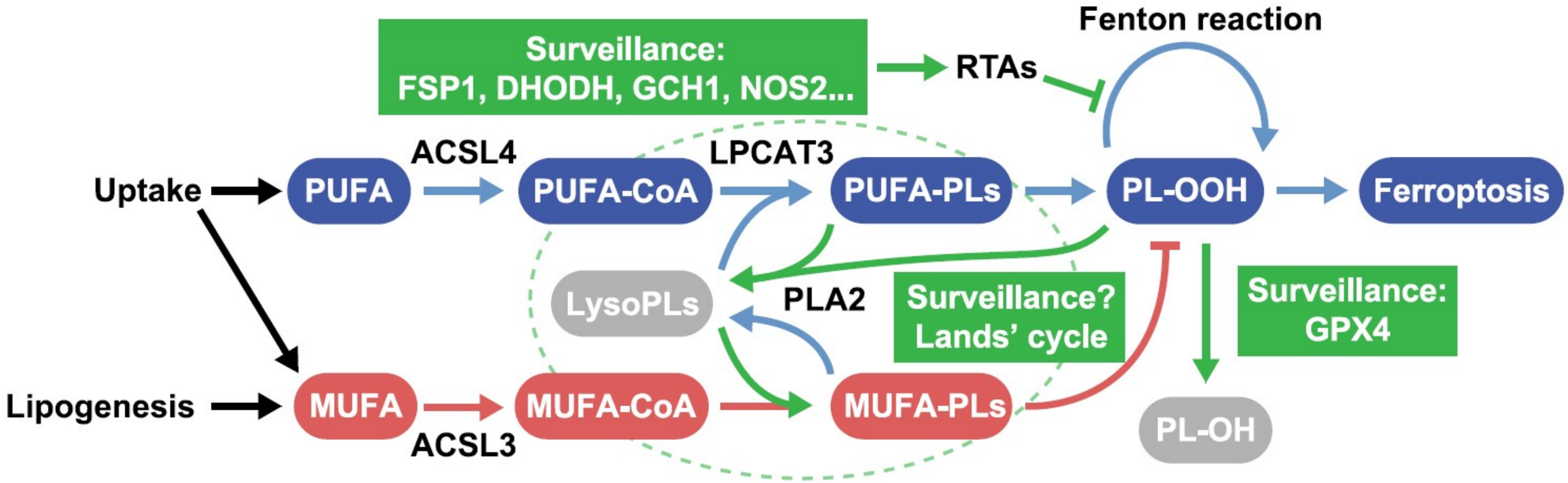


- Lysosomes play a key role in iron metabolism
- Ferrous iron (Fe^{2+}) is highly reactive and stored in ferritin nanocages (Fe^{3+})
- Mitochondria are the main site of iron utilization

Created with BioRender.com

Ferroptosis inhibition

Ferroptosis surveillance



Mono Unsaturated Fatty Acid

Poly Unsaturated Fatty acid

https://doi.org/10.1016/j.molcel.2022.03.022

Article | Published: 21 October 2019

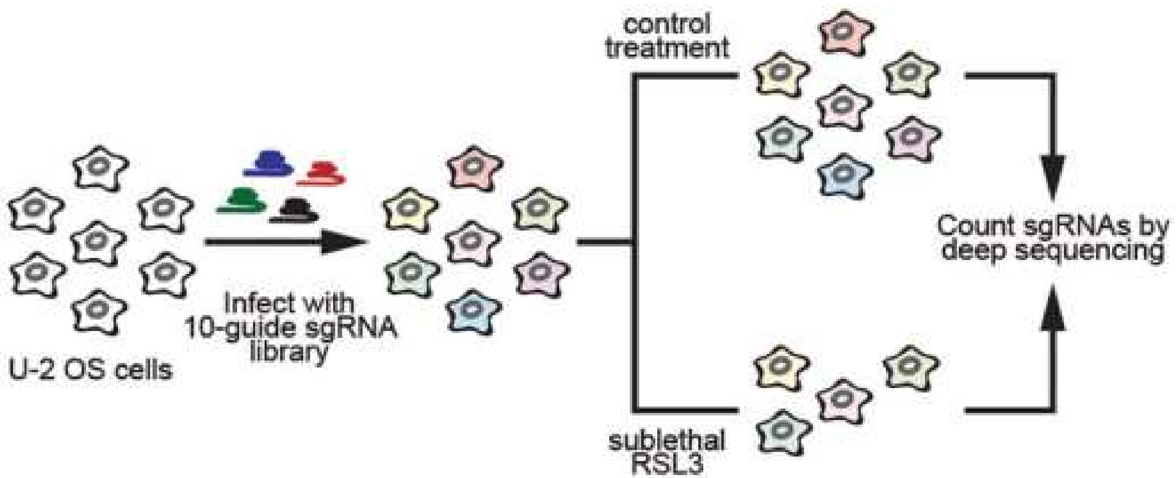
The CoQ oxidoreductase FSP1 acts parallel to GPX4 to inhibit ferroptosis

Kirill Bersuker, Joseph M. Hendricks, Zhipeng Li, Leslie Magtanong, Breanna Ford, Peter H. Tang, Melissa A. Roberts, Bingqi Tong, Thomas J. Maimone, Roberto Zoncu, Michael C. Bassik, Daniel K. Nomura, Scott J. Dixon & James A. Olzmann

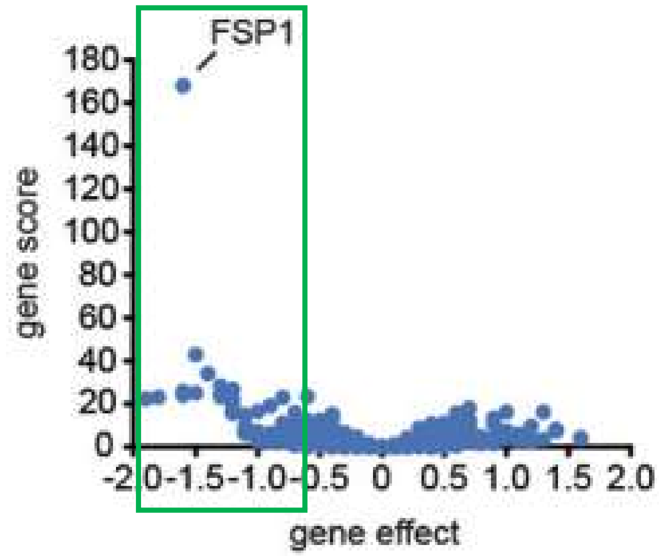
Nature **575**, 688–692 (2019) | Cite this article

Ferroptosis inhibition by FSP1

FSP1 was identified as a ferroptosis inhibitor in a sgRNA screen on U-2 OS cancer cells treated with RSL3

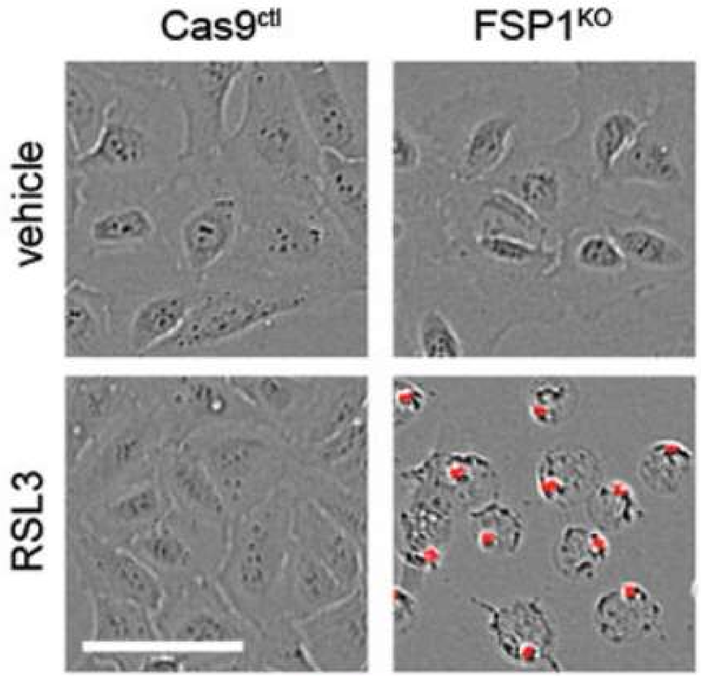


The loss of these genes sensitizes cell to RSL3



Bersuker *et al*, 2019.

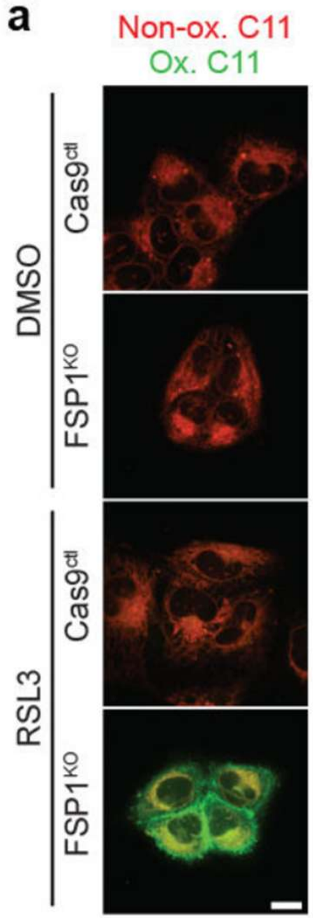
Ferroptosis inhibition by FSP1



Sublethal dose of RSL3
100 nM / 48 h

SYTOX

The loss of FSP1 promotes lipid peroxidation and ferroptosis.



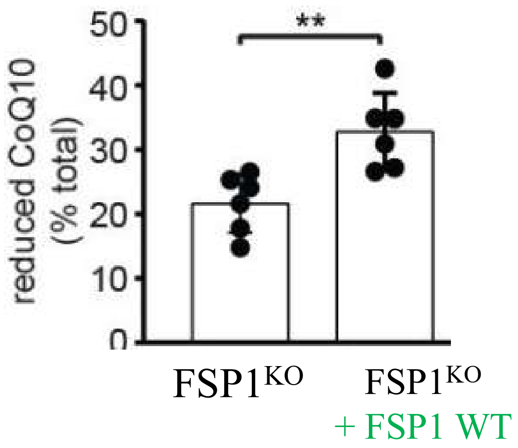
RSL3 treatment (75 min)

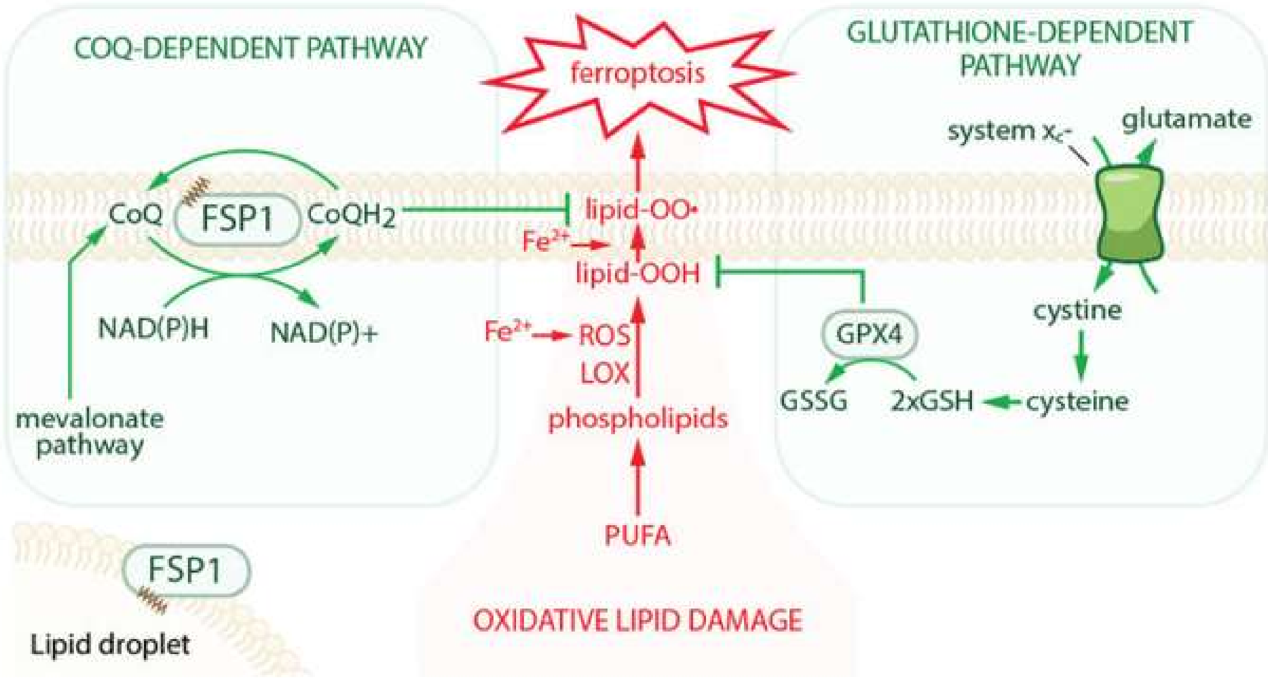
BODIPY 581/591 C11
(lipid peroxidation)

Bersuker *et al*, 2019.

Ferroptosis inhibition by FSP1

FSP1 reduces CoQ10, generating a radical-trapping agent (reduced CoQ10)

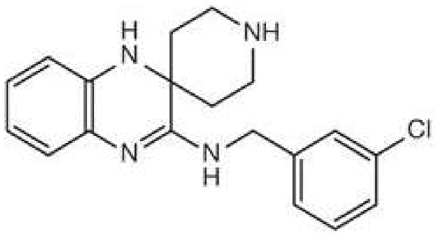


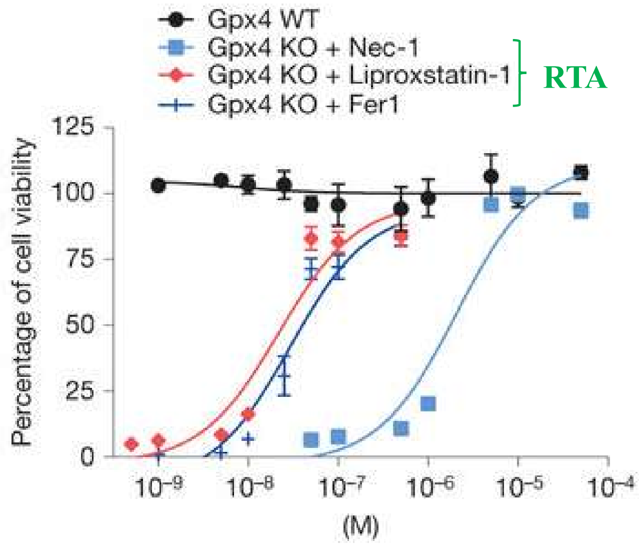


Bersuker *et al*, 2019.

Ferroptosis is inhibited by radical-trapping agents (RTAs)

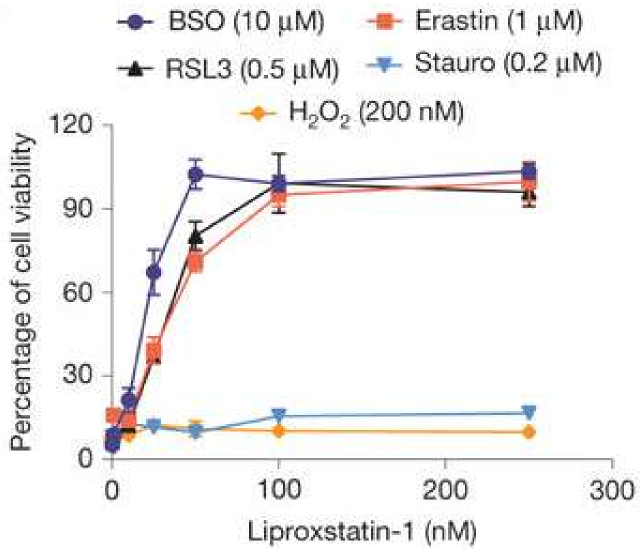
Liproxstatin-1





Nec-1: necroptosis inhibitor
Fer1: ferrostatin (radical-trapping agent)

GPX4 KO



Erastin, RSL3, BSO: ferroptosis inducers
Staurosporine: apoptosis inducer

GSH depletion / GPX4 inactivation

Conclusions?

Adapted from Friedmann Angeli *et al*, 2014

Part 2: What is the role of mitochondria in ferroptosis ?
OPA1 as a regulator of sensitivity to ferroptosis

CellPress Molecular Cell

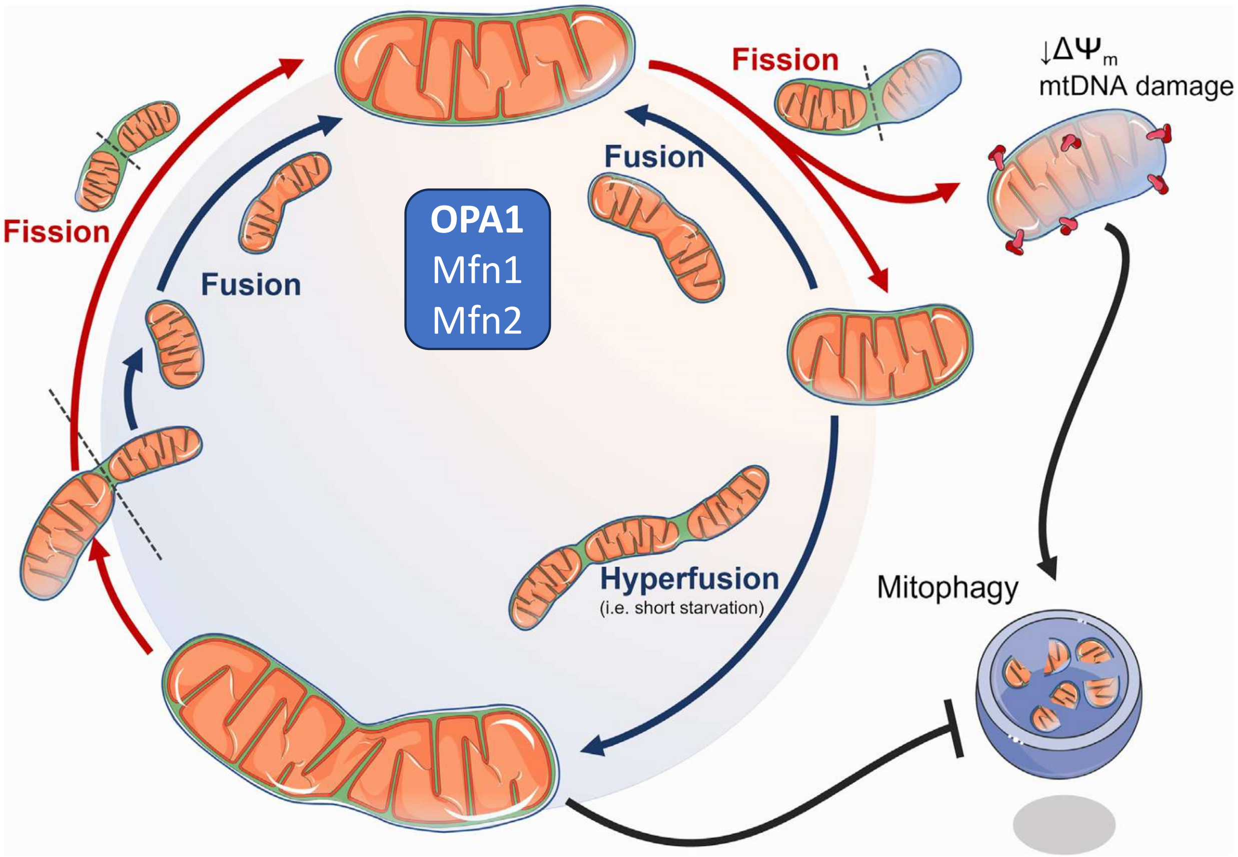
Article

OPA1 promotes ferroptosis by augmenting mitochondrial ROS and suppressing an integrated stress response

Felix G. Liang,[1,2,3] Fereshteh Zandkarimi,[4] Jaehoon Lee,[1,3] Joshua L. Axelrod,[1,2,3] Ryan Pekson,[1,3] Yisang Yoon,[6,7] Brent R. Stockwell,[4,5] and Richard N. Kitsis[1,2,3,8,*]

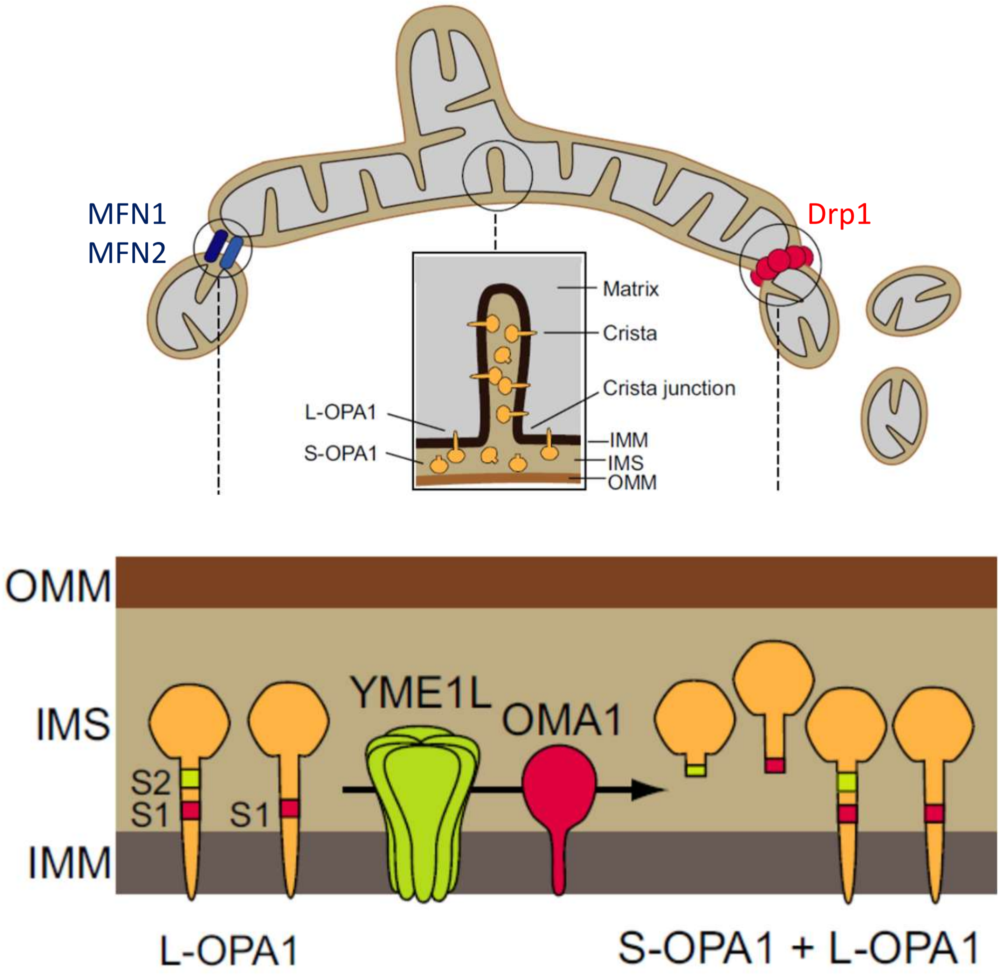
[1]Departments of Medicine, Albert Einstein College of Medicine, Bronx, NY, USA
[2]Departments of Cell Biology, Albert Einstein College of Medicine, Bronx, NY, USA
[3]Wilf Family Cardiovascular Research Institute, Albert Einstein College of Medicine, Bronx, NY, USA
[4]Department of Chemistry, Columbia University, New York, NY, USA
[5]Department of Biological Sciences, Columbia University, New York, NY, USA
[6]Department of Physiology, Medical College of Georgia, Augusta University, Augusta, GA, USA
[7]Deceased
[8]Lead contact
*Correspondence: richard.kitsis@einsteinmed.edu
https://doi.org/10.1016/j.molcel.2024.07.020

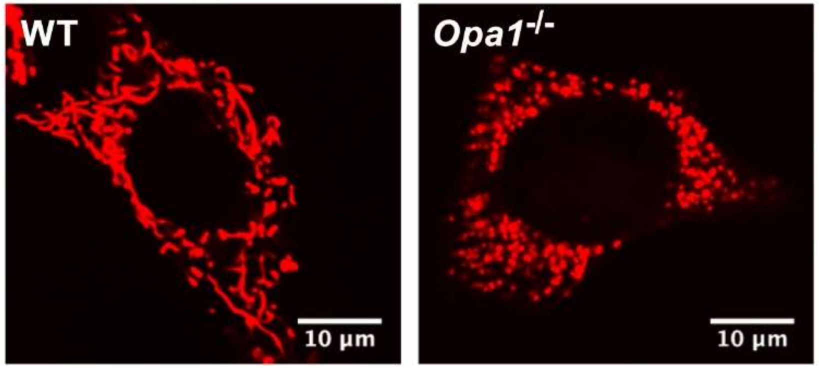
Fusion and fission control mitochondrial shape and activity



Quintana-Cabrera and Scorrano. Molecular Cell (2023).
https://doi.org/10.1016/j.molcel.2023.02.012

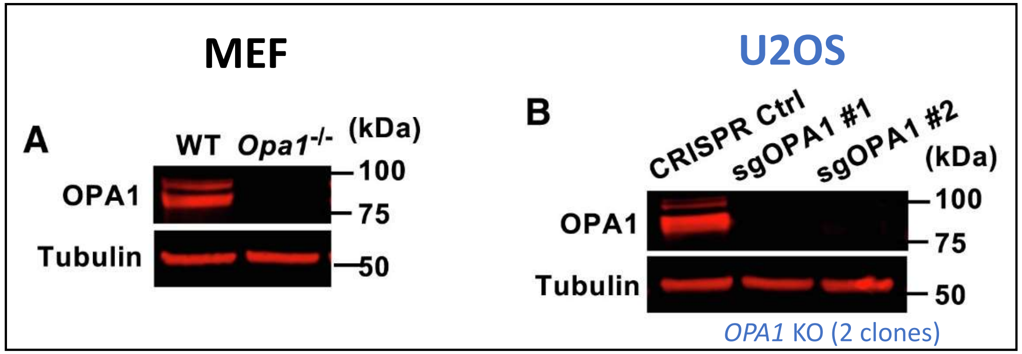
OPA1 is required for mitochondrial fusion





Adapted from MacVicar and Langer, JCellScience (2016) and Liang et al. Molecular Cell (2024)

The loss of OPA1 renders cells resistant to cell death induced by cysteine depletion



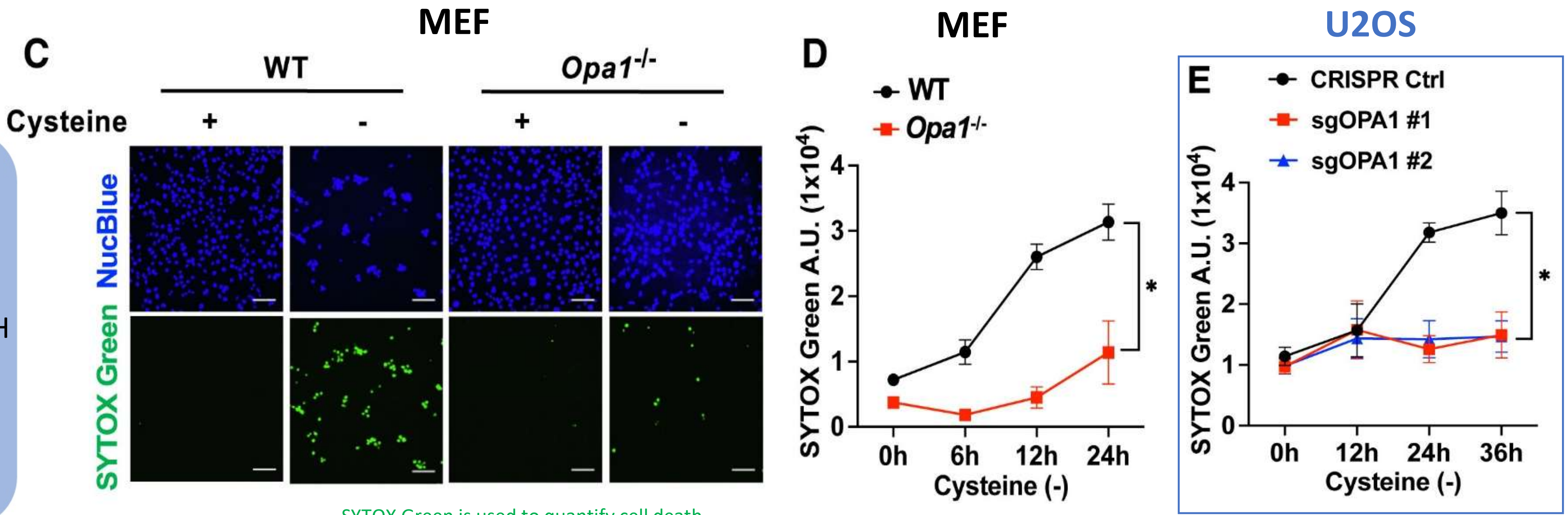
Cys depletion

↓

Low intracellular GSH

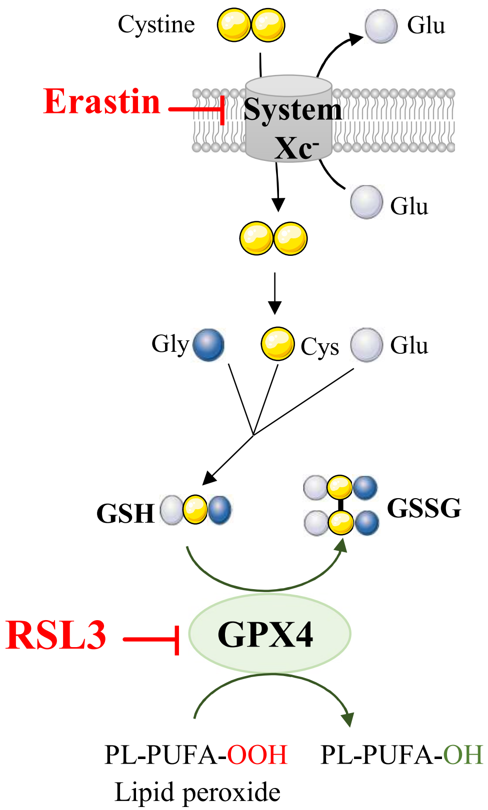
↓

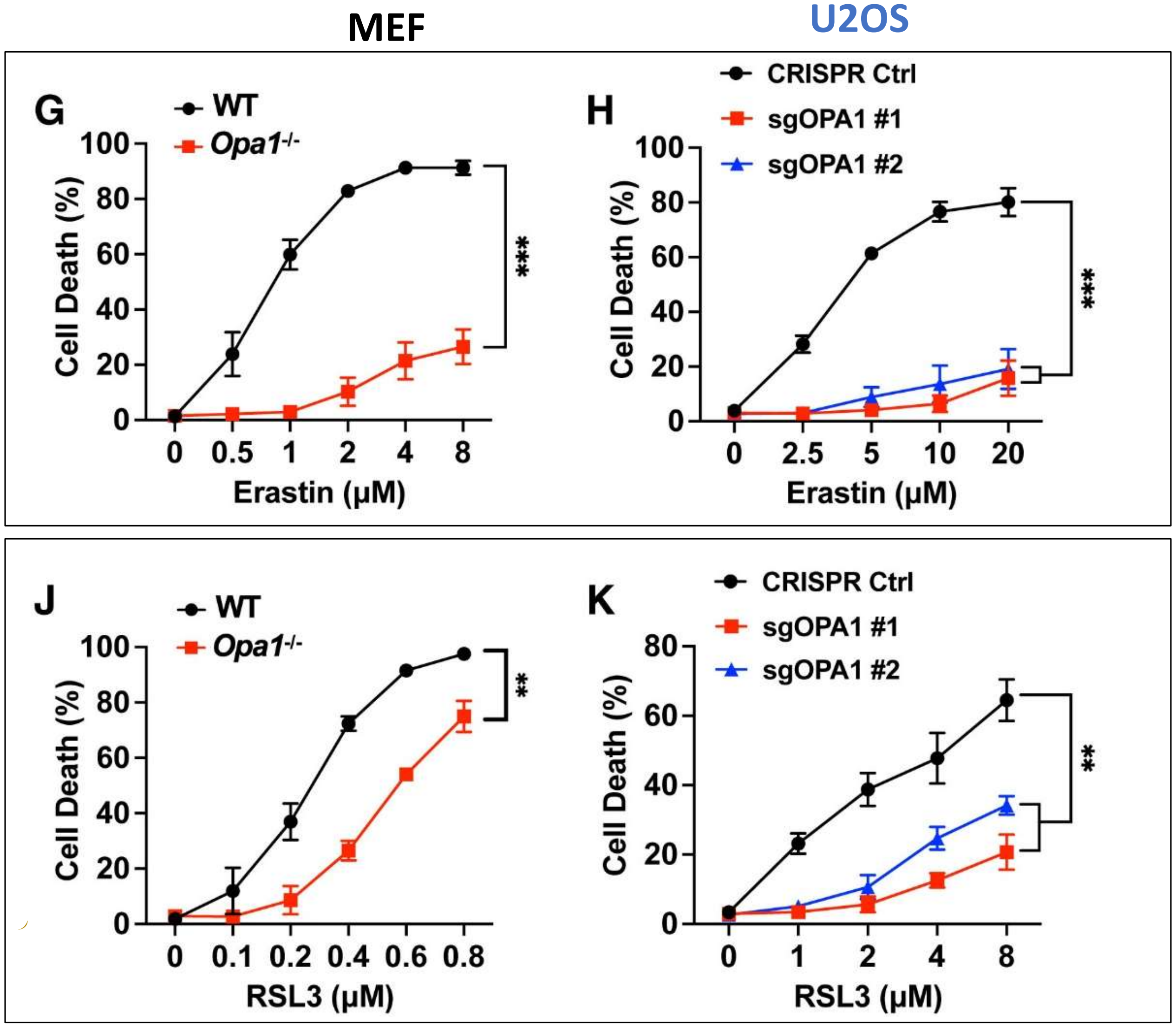
Cell death



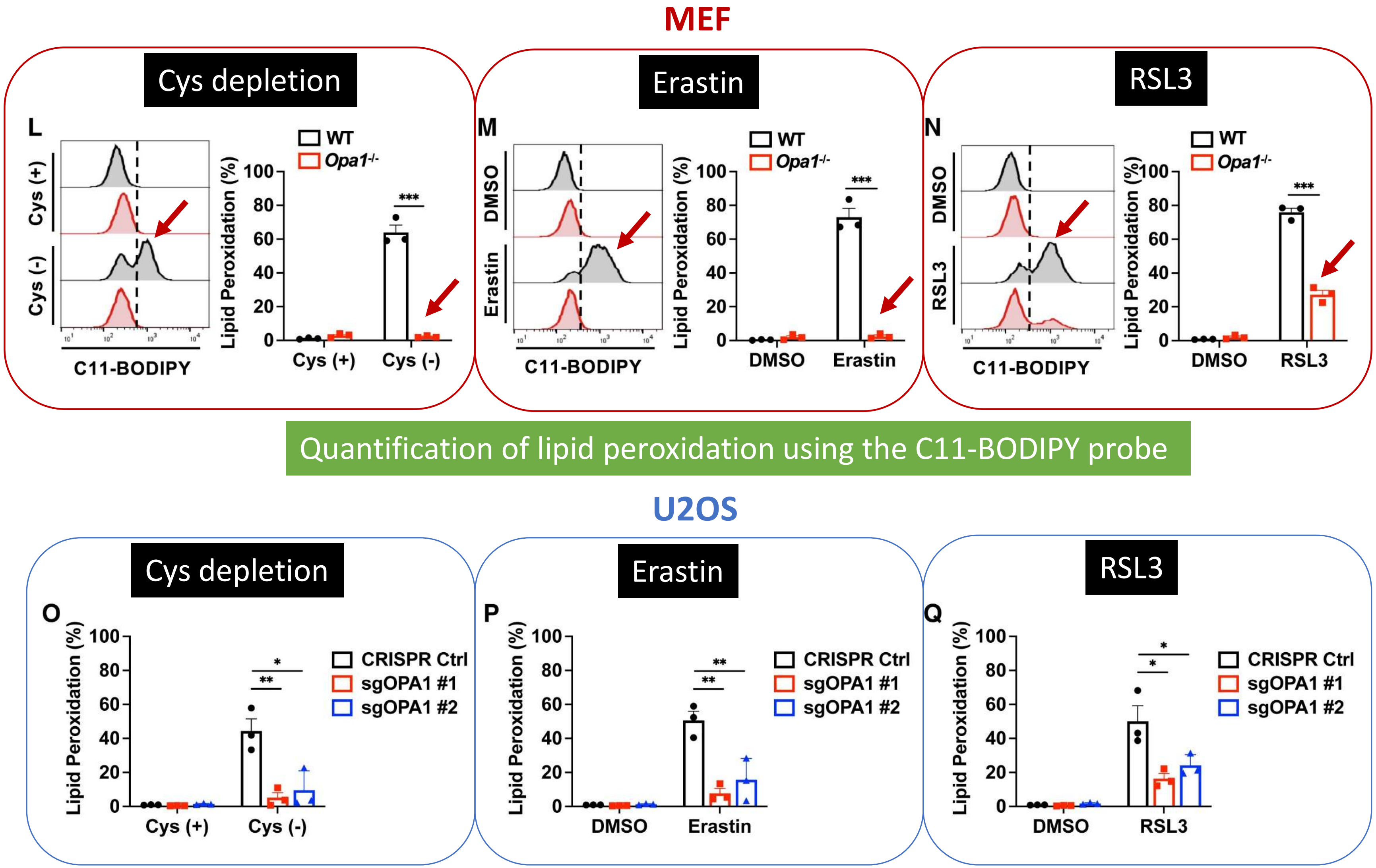
SYTOX Green is used to quantify cell death

The loss of OPA1 renders cells resistant to ferroptosis

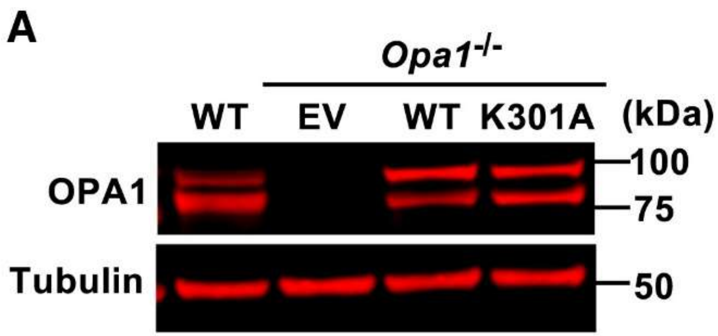




The loss of OPA1 renders cells resistant to ferroptosis

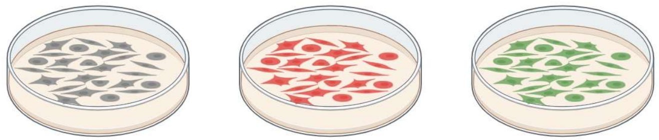


Is the GTPase activity of OPA1 required for sensitizing cells to ferroptosis?

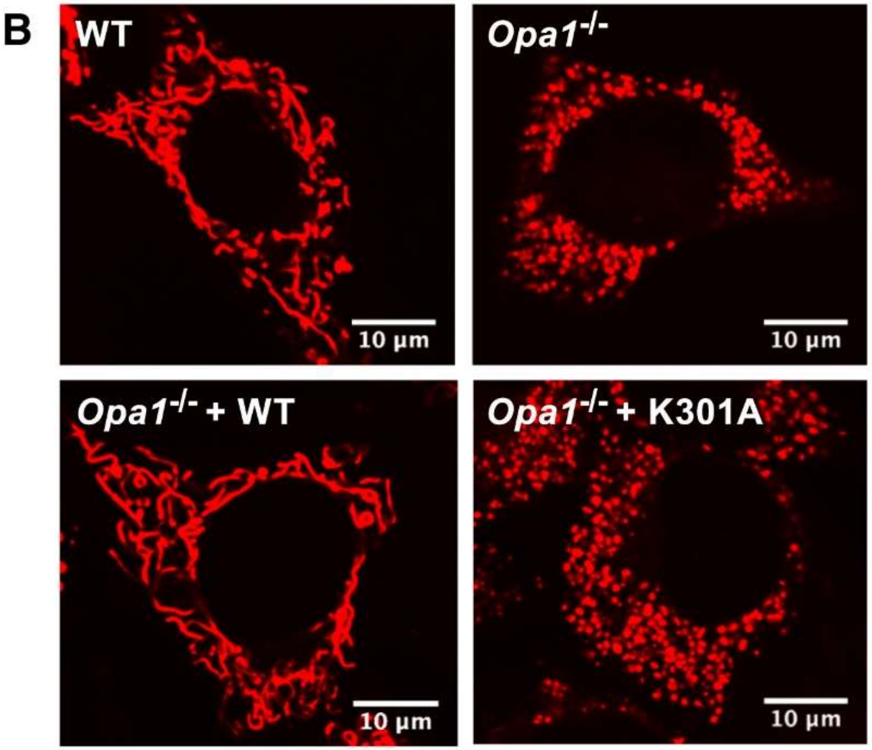


EV: empty vector
OPA1 K301A is a GTPase-defective mutant

Opa1-/- MEF cells



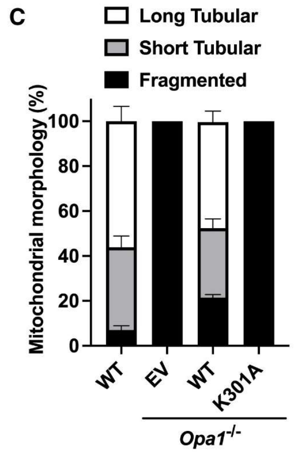
EV   wtOPA1   OPA1 K301A



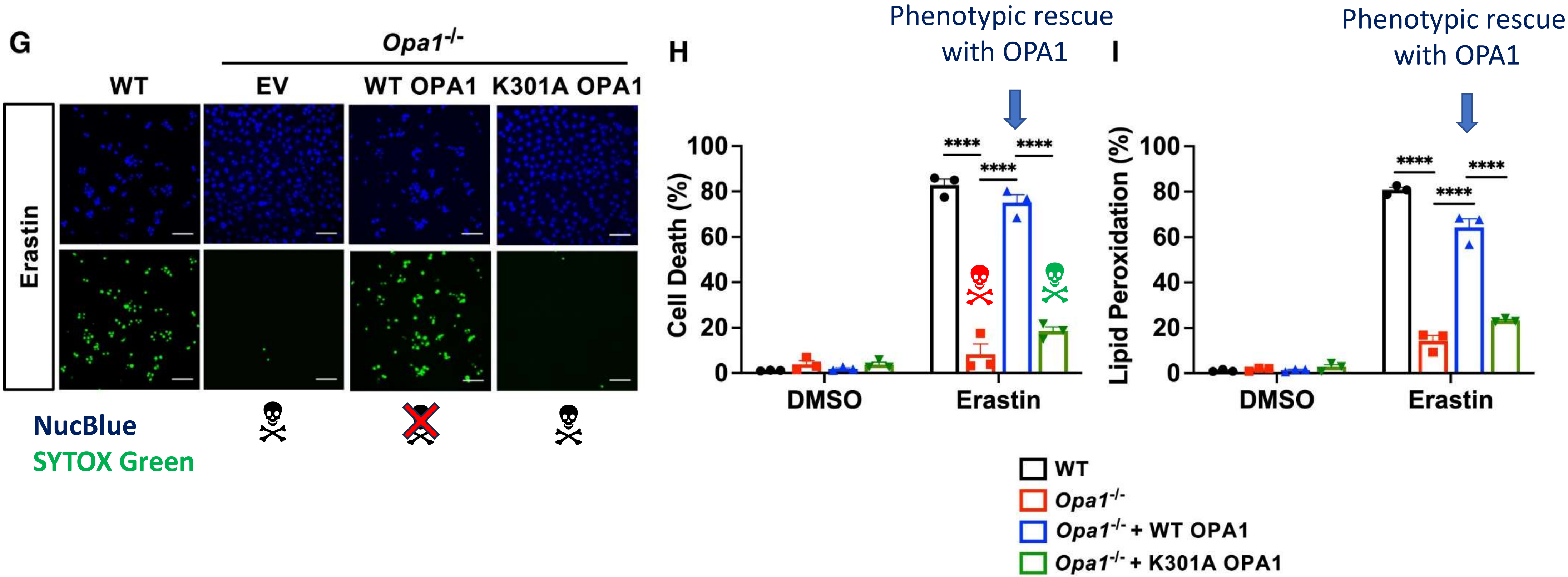
Phenotypic rescue with OPA1

No phenotypic rescue with OPA1 K301A

Fragmented mitochondrial network



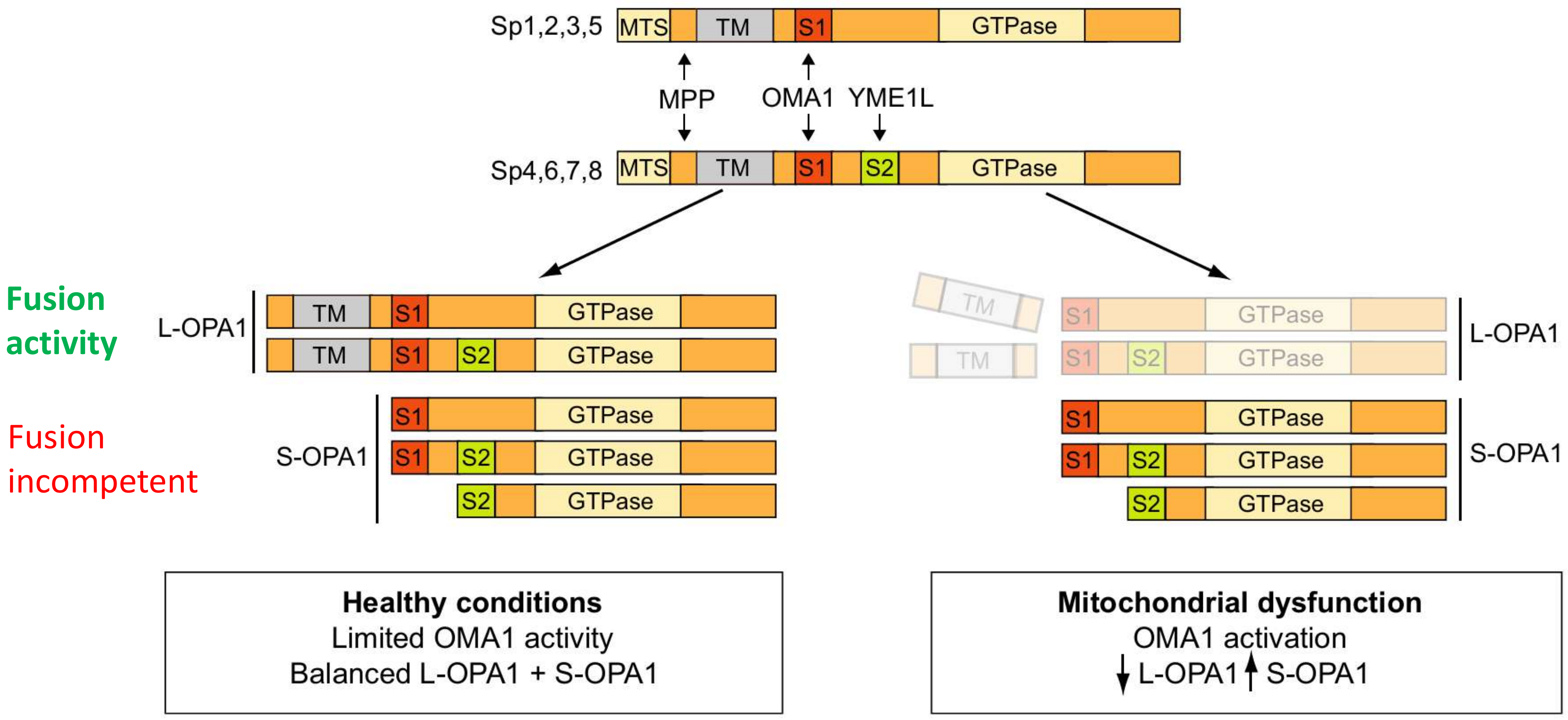
Is the GTPase activity of OPA1 required for sensitizing cells to ferroptosis?



Similar results were obtained with cysteine depletion and RSL3

Role of OPA1 processing

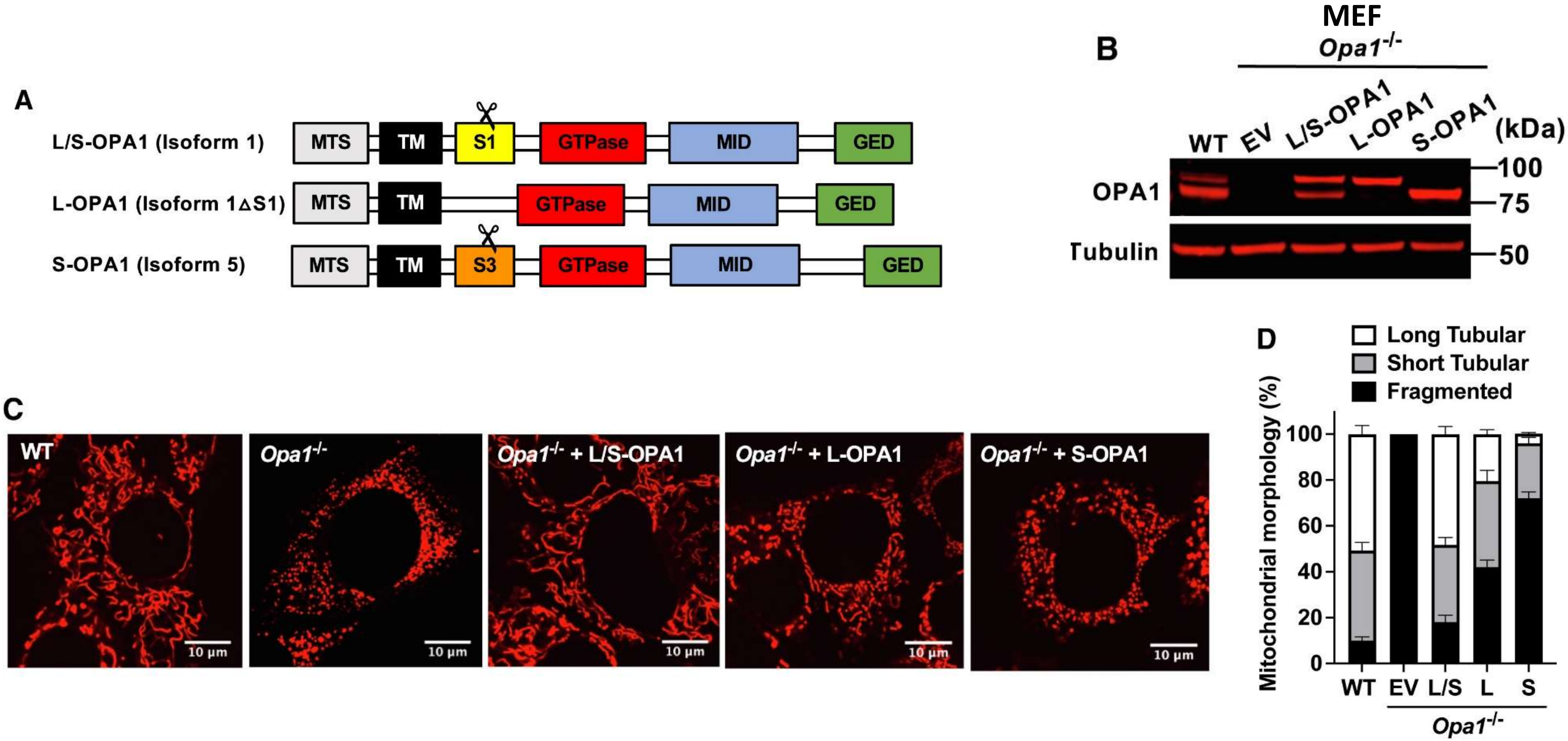
8 variants resulting from alternative splicing (Sp1-8)



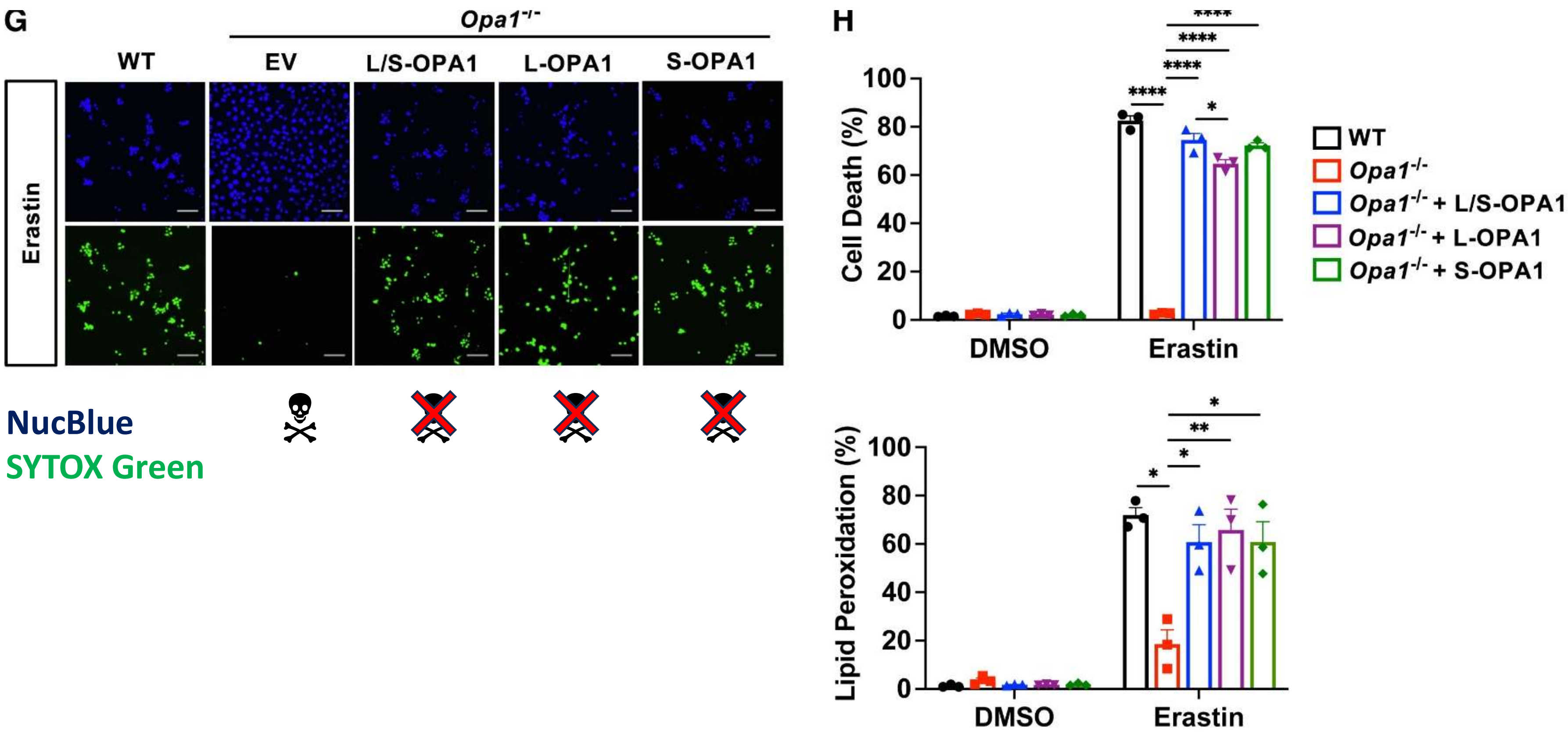
MPP: mitochondrial processing peptidase
Sp: splicing variant

Thomas MacVicar, Thomas Langer; OPA1 processing in cell death and disease – the long and short of it. *J Cell Sci* 15 June 2016; 129 (12): 2297–2306. doi: https://doi.org/10.1242/jcs.159186

OPA1 proteolysis regulates mitochondrial shape

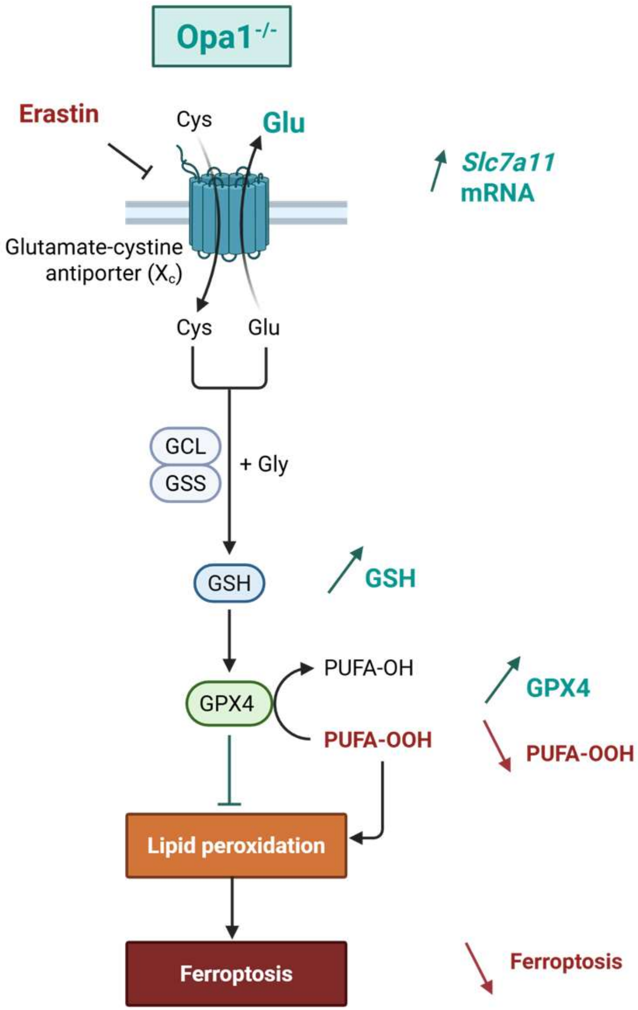


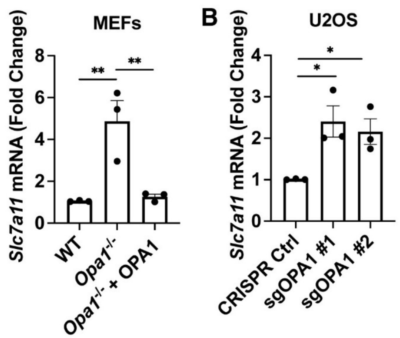
The ability of OPA1 to sensitize cells to ferroptosis is independent of its ability to promote mitochondrial fusion

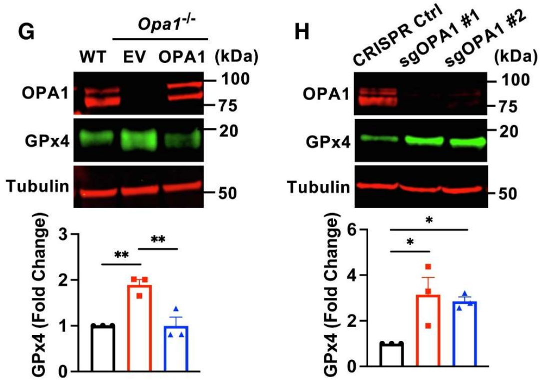


Long and short forms of OPA1 sensitize cells to ferroptosis

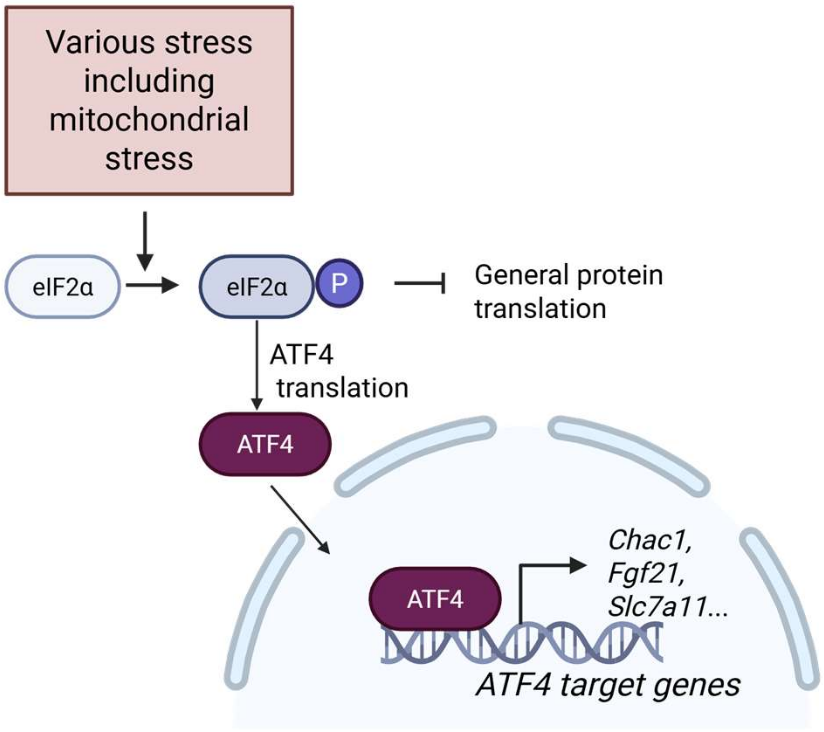
Mechanisms underlying OPA1-mediated sensitization to ferroptosis

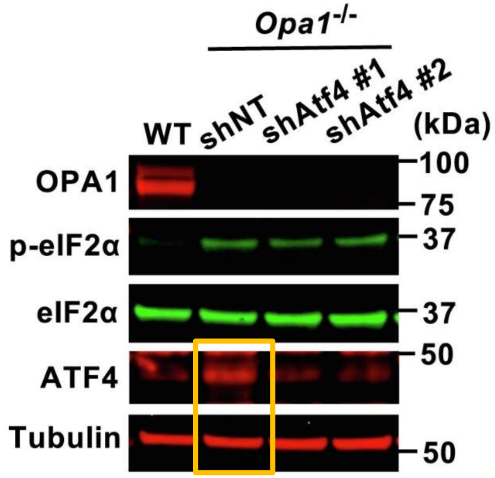




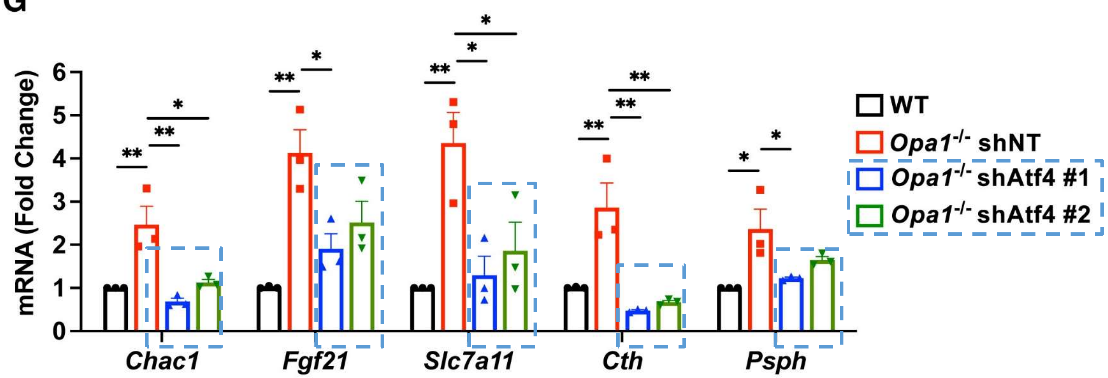


ATF4 mediates *Slc7a11* upregulation in *Opa1*$^{-/-}$ cells





G



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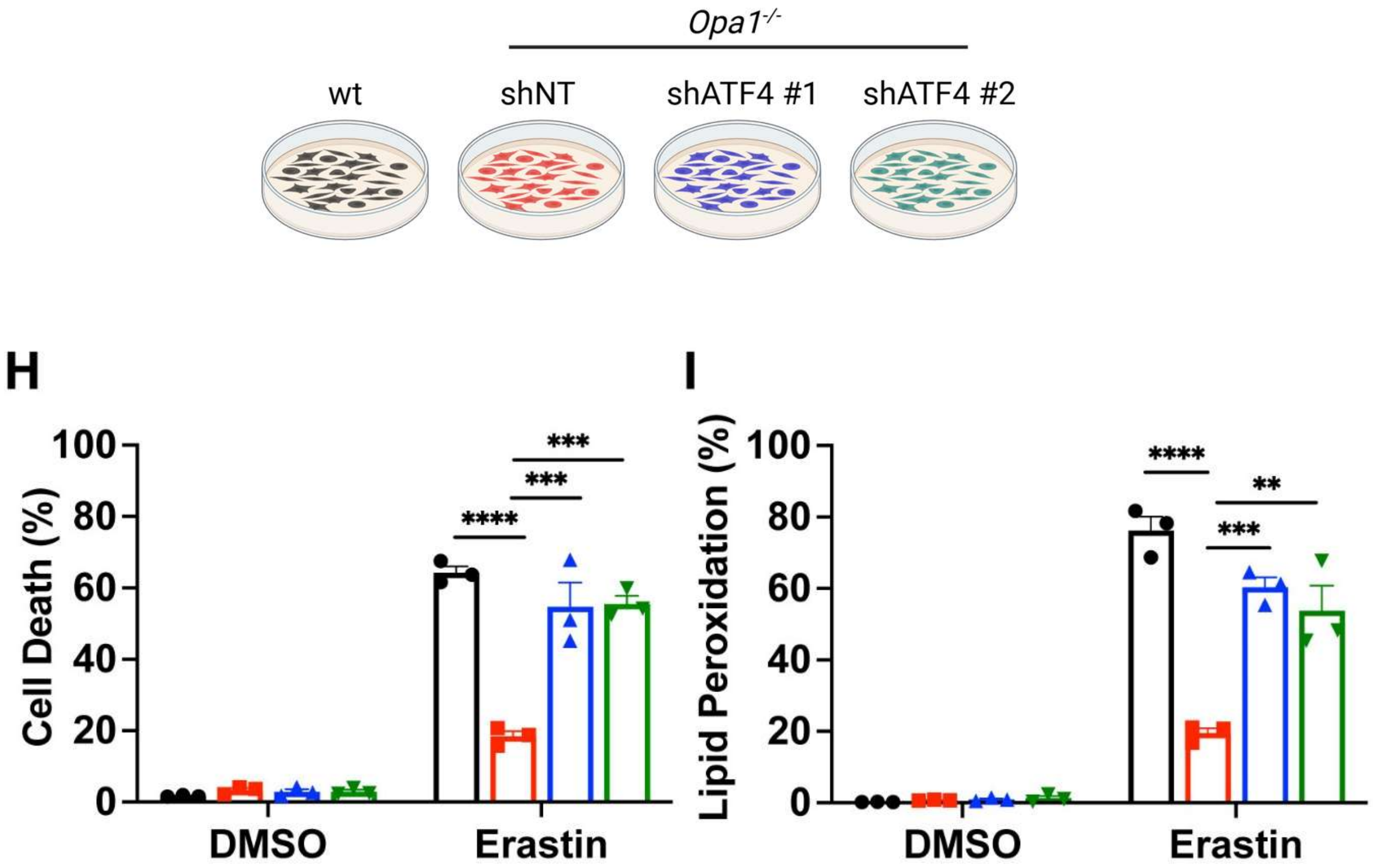
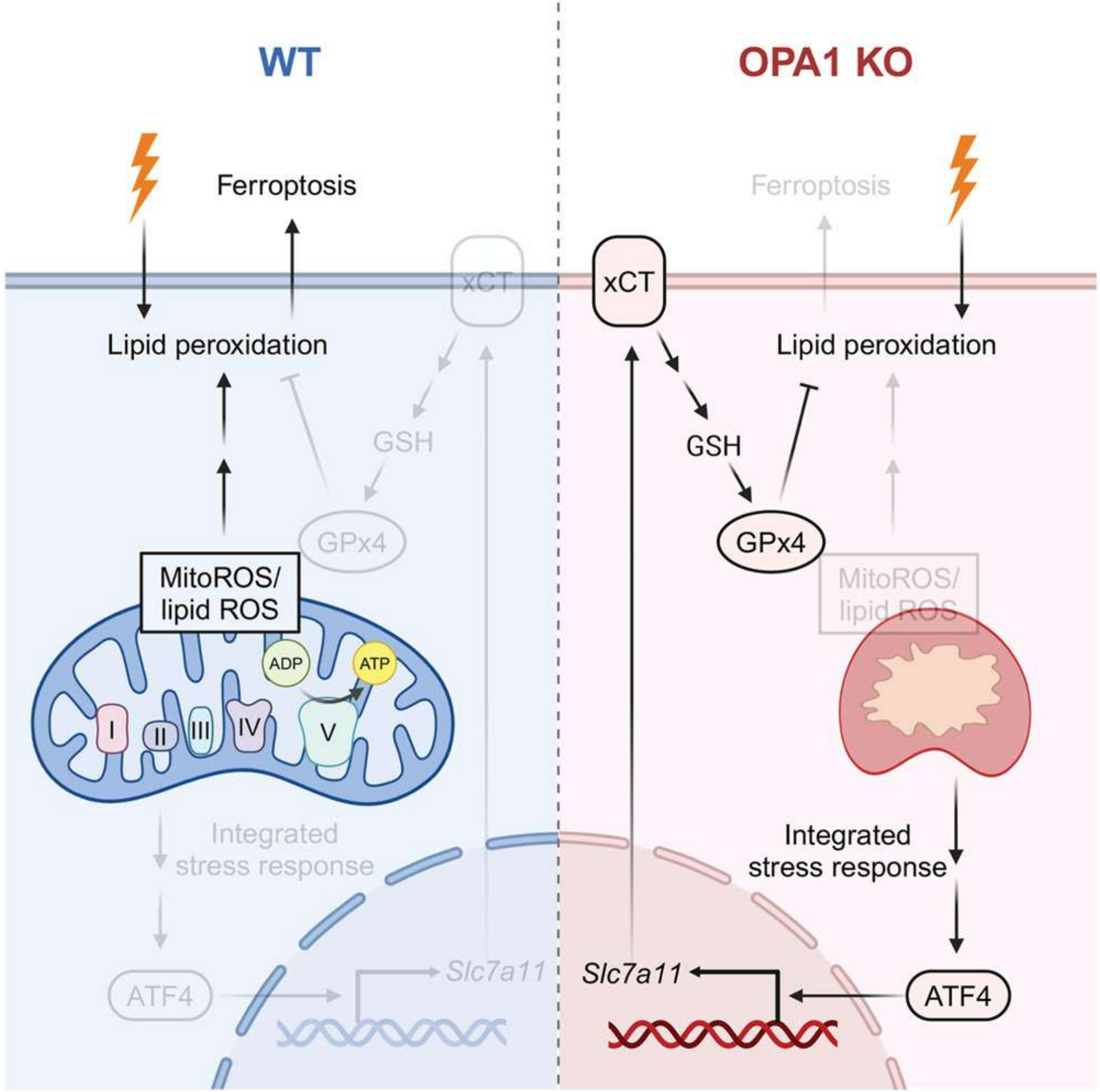
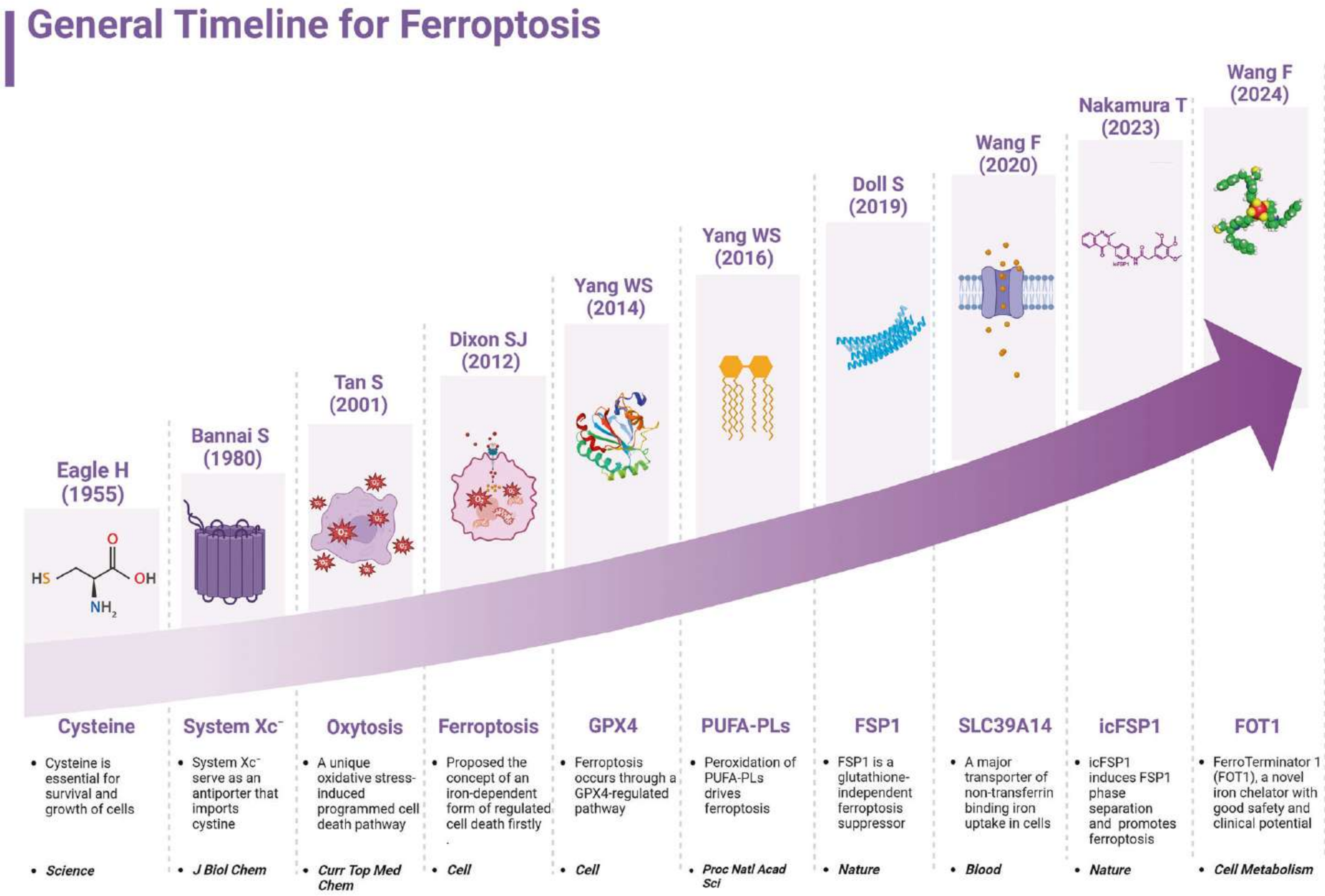
ATF4 promotes resistance to ferroptosis in *Opa1*$^{-/-}$ cells




WT
OPA1 KO
Ferroptosis
Lipid peroxidation
xCT
GSH
GPx4
MitoROS/
lipid ROS
ADP
ATP
I
II
III
IV
V
Integrated
stress response
ATF4
Slc7a11
Ferroptosis
xCT
Lipid peroxidation
GSH
GPx4
MitoROS/
lipid ROS
Integrated
stress response
Slc7a11
ATF4

Take home message

- Ferroptosis is a physiological cell death
- Lipid peroxidation is the main feature of ferroptosis
- An imbalance in iron homeostasis can trigger ferroptosis
- Ferroptosis arises when surveillance pathways are defective
- GPX4 is a central regulator of ferroptosis
- FINs have different modes of action
- Many regulators of ferroptosis have been identified and could serve as targets for therapy



Ru *et al.*, 2024 https://doi.org/10.1038/s41392-024-01969-z